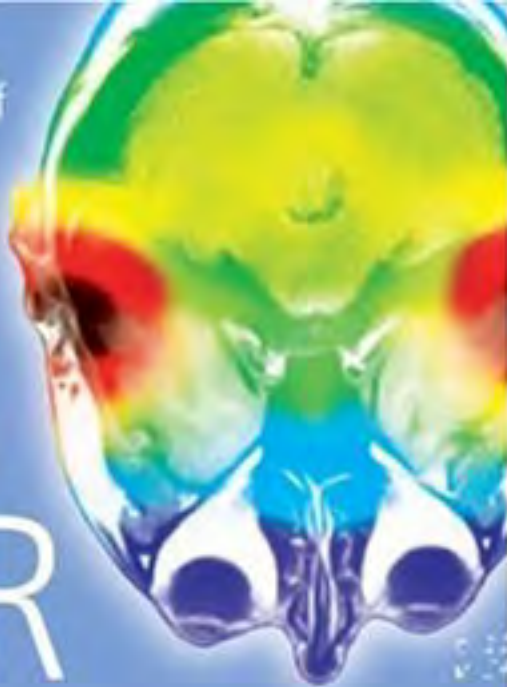


Robin A. de Graaf



*in vivo*  
**NMR**  
**Spectroscopy**

Principles and Techniques

Second Edition

 **WILEY**



# ***In Vivo* NMR Spectroscopy – 2nd Edition**

# *In Vivo* **NMR Spectroscopy** – 2nd Edition **Principles and Techniques**

ROBIN A. DE GRAAF  
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# Preface

Since the first edition of this textbook, published in 1998, the field of *in vivo* NMR spectroscopy has seen continued development of new techniques and applications, while at the same time some of the older techniques have become obsolete. One of the driving forces to write a second edition was to review some of these novel developments, such as hyperpolarized NMR, dynamic  $^{13}\text{C}$  NMR, automated shimming and parallel acquisitions. To maintain the flow of the book, several of the older techniques that have limited merits in modern *in vivo* NMR were removed. A second driving force was provided by the need for a textbook to be used in conjunction with a teaching course on *in vivo* NMR. In order to pursue this objective, most techniques are described from an educational point of view, without losing the practical aspects appreciated by experimental NMR spectroscopists. Furthermore, each chapter is concluded with a number of exercises designed to review, but often also to extend, the presented NMR principles and techniques.

Many of the ideas and changes that formed the basis for this second edition came from numerous discussions with colleagues. I would like to thank Douglas Rothman, Terry Nixon, Graeme Mason, Kevin Behar, Peter Brown and Kevin Koch for many fruitful discussions. Special thanks go to Christoph Juchem for his many insightful comments and careful reading of all chapters.

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# Abbreviations and Symbols

|         |                                                                    |
|---------|--------------------------------------------------------------------|
| AC      | Alternating current                                                |
| Ace     | Acetate                                                            |
| ADC     | Analog-to-digital converter                                        |
| ADC     | Apparent diffusion coefficient                                     |
| ADP     | Adenosine diphosphate                                              |
| AFP     | Adiabatic full passage                                             |
| AHP     | Adiabatic half passage                                             |
| Ala     | Alanine                                                            |
| Asc     | Ascorbic acid                                                      |
| Asp     | Aspartate                                                          |
| ATP     | Adenosine triphosphate                                             |
| BAPTA   | 1,2-Bis-(o-aminophenoxy)ethane- <i>N,N,N',N'</i> -tetraacetic acid |
| BHB     | $\beta$ -Hydroxybutyrate                                           |
| BIR     | B <sub>1</sub> -insensitive rotation                               |
| BISEP   | B <sub>1</sub> -insensitive spectral editing pulse                 |
| BOLD    | Blood oxygen level dependent                                       |
| BPP     | Bloembergen–Purcell–Pound                                          |
| BURP    | Band-selective pulses with uniform response and pure phase         |
| CHES    | Chemical shift selective                                           |
| Cho     | Choline containing compounds                                       |
| CK      | Creatine kinase                                                    |
| COSY    | Correlation spectroscopy                                           |
| CPMG    | Carr–Purcell–Meiboom–Gill                                          |
| Cr      | Creatine                                                           |
| CRLB    | Cramer–Rao lower bound                                             |
| CSF     | Cerebrospinal fluid                                                |
| CT      | Constant time                                                      |
| CW      | Continuous wave                                                    |
| CYCLOPS | Cyclically ordered phase sequence                                  |
| 1D      | One-dimensional                                                    |

|         |                                                          |
|---------|----------------------------------------------------------|
| 2D      | Two-dimensional                                          |
| 3D      | Three-dimensional                                        |
| DANTE   | Delays alternating with nutation for tailored excitation |
| dB      | Decibel                                                  |
| DC      | Direct current                                           |
| DEFT    | Driven equilibrium Fourier transform                     |
| DEPT    | Distortionless enhancement by polarization transfer      |
| DMb     | Deoxymyoglobin                                           |
| DNA     | Deoxyribonucleic acid                                    |
| DNP     | Dynamic nuclear polarization                             |
| DQC     | Double quantum coherence                                 |
| DSS     | 2,2-Dimethyl-2-silapentane-5-sulfonate                   |
| EA      | Ethanolamine                                             |
| EMCL    | Extramyocellular lipids                                  |
| EMF     | Electromotive force                                      |
| EPI     | Echo planar imaging                                      |
| FDG     | 2-Fluoro-2-deoxy-glucose                                 |
| FDG-6P  | 2-Fluoro-2-deoxy-glucose-6-phosphate                     |
| FID     | Free induction decay                                     |
| FLASH   | Fast low-angle shot                                      |
| FFT     | Fast Fourier transformation                              |
| FOCI    | Frequency offset corrected inversion                     |
| FOV     | Field of view                                            |
| FSW     | Fourier series windows                                   |
| FT      | Fourier transformation                                   |
| FWHM    | Frequency width at half maximum                          |
| 5-FU    | 5-Fluorouracil                                           |
| 5'dFUrd | 5'-Deoxy-fluorouridine                                   |
| GABA    | $\gamma$ -Aminobutyric acid                              |
| GE      | Gradient echo                                            |
| Glc     | Glucose                                                  |
| Gln     | Glutamine                                                |
| Glu     | Glutamate                                                |
| Glx     | Glutamine and glutamate                                  |
| Gly     | Glycine                                                  |
| GPC     | Glycerophosphorylcholine                                 |
| GPE     | Glycerophosphorylethanolamine                            |
| GSH     | Glutathione (reduced form)                               |
| HLSVD   | Hankel Lanczos singular value decomposition              |
| HMPT    | Hexamethylphosphorotriamide                              |
| HMQC    | Heteronuclear multiple quantum correlation               |
| HSQC    | Heteronuclear single quantum correlation                 |
| INEPT   | Insensitive nuclei enhanced by polarization transfer     |
| IMCL    | Intramyocellular lipids                                  |
| IR      | Inversion recovery                                       |
| ISIS    | Image-selected <i>in vivo</i> spectroscopy               |

|                |                                                                |
|----------------|----------------------------------------------------------------|
| IT             | Inversion transfer                                             |
| JR             | Jump-return                                                    |
| Lac            | Lactate                                                        |
| Mb             | Myoglobin                                                      |
| MEOP           | Metastability exchange optical pumping                         |
| mI             | <i>Myo</i> -inositol                                           |
| MLEV           | Malcolm Levitt                                                 |
| MQC            | Multiple quantum coherence                                     |
| MRI            | Magnetic resonance imaging                                     |
| MRS            | Magnetic resonance spectroscopy                                |
| MRSI           | Magnetic resonance spectroscopic imaging                       |
| MT             | Magnetization transfer                                         |
| MTC            | Magnetization transfer contrast                                |
| NAA            | <i>N</i> -Acetyl aspartate                                     |
| NAAG           | <i>N</i> -Acetyl aspartyl glutamate                            |
| NAD(H)         | Nicotinamide adenine dinucleotide oxidized (reduced)           |
| NADP(H)        | Nicotinamide adenine dinucleotide phosphate oxidized (reduced) |
| NDP            | Nucleoside diphosphate                                         |
| nOe            | Nuclear Overhauser effect                                      |
| NOESY          | Nuclear Overhauser effect spectroscopy                         |
| NMR            | Nuclear magnetic resonance                                     |
| NTP            | Nucleoside triphosphate                                        |
| OVS            | Outer volume suppression                                       |
| PCA            | Perchloric acid                                                |
| PCr            | Phosphocreatine                                                |
| PDE            | Phosphodiesterases                                             |
| PE             | Phosphorylethanolamine                                         |
| PET            | Positron emission tomography                                   |
| PHIP           | Para-hydrogen-induced polarization                             |
| P <sub>i</sub> | Inorganic phosphate                                            |
| PME            | Phosphomonoesters                                              |
| POCE           | Proton-observed carbon-edited                                  |
| PPM            | Parts per million                                              |
| PRESS          | Point resolved spectroscopy                                    |
| PSF            | Point spread function                                          |
| QUALITY        | Quantification by converting lineshapes to the Lorentzian type |
| RAHP           | Time-reversed adiabatic half passage                           |
| RMS            | Root mean squared                                              |
| RNA            | Ribonucleic acid                                               |
| ROI            | Region of interest                                             |
| RF             | Radiofrequency                                                 |
| SAR            | Specific absorption rate                                       |
| SE             | Spin echo                                                      |
| SEOP           | Spin-exchange optical pumping                                  |
| SI             | Spectroscopic imaging                                          |
| sI             | <i>Scyllo</i> -inositol                                        |

|                   |                                                                              |
|-------------------|------------------------------------------------------------------------------|
| SLR               | Shinnar-Le Roux                                                              |
| S/N               | Signal-to-noise ratio                                                        |
| SNR               | Signal-to-noise ratio                                                        |
| SQC               | Single quantum coherence                                                     |
| SSAP              | Solvent suppression adiabatic pulse                                          |
| ST                | Saturation transfer                                                          |
| STE               | Stimulated echo                                                              |
| STEAM             | Stimulated echo acquisition mode                                             |
| SV                | Single voxel (or volume)                                                     |
| SVD               | Singular value decomposition                                                 |
| Tau               | Taurine                                                                      |
| TCA               | Tricarboxylic acid                                                           |
| tCho              | Total choline                                                                |
| tCr               | Total creatine                                                               |
| Thr               | Threonine                                                                    |
| TMA               | Trimethylammonium                                                            |
| TMS               | Tetramethylsilane                                                            |
| TOCSY             | Total correlation spectroscopy                                               |
| TPPI              | Time proportional phase incrementation                                       |
| Trp               | Tryptophan                                                                   |
| TSP               | 3-(Trimethylsilyl)-propionate                                                |
| Tyr               | Tyrosine                                                                     |
| UV                | Ultraviolet                                                                  |
| Val               | Valine                                                                       |
| VAPOR             | Variable pulse powers and optimized relaxation delays                        |
| VARPRO            | Variable projection                                                          |
| VERSE             | Variable rate selective excitation                                           |
| VOI               | Volume of interest                                                           |
| VNA               | Variable nutation angle                                                      |
| VSE               | Volume selective excitation                                                  |
| WALTZ             | Wideband alternating phase low-power technique<br>for zero residue splitting |
| WEFT              | Water eliminated Fourier transform                                           |
| ZQC               | Zero quantum coherence                                                       |
| a                 | Acceleration (in $\text{m s}^{-2}$ )                                         |
| A                 | Absorption frequency domain signal                                           |
| $A_n, B_n$        | Fourier coefficients                                                         |
| b                 | b-value (in $\text{s m}^{-2}$ )                                              |
| <b>b</b>          | b-value matrix                                                               |
| $B_0$             | External magnetic field (in T)                                               |
| $B_1$             | Magnetic radiofrequency field of the transmitter (in T)                      |
| $B_{1\text{ax}}$  | Axial amplitude of the irradiating $B_1$ field (in T)                        |
| $B_{1\text{max}}$ | Maximum amplitude of the irradiating $B_1$ field (in T)                      |
| $B_{1\text{rad}}$ | Radial amplitude of the irradiating $B_1$ field (in T)                       |
| $B_{1\text{rms}}$ | Root mean square $B_1$ amplitude of a radiofrequency pulse (in T)            |
| $B_{1x}, B_{1y}$  | Real and imaginary components of the irradiating $B_1$ field (in T)          |

|                  |                                                                                                                      |
|------------------|----------------------------------------------------------------------------------------------------------------------|
| $B_2$            | Magnetic, radiofrequency field of the decoupler (in T)                                                               |
| $B_e$            | Effective magnetic field in the laboratory and frequency frames (in T)                                               |
| $B'_e$           | Effective magnetic field in the second rotating frame (in T)                                                         |
| $B_{loc}$        | Local magnetic field (in T)                                                                                          |
| $C$              | Capacitance (in F)                                                                                                   |
| $C$              | Correction factor for calculating absolute concentrations                                                            |
| $D$              | Dispersion frequency domain signal                                                                                   |
| $D$              | (Apparent) diffusion coefficient (in $m^2 s^{-1}$ )                                                                  |
| $\mathbf{D}$     | (Apparent) diffusion tensor                                                                                          |
| $E$              | Energy (in J)                                                                                                        |
| $E_a$            | Activation energy (in J)                                                                                             |
| $f$              | Ratio of immobile spins to mobile spins                                                                              |
| $f$              | Filling factor                                                                                                       |
| $f_{ab}$         | Fraction of magnetization $M_a$ ( $a = x$ or $y$ ) converted to magnetization $M_b$ ( $b = x$ or $y$ ) by a RF pulse |
| $f_B(t)$         | Normalized radiofrequency amplitude modulation function                                                              |
| $f(t)$           | Normalized radiofrequency frequency modulation function                                                              |
| $F$              | Nyquist frequency (in $s^{-1}$ )                                                                                     |
| $F$              | Noise figure (in dB)                                                                                                 |
| $F$              | Force (in $kg m s^{-2}$ )                                                                                            |
| $G(t)$           | Correlation function                                                                                                 |
| $G$              | Magnetic field gradient strength (in $T m^{-1}$ )                                                                    |
| $h$              | Planck's constant ( $6.626208 \times 10^{-34} J s$ )                                                                 |
| $\mathbf{H}$     | Hadamard matrix                                                                                                      |
| $I$              | Imaginary time- or frequency domain signal                                                                           |
| $I$              | Refocused component                                                                                                  |
| $I$              | Spin quantum number                                                                                                  |
| $I_0$            | Boltzmann equilibrium magnetization for spin $I$                                                                     |
| $I_{nm}$         | Shim current for shim coil of order $nm$                                                                             |
| $J$              | Spin-spin or scalar coupling constant (in Hz)                                                                        |
| $J_0$            | Zero-order Bessel function                                                                                           |
| $J(\omega)$      | Spectral density function                                                                                            |
| $k$              | Boltzmann equilibrium constant ( $1.38066 \times 10^{-23} J K^{-1}$ )                                                |
| $k$              | $k$ -space variable (in $m^{-1}$ )                                                                                   |
| $k_f$            | $k$ -space variable in frequency-encoding direction (in $m^{-1}$ )                                                   |
| $k_p$            | $k$ -space variable in phase-encoding direction (in $m^{-1}$ )                                                       |
| $k_{AB}, k_{BA}$ | Unidirectional rate constants (in $s^{-1}$ )                                                                         |
| $k_{for}$        | Forward, unidirectional rate constant (in $s^{-1}$ )                                                                 |
| $k_{rev}$        | Reversed, unidirectional rate constant (in $s^{-1}$ )                                                                |
| $L$              | Inductance (in H)                                                                                                    |
| $L$              | Angular momentum (in $kg m^2 s^{-1}$ )                                                                               |
| $m$              | Mass (in kg)                                                                                                         |
| $m$              | Magnetic quantum number                                                                                              |
| $M$              | Magnitude-mode frequency domain signal                                                                               |
| $M$              | Mutual inductance (in H)                                                                                             |
| $M$              | Macroscopic magnetization                                                                                            |

|                    |                                                                                                                                        |
|--------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| $M_0$              | Macroscopic equilibrium magnetization                                                                                                  |
| $M_x, M_y, M_z$    | Orthogonal components of the macroscopic magnetization                                                                                 |
| $n$                | Total number of nuclei in a macroscopic sample                                                                                         |
| $n_\alpha n_\beta$ | Populations of the $\alpha$ and $\beta$ spin states                                                                                    |
| $N$                | Noise                                                                                                                                  |
| $N$                | Number of phase-encoding increments                                                                                                    |
| $p$                | Linear momentum (in $\text{kg m s}^{-1}$ )                                                                                             |
| $p$                | Order of coherence                                                                                                                     |
| $P_z$              | Component of angular momentum in z-direction                                                                                           |
| $Q$                | Quality factor                                                                                                                         |
| $r$                | Distance (in m)                                                                                                                        |
| $R$                | Composite pulse (sequence)                                                                                                             |
| $R$                | Product of bandwidth and pulselength                                                                                                   |
| $R$                | Real time- or frequency-domain signal                                                                                                  |
| $R$                | Resistance (in $\Omega$ )                                                                                                              |
| $\mathbf{R}$       | Rotation matrix                                                                                                                        |
| $R_{1A}, R_{1B}$   | Longitudinal relaxation rate constants for spins A and B in the absence of chemical exchange or cross relaxation (in $\text{s}^{-1}$ ) |
| $R_2$              | Transverse relaxation rate (in $\text{s}^{-1}$ )                                                                                       |
| $R_A, R_B$         | Longitudinal relaxation rate constants for spins A and B in the presence of chemical exchange (in $\text{s}^{-1}$ )                    |
| $R_H$              | Hydrodynamic radius (in m)                                                                                                             |
| $S$                | Measured NMR signal                                                                                                                    |
| $S(k)$             | Spatial frequency sampling function                                                                                                    |
| $t$                | Time (in s)                                                                                                                            |
| $t_1$              | Incremented time in 2D NMR experiments (in s)                                                                                          |
| $t_{1\text{max}}$  | Maximum $t_1$ period in constant time 2D NMR experiments (in s)                                                                        |
| $t_2$              | Detection period in 2D NMR experiments (in s)                                                                                          |
| $t_{\text{diff}}$  | Diffusion time (in s)                                                                                                                  |
| $t_{\text{null}}$  | Time of zero-crossing (nulling) during an inversion recovery experiment (in s)                                                         |
| $T$                | Absolute temperature (in K)                                                                                                            |
| $T$                | Torque (in $\text{kg m}^2 \text{s}^{-2}$ )                                                                                             |
| $T$                | Pulse length (in s)                                                                                                                    |
| $T_1$              | Longitudinal relaxation time constant (in s)                                                                                           |
| $T_1^*$            | Apparent longitudinal relaxation time constant (in s)                                                                                  |
| $T_{1,\text{obs}}$ | Observed, longitudinal relaxation time constant (in s)                                                                                 |
| $T_2$              | Transverse relaxation time constant (in s)                                                                                             |
| $T_2^*$            | Apparent transverse relaxation time constant (in s)                                                                                    |
| $T_{2,\text{obs}}$ | Observed, transverse relaxation time constant (in s)                                                                                   |
| $T_{\text{acq}}$   | Acquisition time (in s)                                                                                                                |
| $TE$               | Echo time (in s)                                                                                                                       |
| $TE_{\text{CPMG}}$ | Echo time in a CPMG experiment (in s)                                                                                                  |
| $TI$               | Inversion time (in s)                                                                                                                  |
| $TI1$              | First inversion time (in s)                                                                                                            |
| $TI2$              | Second inversion time (in s)                                                                                                           |

|                   |                                                                                                         |
|-------------------|---------------------------------------------------------------------------------------------------------|
| TM                | Delay time between the second and third 90° pulses in STEAM (in s)                                      |
| TR                | Repetition time (in s)                                                                                  |
| v                 | Velocity (in m s <sup>-1</sup> )                                                                        |
| W                 | Transition probability (in s <sup>-1</sup> )                                                            |
| W <sub>nm</sub>   | Angular function of spherical polar coordinates                                                         |
| W(k)              | Spatial frequency weighting function                                                                    |
| x                 | Molar fraction                                                                                          |
| X <sub>C</sub>    | Capacitive reactance (in Ω)                                                                             |
| X <sub>L</sub>    | Inductive reactance (in Ω)                                                                              |
| Z                 | Impedance (in Ω)                                                                                        |
| α                 | Nutation angle (in rad)                                                                                 |
| β                 | Precession angle of magnetization perpendicular to the effective magnetic field B <sub>e</sub> (in rad) |
| γ                 | Gyromagnetic ratio (in rad T <sup>-1</sup> s <sup>-1</sup> )                                            |
| δ                 | Chemical shift (in ppm)                                                                                 |
| δ                 | Gradient duration (in s)                                                                                |
| Δ                 | Separation between a pair of gradients (in s)                                                           |
| ΔB <sub>0</sub>   | Magnetic field shift (in T)                                                                             |
| Δν <sub>1/2</sub> | Full width at half maximum of an absorption line (in Hz)                                                |
| Δω                | Frequency offset (in Hz)                                                                                |
| Δω <sub>max</sub> | Maximum frequency modulation of an adiabatic radiofrequency pulse (in Hz)                               |
| ΔΩ                | Frequency offset (in Hz)                                                                                |
| ε                 | Gradient risetime for a trapezoidal magnetic field gradient (in s)                                      |
| η                 | Nuclear Overhauser enhancement                                                                          |
| η                 | Viscosity (in N s m <sup>-2</sup> )                                                                     |
| θ                 | Nutation angle (in rad)                                                                                 |
| μ                 | Magnetic moment (in A m <sup>2</sup> )                                                                  |
| μ <sub>0</sub>    | Permeability constant in vacuum (4π 10 <sup>-7</sup> kg m s <sup>-2</sup> A <sup>-2</sup> )             |
| μ <sub>e</sub>    | Electronic magnetic moment (in A m <sup>2</sup> )                                                       |
| μ <sub>z</sub>    | Component of magnetic moment in z direction                                                             |
| ν <sub>0</sub>    | Larmor frequency (in Hz)                                                                                |
| ν <sub>A</sub>    | Frequency of a unprotonated compound A (in Hz)                                                          |
| ν <sub>HA</sub>   | Frequency of a protonated compound HA (in Hz)                                                           |
| ν <sub>ref</sub>  | Reference frequency (in Hz)                                                                             |
| ξ                 | Electromotive force (in V)                                                                              |
| σ                 | Magnetic shielding constant (in ppm)                                                                    |
| σ                 | Density matrix                                                                                          |
| τ <sub>c</sub>    | Rotation correlation time (in s)                                                                        |
| τ <sub>m</sub>    | Mixing time in 2D NMR experiments (in s)                                                                |
| φ                 | Phase (in rad)                                                                                          |
| φ <sub>c</sub>    | Phase correction (in rad)                                                                               |
| φ <sub>0</sub>    | Zero-order (constant) phase (in rad)                                                                    |
| φ <sub>1</sub>    | First-order (linear) phase (in rad)                                                                     |
| χ                 | Magnetic susceptibility                                                                                 |
| ω <sub>0</sub>    | Larmor frequency (in rad s <sup>-1</sup> )                                                              |
| [ ]               | Concentration (in M)                                                                                    |

# 1

## Basic Principles

### 1.1 Introduction

The field of spectroscopy is in general concerned with the interaction between matter and electromagnetic radiation. Atoms and molecules have a range of discrete energy levels corresponding to different electronic, vibrational or rotational states. The interaction between atoms and electromagnetic radiation is characterized by the absorption and emission of photons, such that the energy of the photons exactly matches an energy level difference in the atom. Since the energy of a photon is proportional to the frequency, the different forms of spectroscopy are often distinguished on the basis of the frequencies involved. For instance, absorption and emission between electronic states of the outer electrons typically require frequencies in the ultraviolet (UV) range, hence giving rise to UV spectroscopy. Molecular vibrational modes are characterized by frequencies just below visible red light and are thus studied with infrared (IR) spectroscopy. Nuclear magnetic resonance (NMR) spectroscopy uses radiofrequencies, which are typically in the range of 10–800 MHz.

NMR is the study of the magnetic properties (and energies) of nuclei. The absorption and emission of electromagnetic radiation can be observed when the nuclei are placed in a (strong) external magnetic field. Purcell, Torrey and Pound [1] at MIT, Cambridge and Bloch, Hansen and Packard [2] at Stanford simultaneously, but independently discovered NMR in 1946. In 1952 Bloch and Purcell shared the Nobel Prize for physics in recognition of their pioneering achievements [1–4]. At this stage, NMR was purely an experiment for physicists to determine the nuclear magnetic moments of nuclei. NMR could only develop into one of the most versatile forms of spectroscopy after the discovery that nuclei within the same molecule absorb energy at different resonance frequencies. These so-called chemical shift effects, which are directly related to the chemical environment of the nuclei, were first observed in 1950 by Proctor and Yu [5], and independently by Dickinson [6].

In the first two decades, NMR spectra were recorded in a continuous wave mode in which the magnetic field strength or the radiofrequency (RF) was swept through the spectral area



of interest, whilst keeping the other fixed. In 1966, NMR was revolutionized by Ernst and Anderson [7] who introduced pulsed NMR in combination with Fourier transformation. Pulsed or Fourier transform NMR is at the heart of all modern NMR experiments.

The induced energy level difference of nuclei in an external magnetic field is very small when compared with the thermal energy, making it that the energy levels are almost equally populated. As a result the absorption of photons is very low, making NMR a very insensitive technique when compared with the other forms of spectroscopy. However, the low energy absorption makes NMR also a noninvasive and nondestructive technique, ideally suited for *in vivo* measurements. It is believed that, by observing the water signal from his own finger, Bloch was the first to use NMR on a living system. Soon after the discovery of NMR, others showed the utility of using NMR to study living objects. In 1950, Shaw and Elsken [8] used proton NMR to investigate the water content of vegetable material. Odebal and Lindstrom [9] obtained proton NMR signals from a number of mammalian preparations in 1955. Continued interest in defining and explaining the properties of water in biological tissues led to the promising report of Damadian in 1971 [10] that NMR properties (relaxation times) of malignant tumorous tissue significantly differs from normal tissue, suggesting that (proton) NMR may have diagnostic value. In the early 1970s, the first experiments of NMR spectroscopy on intact living tissues were reported. Moon and Richards [11] used  $^{31}\text{P}$  NMR on intact red blood cells and showed how the intracellular pH can be determined from chemical shift differences. In 1974, Hoult *et al.* [12] reported the first study of  $^{31}\text{P}$  NMR to study intact, excised rat hind leg.

Around the same time reports on *in vivo* NMR spectroscopy appeared, Lauterbur [13] and Mansfield and Grannell [14] described the first reports of a major application of modern NMR, namely *in vivo* NMR imaging or magnetic resonance imaging (MRI). By applying position dependent magnetic fields in addition to the static magnetic field, they were able to reconstruct the spatial distribution of the spins in the form of an image. Lauterbur and Mansfield shared the 2003 Nobel Prize in medicine. *In vivo* NMR spectroscopy or magnetic resonance spectroscopy (MRS) and MRI have evolved from relatively simple one or two RF pulse sequences to complex techniques involving spatial localization, water and lipid suppression and spectral editing for MRS and time-varying magnetic field gradients, ultra fast and multiparametric acquisition schemes for MRI.

In this chapter the basic phenomenon of NMR is considered. After establishing the Larmor resonance condition with a combination of classical and quantum mechanical arguments, the NMR phenomenon is approached from a more practical point of view with the aid of the macroscopic Bloch equations. The phenomena of chemical shift, scalar coupling and spin echoes will be described, as well as some elementary processing of the NMR signal.

## 1.2 Classical Description

NMR is based on the concept of nuclear spin. Before discussing the properties of nuclear spins, some relations from classical physics will be introduced which will simplify further discussions. Although classical physics is incapable of describing the quantum mechanical spin, it can be used to create a familiar frame of reference in which the existence of a spin angular momentum can be visualized.

Motion (linear or rotational) always has a corresponding momentum (linear or angular). For an object of mass  $m$  and velocity  $\mathbf{v}$ , the linear momentum  $\mathbf{p}$  is given by:

$$\mathbf{p} = m\mathbf{v} \quad (1.1)$$

Conceptually, momentum can be thought of as the tendency for an object to continue its motion. The momentum only changes when an external force  $\mathbf{F}$  is applied, in accordance with Newton's second law:

$$\mathbf{F} = \left( \frac{d\mathbf{p}}{dt} \right) = m\mathbf{a} \quad (1.2)$$

where  $\mathbf{a}$  is the acceleration. In the absence of external forces, the object does not accelerate (or decelerate) and the linear momentum and hence the speed is constant.

Now consider an object rotating with constant velocity about a fixed point at a distance  $\mathbf{r}$ . This motion is described with an angular momentum vector  $\mathbf{L}$ , defined as:

$$\mathbf{L} = \mathbf{r} \times \mathbf{p} \quad (1.3)$$

Therefore, the magnitude of  $\mathbf{L}$  is  $mvr$  and its direction is perpendicular to the plane of motion. Angular momentum can only be changed when an external torque is applied, in analogy with the application of force on a linear momentum. Torque  $\mathbf{T}$  (or rotational force) is defined as the cross product of force and the distance over which the force has to be delivered:

$$\mathbf{T} = \mathbf{r} \times \mathbf{F} = \mathbf{r} \times \left( \frac{d\mathbf{p}}{dt} \right) = \left( \frac{d\mathbf{L}}{dt} \right) \quad (1.4)$$

Now suppose that the rotating object carries an electrical charge so that a current loop is created. According to classical physics this current generates a magnetic field, which is characterized by the magnetic dipole moment,  $\boldsymbol{\mu}$ , a fundamental magnetic quantity associated with the current. In general the magnetic moment  $\boldsymbol{\mu}$  is given by:

$$\boldsymbol{\mu} = [\text{current}][\text{area}] \quad (1.5)$$

For an object of mass  $m$  and charge  $e$  rotating at constant rotational velocity  $\mathbf{v}$  about a fixed point at distance  $\mathbf{r}$ , the magnetic moment  $\boldsymbol{\mu}$  is given by:

$$\boldsymbol{\mu} = \left[ \frac{e\mathbf{v}}{2\pi\mathbf{r}} \right] \pi\mathbf{r}^2 \quad (1.6)$$

Using  $\mathbf{L} = m\mathbf{vr}$ , a fundamental relation between magnetic moment and angular moment is obtained:

$$\boldsymbol{\mu} = \left( \frac{e}{2m} \right) \mathbf{L} = \gamma \mathbf{L} \quad (1.7)$$

where  $\gamma$  is the (classical) gyromagnetic ratio. It turns out that relation (1.7) is valid for any periodic, orbital motion, including microscopic motion of elementary particles. In the next section it is shown that relation (1.7) is also obtained when using quantum mechanical arguments. When the rotating object is placed in an external magnetic field  $\mathbf{B}_0$ , the loop will feel a torque given by:

$$\mathbf{T} = \boldsymbol{\mu} \times \mathbf{B}_0 \quad (1.8)$$

Combining Equations (1.4), (1.7) and (1.8) gives:

$$\left(\frac{d\boldsymbol{\mu}}{dt}\right) = \gamma\boldsymbol{\mu} \times \mathbf{B}_0 \quad (1.9)$$

Since the amplitude of  $\boldsymbol{\mu}$  is constant, the differential equation in Equation (1.9) expresses the fact that  $\boldsymbol{\mu}$  changes its orientation relative to  $\mathbf{B}_0$ , i.e.  $\boldsymbol{\mu}$  rotates (precesses) about  $\mathbf{B}_0$ . Alternatively, a precession of  $\boldsymbol{\mu}$  about  $\mathbf{B}_0$  can be described by:

$$\left(\frac{d\boldsymbol{\mu}}{dt}\right) = \boldsymbol{\mu} \times \boldsymbol{\omega}_0 \quad (1.10)$$

Combining Equations (1.9) and (1.10) results in the famous Larmor equation:

$$\boldsymbol{\omega}_0 = \gamma\mathbf{B}_0$$

or

$$\nu_0 = \left(\frac{\boldsymbol{\omega}_0}{2\pi}\right) = \left(\frac{\gamma}{2\pi}\right)\mathbf{B}_0 \quad (1.11)$$

The precession (or Larmor) frequency  $\nu_0$  is thus directly proportional to the applied magnetic field  $\mathbf{B}_0$  and also to the gyromagnetic ratio  $\gamma$  (or  $\mu$ ), which is characteristic for the nucleus under investigation.

A magnetic moment in an external magnetic field also has an associated magnetic energy defined as:

$$E = -\boldsymbol{\mu} \cdot \mathbf{B}_0 = -\mu B_0 \cos \theta \quad (1.12)$$

where  $\theta$  is the angle between the magnetic moment  $\boldsymbol{\mu}$  and the external magnetic field  $\mathbf{B}_0$ . Equation (1.12) indicates that the magnetic energy is minimized when  $\boldsymbol{\mu}$  is parallel with  $\mathbf{B}_0$  ( $\theta = 0^\circ$ ) and maximized when  $\boldsymbol{\mu}$  is antiparallel with  $\mathbf{B}_0$  ( $\theta = 180^\circ$ ). According to Equation (1.12), the classical magnetic moment may assume any orientation ( $0^\circ \leq \theta \leq 180^\circ$ ), with energy varying between  $+\mu B_0$  and  $-\mu B_0$ . Therefore, even though classical mechanics can create a familiar picture of the relation between angular momentum, magnetic moment and Larmor frequency, it cannot explain how the general resonance condition for spectroscopy,  $\Delta E = h\nu$ , relates to the magnetic energy associated with the magnetic moment. A quantum mechanical treatment is necessary to obtain information about the interaction of electromagnetic waves and nuclear spins. In the next section basic quantum mechanical concepts are introduced, after which the NMR resonance condition is derived.

### 1.3 Quantum Mechanical Description

One of the fundamental postulates in quantum mechanics is that the angular momentum of elementary particles (be it protons, neutrons, or electrons) is limited to discrete values, i.e. the angular momentum  $\mathbf{L}$  is quantized and its amplitude is given by:

$$L = \left(\frac{h}{2\pi}\right) \sqrt{I(I+1)} \quad (1.13)$$

where  $I$  is the spin quantum number, which can only be integral or half-integral and  $h$  is Planck's constant. Since angular momentum is a vector, the full description of  $\mathbf{L}$  must

involve its amplitude, given by Equation (1.13), and its direction. In quantum mechanics the direction of angular momentum is specified by a second quantum number  $m$ , which can only have certain discrete orientations with respect to a given direction. The component of angular momentum in the  $z$  direction,  $L_z$ , is given by:

$$L_z = \left( \frac{h}{2\pi} \right) m \quad (1.14)$$

Quantum mechanics shows that  $m$  can have  $2I+1$  values, given by:

$$m = I, I-1, I-2, \dots, -I \quad (1.15)$$

For protons, neutrons and electrons, the spin quantum number  $I$  equals  $1/2$ . For nuclei,  $I$  cannot simply be calculated by summation of its individual components. However, by using the atomic mass and the charge number,  $I$  can be deduced from the following rules:

1. For nuclei with an odd mass number,  $I$  is half-integral ( $1/2, 3/2, 5/2, \dots$ , e.g.  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{23}\text{Na}$ ,  $^{31}\text{P}$ ).
2. For nuclei with an even mass number and an even charge number,  $I$  is zero (e.g.  $^{12}\text{C}$ ,  $^{16}\text{O}$ ,  $^{32}\text{S}$ ).
3. For nuclei with an even mass and an odd charge number  $I$  is an integral number ( $1, 2, \dots$ , e.g.  $^2\text{H}$ ,  $^{14}\text{N}$ ).

By analogy with Equation (1.7), elementary particles also have a magnetic moment  $\mu$  which is related to the angular momentum  $\mathbf{L}$  through:

$$\mu = \gamma \mathbf{L} \quad (1.16)$$

where  $\gamma$  is again the gyromagnetic ratio. Since the angular momentum is quantized, the magnetic moment will also be quantized. The component of the magnetic moment along the longitudinal  $z$  axis is given [by analogy with Equation (1.14)] by:

$$\mu_z = \gamma \left( \frac{h}{2\pi} \right) m \quad (1.17)$$

where  $m$  is given by Equation (1.15). In an external magnetic field  $\mathbf{B}_0$ , the particle acquires a magnetic energy given by Equation (1.12). Combining this classical description of the magnetic energy with the quantum mechanical formulation of magnetic moment gives:

$$E = -\mu_z B_0 = -\gamma \left( \frac{h}{2\pi} \right) m B_0 \quad (1.18)$$

Since  $m$  is a discrete quantum number [see Equation (1.15)], the energy levels are also quantized. For a particle of spin  $I = 1/2$ , there are only two energy levels ( $m = -1/2$  and  $+1/2$ ) and the energy difference  $\Delta E$  is given by (see Figure 1.1):

$$\Delta E = \gamma \left( \frac{h}{2\pi} \right) B_0 \quad (1.19)$$

The resonance phenomenon in NMR is achieved by applying an oscillating magnetic field perpendicular to  $\mu_z$  with a frequency  $\nu_0$ , such that the energy equals the magnetic energy

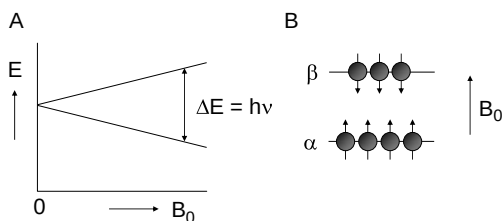


**Figure 1.1** (A) The nuclear spin energy for a spin-1/2 nucleus as a function of the external magnetic field strength  $B_0$ . (B) The lower energy level ( $\alpha$  spin state) corresponds to magnetic moments parallel with  $B_0$ , while spins in the higher energy level ( $\beta$  spin state) have an antiparallel alignment with  $B_0$ . For all currently available magnets, the energy level difference between the two spin states corresponds to electromagnetic radiation in the RF range.

given by Equation (1.19), i.e. the energy of the electromagnetic wave is given by:

$$\Delta E = h\nu_0 \quad (1.20)$$

Combining Equations (1.19) and (1.20) will give the earlier derived Larmor equation:

$$\nu_0 = \left( \frac{\gamma}{2\pi} \right) B_0 \quad (1.21)$$

Even though the classical and quantum mechanical descriptions of NMR lead to the same result, they play a different role in the understanding of the technique. Quantum mechanics is the only theory which can quantitatively describe the NMR phenomenon. Classical principles are mainly used to visualize the effects of RF pulses on macroscopic magnetization vectors.

## 1.4 Macroscopic Magnetization

Figure 1.2A shows the precession (at the Larmor frequency) of a magnetic moment around an external magnetic field according to classical principles. Quantization of magnetic moment (and magnetic energy) can readily be incorporated in this picture. For elementary particles the angle  $\theta$  between  $\mu$  and  $B_0$  can no longer be arbitrary as in Section 1.2 but is given by:

$$\cos \theta = \frac{m}{\sqrt{I(I+1)}} \quad (1.22)$$

For a nucleus of spin  $I = 1/2$ ,  $m = +1/2$  or  $-1/2$  yielding an angle  $\theta = 54.74^\circ$  relative to the  $+z$  or  $-z$  axis, respectively. Therefore, the nuclei of spin  $I = 1/2$  are distributed on the surface of two cones, and rotate about  $B_0$  at the Larmor frequency (Figure 1.2B). In the general case of a spin  $I$  nucleus, the magnetic moments will be distributed on  $2I+1$  cones at discrete angles  $\theta$  as defined by Equation (1.22). For a spin  $1/2$  nucleus the two spin states  $m = +1/2$  ( $\mu$  parallel with  $B_0$ ) and  $m = -1/2$  ( $\mu$  antiparallel to  $B_0$ ) are often referred to as the  $\alpha$  and  $\beta$  spin states, respectively.

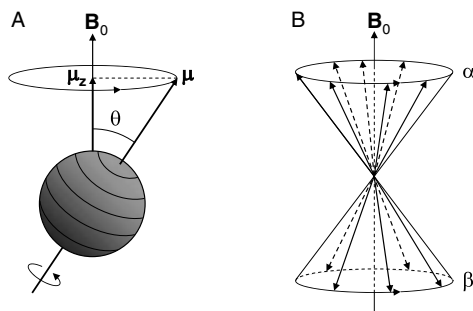


**Figure 1.2** (A) A nuclear spin precessing in an external magnetic field  $B_0$ . The spin magnetic moment  $\mu$  precesses about  $B_0$ , in which the orientation  $\theta$  and the amplitude (along  $z$ )  $\mu_z$  are quantized. (B) In a macroscopic ensemble of nuclear spin-1/2, the spins distribute themselves among two possible orientations according to the Boltzmann equation.

So far, only the behavior of individual nuclear spins has been considered. However, a macroscopic sample contains many spins, which will be randomly distributed on the cones. As a consequence of the small energy difference between the spin states there will be a small difference in the population of these spin states. This population difference can be calculated using the Boltzmann equation. For the situation shown in Figure 1.2B the energy difference  $\Delta E = h\nu$  gives rise to a population distribution given by:

$$\left(\frac{n_\alpha}{n_\beta}\right) = e^{\Delta E/kT} = e^{h\nu/kT} \quad (1.23)$$

where  $n_\alpha$  is the number of spins in the  $\alpha$  (low energy) state,  $n_\beta$  is the number of spins in the  $\beta$  (high energy state),  $k$  is the Boltzmann constant and  $T$  is the absolute temperature. Since at normal temperature,  $h\nu$  is much less than the thermal energy  $kT$ , the exponent in Equation (1.23) can be simplified through an expansion and truncation of a Taylor series to give:

$$\left(\frac{n_\alpha}{n_\beta}\right) = 1 + \left(\frac{h\nu}{kT}\right) \quad (1.24)$$

For a macroscopic sample containing one million nuclear spins at 37 °C ( $T = 310.15$  K) and in a magnetic field of 9.4 T, corresponding to  $\nu = 400$  MHz, the population difference between the  $\alpha$  and  $\beta$  spin states is only 31 spins (corresponding to 0.0031 %). Since the final received signal is proportional to the population difference, NMR is a rather insensitive technique compared with other forms of spectroscopy, where the energy difference is much larger.

The total net magnetic moment (i.e. ‘the magnetization’),  $\mathbf{M}$ , of a macroscopic sample is the resultant of the sum over all individual magnetic moments  $\mu$ . Since the magnetic moments are randomly distributed on the cones, there will be no net component of  $\mathbf{M}$  in the transverse  $xy$  plane (see Figure 1.2B). However, due to the population difference there will be a net component of  $\mathbf{M}$  parallel with  $B_0$  along the  $+z$  axis. At thermal equilibrium

the magnitude of the longitudinal magnetization,  $\mathbf{M}_0$  is:

$$M_0 = \sum_{i=1}^n \mu_i = n_\alpha \mu_z + n_\beta \mu_z = \gamma \left( \frac{\hbar}{4\pi} \right) (n_\alpha - n_\beta) \quad (1.25)$$

Using Equation (1.24),  $(\hbar\nu/kT) \ll 1$  and  $n = n_\alpha + n_\beta$  where  $n$  is the total number of nuclear spins in the macroscopic sample, the population difference  $(n_\alpha - n_\beta)$  is given by:

$$(n_\alpha - n_\beta) \approx \left( \frac{n\hbar\nu}{2kT} \right) \quad (1.26)$$

Therefore, at thermal equilibrium, the amplitude of the macroscopic magnetization vector  $\mathbf{M}_0$  is:

$$M_0 = \left( \frac{\gamma\hbar}{2\pi} \right)^2 \left( \frac{nB_0}{4kT} \right) \quad (1.27)$$

From Equation (1.27) several important features concerning the sensitivity of NMR experiments can be deduced. The quadratic dependence of  $\mathbf{M}_0$  on the gyromagnetic ratio  $\gamma$  implies that nuclei resonating at high frequency [see Equation (1.11)] also generate relatively intense NMR signals. Hydrogen has the highest  $\gamma$  of the commonly encountered nuclei, and has therefore the highest relative intensity. The linear dependence of  $\mathbf{M}_0$  on the magnetic field strength  $\mathbf{B}_0$  implies that higher magnetic fields improve the sensitivity. In fact this argument (and the related increase in chemical shift dispersion) has caused a steady drive towards higher magnetic field strength which now typically range from 1.5 T to 17.5 T (or up to circa 11.7 T for *in vivo* applications). Finally, the inverse proportionality of  $\mathbf{M}_0$  to the temperature  $T$  indicates that sensitivity can be enhanced at lower sample temperatures. Obviously, the latter option is unrealistic for *in vivo* applications.

The actual experimental sensitivity is determined by many factors, like sample volume, gyromagnetic ratio, natural abundance of the nucleus studied, (sample) noise, relaxation parameters and magnetic field strength. Although some factors can be predicted by Equation (1.27), others (e.g. noise) need a more detailed treatment which will be given in Chapter 10. The intrinsic sensitivities of the most relevant nuclei encountered in *in vivo* NMR spectroscopy are summarized in Table 1.1.

## 1.5 Excitation

In NMR experiments, macroscopic samples are studied, containing many individual spins. Figure 1.2B demonstrates clearly how the spin angular moments are distributed on a discrete number of cones. The quantum mechanical representation is convenient to illustrate the spin distribution, but it is not very suitable to illustrate the interaction of the spins with external magnetic fields. Therefore the classical picture of the net macroscopic magnetization vector  $\mathbf{M}_0$  will be used in further discussions.

In order to observe nuclear magnetization, the precessional motion needs to be detected. However, at thermal equilibrium the spins have no phase coherence in the transverse plane and the net longitudinal magnetization is a static vector. Nuclear magnetization can only be observed by rotating the net longitudinal magnetization towards or onto the transverse plane. This can be accomplished by a second magnetic field in the transverse plane

**Table 1.1** NMR properties of commonly encountered nuclei in *in vivo* NMR

| Isotope           | Spin | Gyromagnetic ratio<br>( $10^7 \text{ rad T}^{-1} \text{ s}^{-1}$ ) | NMR frequency<br>at 2.35 T (MHz) | Natural<br>abundance (%) | Relative<br>sensitivity <sup>a</sup> |
|-------------------|------|--------------------------------------------------------------------|----------------------------------|--------------------------|--------------------------------------|
| $^1\text{H}$      | 1/2  | 26.752                                                             | 100.000                          | 99.985                   | 1.00                                 |
| $^2\text{H}$      | 1    | 4.107                                                              | 15.351                           | 0.015                    | $1.45 \times 10^{-6}$                |
| $^3\text{He}$     | 1/2  | -20.380                                                            | 76.181                           | $1.4 \times 10^{-4}$     | $5.75 \times 10^{-7}$                |
| $^7\text{Li}$     | 3/2  | 10.398                                                             | 38.866                           | 92.58                    | 0.272                                |
| $^{13}\text{C}$   | 1/2  | 6.728                                                              | 25.145                           | 1.108                    | $1.76 \times 10^{-4}$                |
| $^{14}\text{N}$   | 1    | 1.934                                                              | 7.228                            | 99.630                   | $1.00 \times 10^{-3}$                |
| $^{15}\text{N}$   | 1/2  | -2.712                                                             | 10.137                           | 0.370                    | $3.86 \times 10^{-6}$                |
| $^{17}\text{O}$   | 5/2  | -3.628                                                             | 13.562                           | 0.037                    | $1.08 \times 10^{-5}$                |
| $^{19}\text{F}$   | 1/2  | 25.181                                                             | 94.094                           | 100.000                  | 0.834                                |
| $^{23}\text{Na}$  | 3/2  | 7.080                                                              | 26.466                           | 100.000                  | $9.27 \times 10^{-2}$                |
| $^{31}\text{P}$   | 1/2  | 10.841                                                             | 40.481                           | 100.000                  | $6.65 \times 10^{-2}$                |
| $^{39}\text{K}$   | 3/2  | 1.250                                                              | 4.672                            | 93.100                   | $4.75 \times 10^{-4}$                |
| $^{129}\text{Xe}$ | 1/2  | -7.452                                                             | 27.856                           | 26.44                    | $5.71 \times 10^{-3}$                |

<sup>a</sup>Relative sensitivity is calculated as the product of NMR sensitivity (proportional to  $|\gamma^3| \times I(I+1)$ ) and the natural abundance.

oscillating in the RF (MHz) range, i.e.  $B_{1\max}\cos(\omega t)$ , where  $B_{1\max}$  is the amplitude of the applied field and  $\omega$  its frequency. In modern Fourier transform NMR, the  $B_1$  field is applied as a RF pulse (i.e. turned on for a finite time  $T$  and turned off again). During the RF pulse, the magnetization will precess about  $\mathbf{B}_0$  and  $\mathbf{B}_1$ . Throughout this chapter and the remainder of the book anticlockwise rotations will be used in accordance with the theory first developed by Bloch [2–4]. The initially longitudinal magnetization experiences a torque from the applied  $\mathbf{B}_1$  field, which results in a rotation of  $\mathbf{M}_0$  towards the transverse plane (Figure 1.3). Because two external magnetic fields act simultaneously

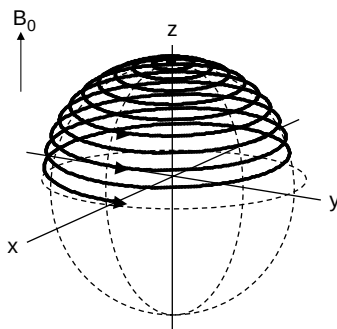


**Figure 1.3** Excitation of magnetization in the nonrotating, laboratory frame  $xyz$ . The longitudinal magnetization  $\mathbf{M}_0$ , initially aligned with the  $z$  axis, will precess about the static magnetic field  $\mathbf{B}_0$  and the irradiating RF field  $\mathbf{B}_1$  in the transverse plane. This results in a rotation towards the transverse plane due to  $\mathbf{B}_1$  and a simultaneous precession at the Larmor frequency about  $\mathbf{B}_0$ . In this case  $\mathbf{B}_1$  was calibrated to rotate  $\mathbf{M}_0$  by  $90^\circ$  away from the  $z$  axis to give complete excitation.



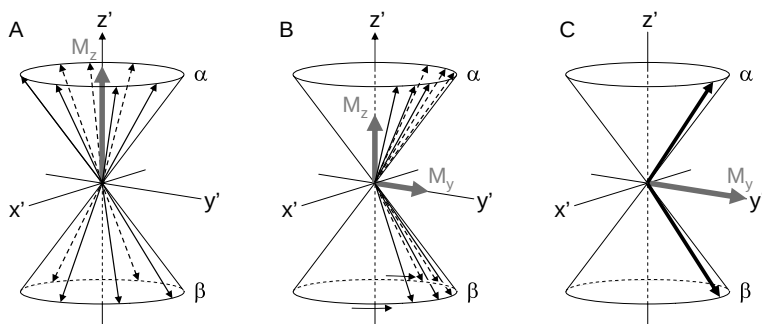


**Figure 1.4** Excitation of magnetization in the rotating frequency frame of reference  $x'y'z'$ . (A) At thermal equilibrium the Boltzmann distribution of individual nuclear spins in a macroscopic sample creates a net magnetization vector along  $+z'$ . Since the individual spins have no phase coherence (i.e. their phases are randomly distributed), there is no net magnetization in the transverse plane. (B) A magnetic field  $B_1$  along  $-x'$  rotates the net macroscopic magnetization towards  $+y'$ . On the microscopic level this is equivalent to the generation of phase coherence between the individual spins. (C) When the magnetic field  $B_1$  is calibrated to give complete excitation, the spins have attained complete phase coherence resulting in a net magnetization vector along  $+y'$ . No magnetization remains along  $z'$ .

on  $M_0$ , the rotation of  $M_0$  during the applied  $B_1$  field appears to be rather complex. In Section 1.6 the concept of rotating frames of reference will be introduced which considerably simplifies the rotations. When the applied  $B_1$  field is applied long enough,  $M_0$  can be completely excited onto the transverse plane or even inverted to the  $-z$  axis, giving rise to so-called  $90^\circ$  excitation and  $180^\circ$  inversion RF pulses, respectively. Following the pulse, the magnetization experiences only the main magnetic field  $B_0$  and will precess around it with the Larmor frequency. For the observant reader the rotation of magnetization towards the transverse plane may seem in violation with the quantum mechanical property of spins to be either parallel or antiparallel with the main magnetic field  $B_0$ . However, a link between individual spins, which can only be parallel or antiparallel to the static magnetic field, and macroscopic transverse magnetization can still be understood with a classical description. Figure 1.4 shows an ensemble of individual spins at thermal equilibrium, i.e. the phase of the spins is random such that the net transverse magnetization is zero. Application of a perpendicular RF magnetic field has two effects on the spins. First, the two spin states become more equally populated as a  $90^\circ$  nutation (rotation) angle is approached and second the spins come into a state of phase coherence [15], i.e. the external magnetic field forces the phases of the spins to attain coherence thereby generating transverse magnetization. The transverse magnetization coherently rotates about  $B_0$  at the Larmor frequency  $\nu_0$  and induces an electromotive force (emf) in the receiver coil surrounding the sample. The amplitude of the induced emf is determined by Faraday's law of electromagnetic induction. After amplification this induced emf gives directly rise to the NMR signal. Sections 1.7 and 1.9 will deal with the processing of NMR signal to recognizable spectra and Chapter 10 will deal with the theory of detection systems and coils.

## 1.6 Bloch Equations

In Section 1.2 it was shown that, when placed in a magnetic field  $\mathbf{B}$ , a magnetic moment  $\boldsymbol{\mu}$  experiences a torque which is proportional to the time derivative of the angular momentum [Equations (1.4) and (1.8)]. Utilizing the fact that the magnetization is the sum over all magnetic moments, i.e. Equation (1.25), the expression of motion for a single magnetic moment can be generalized for the total magnetization, giving:

$$\frac{d\mathbf{M}(t)}{dt} = \mathbf{M}(t) \times \gamma \mathbf{B}(t) \quad (1.28)$$

where  $\mathbf{B}(t)$  may include time-varying components in addition to the static magnetic field  $\mathbf{B}_0$ . At thermal equilibrium, in the absence of additional (time-varying) magnetic fields, Equation (1.28) simply expresses the fact that the z component of the magnetization  $\mathbf{M}$  is constant, i.e.:

$$\frac{dM_z(t)}{dt} = 0 \quad (1.29)$$

No net x and y components of  $\mathbf{M}$  exist at thermal equilibrium, and therefore no NMR signal can be detected. As qualitatively illustrated in Figure 1.3, the longitudinal magnetization  $\mathbf{M}_z$  must be rotated onto the transverse plane, after which the rotating transverse magnetization will induce signal in a receive coil through Faraday's law of induction. From Equation (1.28) it follows that  $\mathbf{M}_z$  can be perturbed by a second magnetic field perpendicular to  $\mathbf{M}_z$  and since this field is rotating at the Larmor frequency in the RF range of the electromagnetic spectrum, it is often referred to as a RF magnetic field.

The magnetic component of a RF field that is linearly polarized along the x axis in the laboratory frame can be written as:

$$\mathbf{B}_1(t) = 2B_{1\max} \cos \omega t [\mathbf{x}] \quad (1.30)$$

where  $B_{1\max}$  is the maximum amplitude of the applied field,  $\omega$  is the angular transmitter or carrier frequency of the RF field and  $[\mathbf{x}]$  represents a unit vector along the x axis. The linearly polarized field can be decomposed into two circularly polarized fields rotating in opposite direction about the z axis (Figure 1.5) according to:

$$\mathbf{B}_1(t) = B_{1\max} (\cos \omega t [\mathbf{x}] + \sin \omega t [\mathbf{y}]) + B_{1\max} (\cos \omega t [\mathbf{x}] - \sin \omega t [\mathbf{y}]) \quad (1.31)$$

Only the field rotating in the same sense as the magnetic moment interacts significantly with the magnetic moment. The counter rotating field influences the spins to the order  $(B_1/2B_0)^2$ , which is typically a very small number known as the Bloch–Siegert shift [16]. Since under most conditions the counter rotating field can be ignored, the linearly polarized RF field of Equation (1.31) is then equivalent to a rotating magnetic field given by:

$$\mathbf{B}_{1x}(t) = B_{1\max} (\cos \omega t [\mathbf{x}] - \sin \omega t [\mathbf{y}]) = B_{1x} \cos \omega t + B_{1y} \sin \omega t \quad (1.32)$$

A similar expression can be derived for  $B'_{1y}(t)$ .

In the presence of two magnetic fields  $\mathbf{B}_0$  and  $\mathbf{B}_1$ , Equation (1.28) can be expanded to yield the Bloch equations in the laboratory frame of reference in the absence of

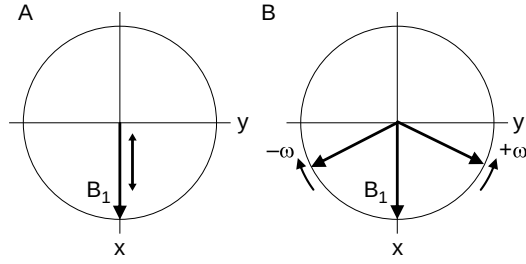


**Figure 1.5** Decomposition of a linear oscillating magnetic field (A) into two rotating magnetic fields (B) with frequencies  $-\omega$  and  $+\omega$ , respectively.

relaxation [3]:

$$\frac{dM_x(t)}{dt} = \gamma[M_y(t)B_0 - M_z(t)B_{1y}] \quad (1.33)$$

$$\frac{dM_y(t)}{dt} = \gamma[M_z(t)B_{1x} - M_x(t)B_0] \quad (1.34)$$

$$\frac{dM_z(t)}{dt} = \gamma[M_x(t)B_{1y} - M_y(t)B_{1x}] \quad (1.35)$$

Relaxation is the process of return to thermal equilibrium after a perturbation. Components of the magnetization  $\mathbf{M}$  (i.e.  $M_x$ ,  $M_y$  and  $M_z$ ) return to thermal equilibrium in an exponential manner. The components perpendicular (i.e.  $M_x$  and  $M_y$ ) and parallel (i.e.  $M_z$ ) to  $\mathbf{B}_0$  relax with different time constants. The relaxation process can be written as:

$$\frac{dM_x(t)}{dt} = -\frac{M_x(t)}{T_2} \quad (1.36)$$

$$\frac{dM_y(t)}{dt} = -\frac{M_y(t)}{T_2} \quad (1.37)$$

$$\frac{dM_z(t)}{dt} = -\frac{M_z(t) - M_0}{T_1} \quad (1.38)$$

$T_1$  and  $T_2$  are relaxation time constants.  $T_1$  is the longitudinal relaxation time (or spin-lattice relaxation time) and describes the return of longitudinal magnetization after a perturbation.  $T_1$  relaxation is in principle a process in which energy from the spins is transferred to the surrounding ‘lattice’ (which can be either solid or liquid).  $T_2$  is the transverse relaxation time (or spin-spin relaxation time) and describes the disappearance of transverse magnetization.  $T_2$  relaxation is an entropy-process, since spins exchange energy between themselves (there is no net energy transfer) causing a decrease in phase coherence (i.e. an increase in global chaos or entropy).  $T_1$  and  $T_2$  relaxation processes in biological tissues are discussed in detail in Chapter 3. Combining Equations (1.33)–(1.35) and Equations (1.36)–(1.38) yields the complete Bloch equations in the laboratory

frame [3]:

$$\frac{dM_x(t)}{dt} = \gamma[M_y(t)B_0 - M_z(t)B_{1y}] - \frac{M_x(t)}{T_2} \quad (1.39)$$

$$\frac{dM_y(t)}{dt} = \gamma[M_z(t)B_{1x} - M_x(t)B_0] - \frac{M_y(t)}{T_2} \quad (1.40)$$

$$\frac{dM_z(t)}{dt} = \gamma[M_x(t)B_{1y} - M_y(t)B_{1x}] - \frac{(M_z(t) - M_0)}{T_1} \quad (1.41)$$

Until this point the NMR experiment has been described in a Cartesian frame fixed with respect to the laboratory (i.e. the ‘laboratory’ frame). It turns out to be more convenient to describe NMR in a rotating frame. Therefore, consider a new set of Cartesian axes ( $x'$ ,  $y'$  and  $z'$ ) rotating about the static magnetic field  $\mathbf{B}_0$  with frequency  $\omega$ . The  $z$  and  $z'$  axes of the laboratory and rotating frames, respectively, are collinear with the external magnetic field  $\mathbf{B}_0$ . The components of the magnetization in the rotating frame are given by:

$$M'_x = M_x \cos \omega t + M_y \sin \omega t \quad (1.42)$$

$$M'_y = M_y \cos \omega t - M_x \sin \omega t \quad (1.43)$$

$$M'_z = M_z \quad (1.44)$$

Therefore, using Equations (1.42)–(1.44) the Bloch equations in the rotating frame can be calculated from Equations (1.39)–(1.41), i.e.:

$$\frac{dM'_x(t)}{dt} = -(\omega_0 - \omega)M'_y(t) - \gamma B'_{1y}M'_z(t) - \frac{M'_x(t)}{T_2} \quad (1.45)$$

$$\frac{dM'_y(t)}{dt} = (\omega_0 - \omega)M'_x(t) + \gamma B'_{1x}M'_z(t) - \frac{M'_y(t)}{T_2} \quad (1.46)$$

$$\frac{dM'_z(t)}{dt} = \gamma B'_{1y}M'_x(t) - \gamma B'_{1x}M'_y(t) - \frac{(M'_z(t) - M_0)}{T_1} \quad (1.47)$$

where the definitions of  $B'_{1x}$  and  $B'_{1y}$  as specified in Equation (1.32) are used. In all following text the prime will be omitted as it is assumed that the magnetization vector evolves in the rotating frame of reference. The conversion to a rotating frame of reference has consequences for the magnetic field vectors encountered in that frame. In a frame that rotates with a frequency equal to the frequency of  $\mathbf{B}_1$ ,  $\mathbf{B}_1$  appears static. Furthermore, the precessional motion of the magnetization (i.e.  $\omega_0 = -\gamma B_0$ ) appears to be reduced to a value  $(\omega_0 - \omega)$ . Figure 1.6A shows the generation of transverse magnetization for  $\omega = \omega_0$ . Since the vectors are drawn in the rotating frame of reference, the magnetization simply precesses about the applied  $\mathbf{B}_1$  field towards the transverse plane. Comparison with Figure 1.3, which shows the same situation in the laboratory frame illustrates the clarity of a rotating frame. It is convenient to define an effective magnetic field  $\mathbf{B}_e$ , which is the vector sum of  $(\omega_0 - \omega)/\gamma$  and  $\mathbf{B}_1$ , since the magnetization precesses about the effective field. The magnitude of  $\mathbf{B}_e$  is given by

$$B_e = |\mathbf{B}_e| = \sqrt{B_1^2 + \left(\frac{\omega_0 - \omega}{\gamma}\right)^2} \quad (1.48)$$

On-resonance (i.e. when the frequency of the applied RF pulse  $\omega$  equals the Larmor frequency  $\omega_0$ ), Equation (1.48) reduces to  $\mathbf{B}_e = \mathbf{B}_1$  and the magnetization simply rotates

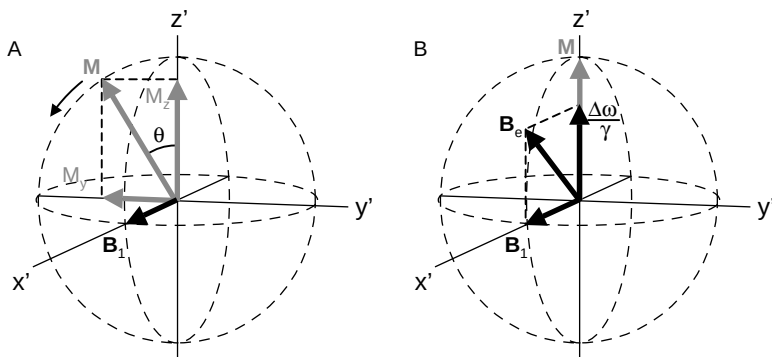


**Figure 1.6** Magnetic field vectors encountered in the rotating frame of reference  $x'y'z'$  during excitation. (A) On-resonance, the effective, external magnetic field vector equals the magnetic field vector  $\mathbf{B}_1$  along  $x'$ . The longitudinal magnetization experiences a torque and will rotate towards the transverse plane through an angle  $\theta$ . (B) Off-resonance, the frequency of the magnetic field  $\mathbf{B}_1$  no longer equals the Larmor frequency, resulting in an additional magnetic field vector  $\Delta\omega/\gamma$  along  $z'$ . The effective magnetic field  $\mathbf{B}_e$  then equals the vector sum of  $\mathbf{B}_1$  and  $\Delta\omega/\gamma$ . The longitudinal magnetization will experience a torque from this effective field, resulting in a more complex rotation about  $\mathbf{B}_e$ .

about  $\mathbf{B}_1$  as shown in Figure 1.6A. In the event of a nonvanishing off-resonance vector (i.e.  $\omega \neq \omega_0$ ), the effective magnetic field  $\mathbf{B}_e$  is tilted from the transverse plane (Figure 1.6B). The magnetization will precess about  $\mathbf{B}_e$ , leading to a more complex rotation when compared with the on-resonance situation. Off-resonance effects during RF pulses (excitation) are discussed in detail in Chapter 5. For the remainder of this chapter it will be assumed that off-resonance effects are negligible.

## 1.7 Fourier Transform NMR

Following a RF pulse which is calibrated to rotate  $\mathbf{M}_0$  by  $90^\circ$  (i.e. complete excitation), the magnetization is placed in the transverse plane of the rotating frame of reference. The magnetization precesses about  $\mathbf{B}_0$  at the Larmor frequency and induces an emf in a receiving coil positioned in the transverse plane. Because of  $T_2$  relaxation, the transverse magnetization and consequently the emf will decrease as a function of time. However, macroscopic and microscopic inhomogeneity in the main magnetic field  $\mathbf{B}_0$  will create a distribution of locally different  $\mathbf{B}_0$  magnetic fields across the sample, leading to a distribution of Larmor frequencies. When a macroscopic sample is considered, this distribution leads to a more rapid loss of transverse magnetization than caused by pure  $T_2$  relaxation. The origin and compensation of  $\mathbf{B}_0$  inhomogeneity is discussed in great detail in Chapter 10. For a sample with uniform proton density and  $T_2$  relaxation constants, the acquired signal in the presence of magnetic field inhomogeneity can be described by:

$$M_{xy}(t) = M_{xy}(0)e^{-t/T_2} \int_{\mathbf{r}} e^{+i\gamma \Delta B_0(\mathbf{r})t} d\mathbf{r} = M_{xy}(0)e^{-t/T_2^*} \quad (1.49)$$

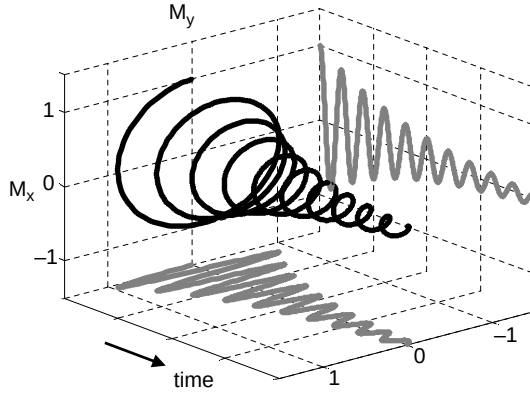


**Figure 1.7** The free induction decay (FID) of nuclear magnetization following an excitation pulse. The transverse magnetization precesses at the Larmor frequency and decays with a characteristic time constant  $T_2^*$  as time progresses. The complex three-dimensional FID can be completely described by two projections on the  $(M_x, t)$  and  $(M_y, t)$  planes, corresponding to the real and imaginary components of the FID, respectively.

where  $\Delta B_0$  is indicative of  $B_0$  inhomogeneity and equals  $(B_0(\mathbf{r}) - B_{0,\text{nom}})$  where  $B_0(\mathbf{r})$  is the magnetic field strength at position  $\mathbf{r}$  and  $B_{0,\text{nom}}$  represent the nominal magnetic field strength.  $M_{xy}$  is the complex transverse magnetization ( $M_{xy} = M_x + iM_y$ ). Note that even though the  $T_2^*$  relaxation is often presented as a single-exponential decay, in practice it is a multi-exponential decay depending on the local  $B_0$  magnetic field inhomogeneity of individual spins as expressed by Equation (1.49).

The time-dependence of the emf (or signal intensity) is called the free induction decay (FID). The complex motion of the transverse magnetization as function of time can be represented as shown in Figure 1.7. NMR spectrometers separately detect the x and y components of this complex motion (see Chapter 10 for more details) and commonly the projections on the xt and yt planes are shown, which are given by:

$$M_x(t) = M_0 \cos[(\omega_0 - \omega)t + \phi] e^{-t/T_2^*} \quad (1.50)$$

$$M_y(t) = M_0 \sin[(\omega_0 - \omega)t + \phi] e^{-t/T_2^*} \quad (1.51)$$

where  $\phi$  is the phase at  $t = 0$ .  $M_x(t)$  and  $M_y(t)$  are normally referred to as the real and imaginary FIDs, respectively. Although the FIDs hold all the relevant information about the nuclear spins, like their resonance frequencies and relative abundance, they are seldom used directly. Normally the time-domain data (i.e. the FID) is converted to frequency-domain data (i.e. the spectrum) by a Fourier transformation [17]. The Fourier transformation of a time-domain signal  $f(t)$  gives a frequency-domain signal  $F(\omega)$  according to:

$$F(\omega) = \int_{-\infty}^{+\infty} f(t) e^{-i\omega t} dt \quad \text{or} \quad F(\nu) = \int_{-\infty}^{+\infty} f(t) e^{-i2\pi\nu t} dt \quad (1.52)$$

Fourier transformation is a reversible operation, so that a time-domain signal can be calculated from a frequency-domain signal with an inverse Fourier transformation given

by:

$$f(t) = \frac{1}{2\pi} \int_{-\infty}^{+\infty} F(\omega) e^{+i\omega t} d\omega \quad \text{or} \quad f(t) = \int_{-\infty}^{+\infty} F(\nu) e^{+i2\pi\nu t} d\nu \quad (1.53)$$

In principle it is possible to construct a spectrum from one of the components of the complex FID [i.e. either  $M_x(t)$  or  $M_y(t)$ ]. However, in that case negative and positive frequencies can not be discriminated since  $\cos(\omega) = \cos(-\omega)$ . Therefore, both components of the complex FID are measured, using so-called quadrature detection. More details about quadrature detection can be found in Chapter 10, while the characteristics of Fourier transformations are described in Appendix A3. Fourier transformation of the time-domain signals yields the real and imaginary frequency-domain signals (i.e. the spectrum) given by:

$$R(\omega) = A(\omega) \cos \phi - D(\omega) \sin \phi \quad (1.54)$$

$$I(\omega) = A(\omega) \sin \phi + D(\omega) \cos \phi \quad (1.55)$$

where

$$A(\omega) = \frac{M_0 T_2^*}{1 + (\omega_0 - \omega)^2 T_2^{*2}} \quad (1.56)$$

$$D(\omega) = \frac{M_0 (\omega_0 - \omega) T_2^{*2}}{1 + (\omega_0 - \omega)^2 T_2^{*2}} \quad (1.57)$$

$A(\omega)$  and  $D(\omega)$  describe the absorption and dispersion components of a Lorentzian lineshape and are drawn in Figure 1.8A. The width at half height,  $\Delta\nu_{1/2}$ , of the absorption component of a Lorentzian lineshape equals  $1/(\pi T_2^*)$ . The dispersive component is substantially broader, with a net integrated intensity of zero. Therefore, for the best separation (or resolution) of multiple lines in a NMR spectrum, absorption mode spectra are generally desired. However, when  $\phi \neq 0$  a mixture of absorption and dispersion signals is observed (Figure 1.8B) as described by Equations (1.54) and (1.55) (Figure 1.8B). Pure absorption mode spectra can be obtained by ‘phasing’ the observed, mixed  $R(\omega)$  and  $I(\omega)$  spectra according to:

$$A(\omega) = R(\omega) \cos \phi_c + I(\omega) \sin \phi_c \quad (1.58)$$

$$D(\omega) = I(\omega) \cos \phi_c - R(\omega) \sin \phi_c \quad (1.59)$$

By interactively adjusting the phase  $\phi_c$ , absorption mode spectra are obtained when  $\phi_c = \phi$  (Figure 1.8C). Due to hardware imperfections and/or timing errors the phase  $\phi$  may depend upon the resonance frequency  $\omega$ . The simple ‘zero-order’ phase correction of Equations (1.58) and (1.59) is no longer adequate and one needs to resort to higher-order phase corrections as well. On most NMR spectrometers phase correction is performed according to:

$$\phi_c = \phi_0 + (\omega_0 - \omega) \phi_1 \quad (1.60)$$

where  $\phi_0$  and  $\phi_1$  are the zero and first order phase corrections, respectively. The adjustable phase  $\phi_c$  therefore contains contributions from a constant phase correction  $\phi_0$  for all resonances and a linear, frequency-dependent phase correction  $\phi_1$ . For some dedicated

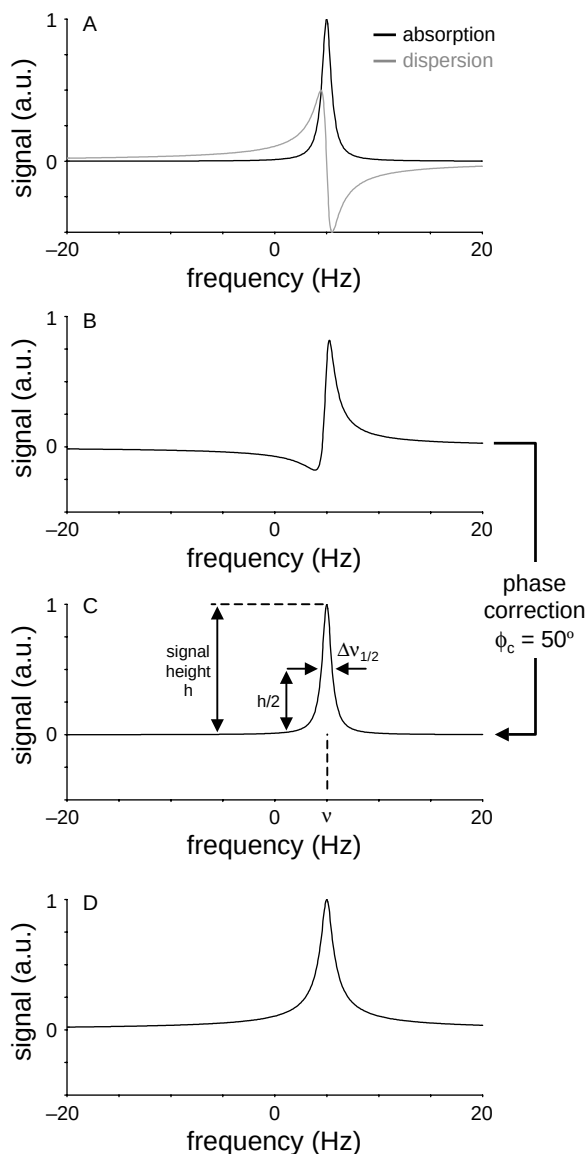


**Figure 1.8** Principle components of a NMR spectrum. (A) Complex Fourier transformation of an exponentially decaying FID gives rise to Lorentzian absorption and dispersion lineshapes. (B) In general, the initial phase of the FID is nonzero, such that a mixture of absorption and dispersion lineshapes is obtained. The dispersive component exhibits broad 'tails' which decreases the spectral resolution. The dispersive component can be eliminated by 'phasing' the spectrum, such that only the absorption component remains as shown in (C). From the phased spectrum, the frequency  $\nu$ , the signal height  $h$  and linewidth at half height  $\Delta\nu_{1/2}$  can be accurately measured. (D) Phase information is completely eliminated when presenting the spectrum in magnitude mode, given by the square root of the sums of the squares of the absorption and dispersion components [i.e. Equation (1.61)]. Because the dispersive component is included the resonance is substantially broader than the pure absorption lineshape.



experiments even higher-order phase corrections may be necessary, but these will not be discussed here.

When the phase of the signal is not relevant, or if the phase can not be adjusted properly with zero- and first-order phase corrections (as in some two-dimensional NMR experiments, see Chapter 8), the signal can be presented in absolute value (or magnitude) mode. An absolute value signal is defined as:

$$M(\omega) = \sqrt{R(\omega)^2 + I(\omega)^2} \quad (1.61)$$

Figure 1.8D shows the absolute-value spectrum of the resonance line shown in Figure 1.8A. Because of the dispersive component, the resonance is much broader than in the corresponding phased spectrum (Figure 1.8C).

## 1.8 Chemical Shift

So far most of the descriptions assumed a macroscopic sample containing only one type of nuclear spin, having a resonance frequency given by Equation (1.11). If the frequency of nuclear spins were solely determined by the resonance condition of Equation (1.11), NMR spectroscopy would be of minor importance in chemistry and medicine. Nuclei of the same element (or isotope) even in different molecules, would resonate at the same frequency because of their identical gyromagnetic ratio. Fortunately, however, the resonance frequency  $\omega$  not only depends on the gyromagnetic ratio  $\gamma$  and the external magnetic field  $B_0$ , but is also highly sensitive to the chemical environment of the nucleus under investigation [5,6]. This is commonly referred to as the chemical shift. The phenomenon of chemical shift is caused by shielding (screening) of nuclei from the external magnetic field by electrons surrounding them. Figure 1.9A shows a schematic representation of the electrons around a nucleus. When placed in an external magnetic field, the electrons will rotate about  $\mathbf{B}_0$  in an opposite sense to the proton spin precession. Since this precession of electrons involves motion of charge, there will be an associated magnetic moment  $\mu_e$ , in analogy to the existence to a nuclear magnetic moment. The electron magnetic moment opposes the primary applied magnetic field  $\mathbf{B}_0$ . Therefore, the electrons will reduce the magnetic field that is sensed by the nucleus. This effect can be expressed in terms of an effective magnetic field  $\mathbf{B}$  at the nucleus:

$$\mathbf{B} = \mathbf{B}_0(1 - \sigma) \quad (1.62)$$

where  $\sigma$  is the shielding (or screening) constant.  $\sigma$  is a dimensionless number [normally expressed in parts per million (ppm)], which depends on the chemical environment of the nucleus. Using Equation (1.62), the resonance condition of Equation (1.11) can be modified to:

$$\nu = \left( \frac{\gamma}{2\pi} \right) B_0(1 - \sigma) \quad (1.63)$$

Most often chemical shifts are not expressed in units of Hertz, since this would make chemical shifts dependent on the magnetic field strength. Instead chemical shifts are

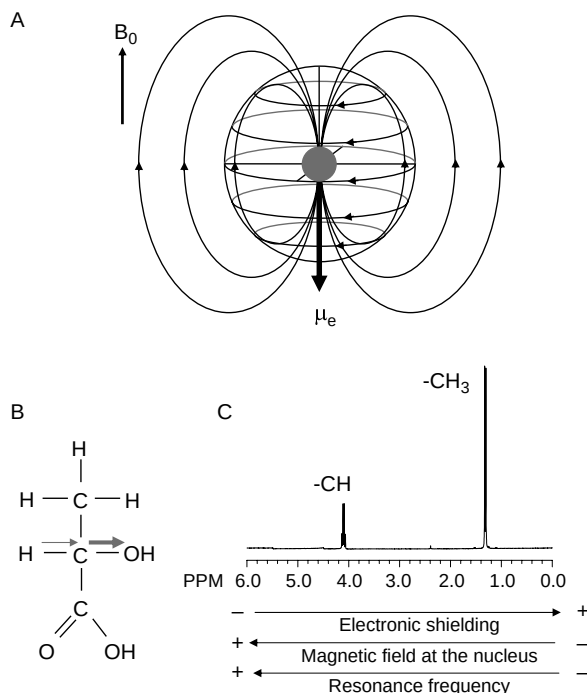


**Figure 1.9** Origin of the chemical shift. (A) The electrons surrounding a nucleus can be regarded as small currents, giving rise to a magnetic moment  $\mu_e$  at the nucleus. Since the magnetic moment opposes the external magnetic field, the effective magnetic field at the nucleus is reduced, thereby leading to a different Larmor frequency and hence a different chemical shift. The reduction of the effective magnetic field by surrounding electrons is often referred to as electronic shielding. (B) The electronegative oxygen atoms in lactate shift the electron density away from the protons, leading to reduced electronic shielding and thus to a higher Larmor frequency. (C) The proton NMR spectrum of lactate is readily explained by the fact that the methine proton is closer to electronegative oxygen atoms than the three methyl protons, thus leading to a higher Larmor frequency and chemical shift.

expressed in terms of ppm. By convention the chemical shift  $\delta$  is defined as:

$$\delta = \frac{\nu - \nu_{\text{ref}}}{\nu_{\text{ref}}} \times 10^6 \quad (1.64)$$

where  $\nu$  and  $\nu_{\text{ref}}$  are the frequencies of the compound under investigation and of a reference compound, respectively. The reference compound should ideally be chemically inert and its chemical shift should be independent of external variables (temperature, ionic strength, shift reagents) and should produce a strong (singlet) resonance signal well separated from all other resonances. A widely accepted reference compound for  $^1\text{H}$  and  $^{13}\text{C}$  NMR is tetramethylsilane (TMS) to which  $\delta = 0$  has been assigned. However, the use of TMS is restricted to NMR on compounds in organic solvents. For aqueous solutions, 3-(trimethylsilyl) propionate (TSP) or 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) are

typically used, of which DSS is more desirable as the chemical shift is temperature and pH independent [18]. Unfortunately, none of these compounds is found in *in vivo* systems and can therefore never be used as internal references. TSP and DSS can in principle be used as external reference compounds, being placed adjacent to the object under investigation. However, under these circumstances the observed chemical shift needs to be corrected for bulk magnetic susceptibility effects as well as macroscopic inhomogeneity of the main magnetic field (see Chapter 10). Differences in susceptibility and local magnetic field strength between the object under investigation and the adjacent external reference make the use of external chemical shift referencing undesirable. For *in vivo* applications other resonances have been used as an internal reference. Commonly used internal references are the methyl resonance of *N*-acetyl aspartate (2.01 ppm) for  $^1\text{H}$  MRS of the brain and the phosphocreatine resonance (0.00 ppm) for  $^{31}\text{P}$  MRS of brain and muscle.

## 1.9 Digital Fourier Transform NMR

The quality and/or information content of NMR spectra is determined by the signal-to-noise ratio (S/N) and the line width of the resonances. This relates through the Fourier transformation directly to the S/N and the  $T_2^*$  relaxation decay of the FID. Next it will be demonstrated that the appearance of NMR spectra can be improved by specific manipulations of the FID.

### 1.9.1 Multi-scan Principle

The S/N can be improved by averaging, i.e. adding the FIDs of  $n$  consecutive, identical experiments leads to an improvement in S/N of a factor  $\sqrt{n}$  [19, 20]. This is because the voltage of the signal  $S$  increases linearly with  $n$ , while for the random processes of noise  $N$  the power increases linearly. Since power is proportional to the square of voltage, the noise voltage increases as  $\sqrt{n}$ , leading to an overall improvement of the S/N of  $n/\sqrt{n} = \sqrt{n}$ . In practice, the improvement in S/N of *in vivo* NMR by time-averaging is limited, since an improvement of a factor 10 requires a prolongation of measurement time by a factor  $10^2 = 100$ . Typically, an *in vivo* NMR experiment is a compromise between sufficient signal-to-noise and the allowable duration of the experiment.

### 1.9.2 Time-domain Filtering

Using Fourier transformation as the only data processing of the NMR time domain signals seldom results in an optimal frequency domain spectrum in terms of S/N, resolution or general appearance. Using the characteristics of the Fourier transformation (see Appendix A3), several manipulations prior to Fourier transformation can be performed on the NMR time domain signal to influence the S/N, resolution or remove broad background signals.

Apodization (or time-domain filtering) is a commonly used manipulation and essentially multiplies a time domain signal with a filter function, according to:

$$f_{\text{filtered}}(t) = f_{\text{original}}(t) \times f_{\text{filter}}(t) \quad (1.65)$$

where  $f_{\text{original}}(t)$  and  $f_{\text{filtered}}(t)$  are the original and filtered time-domain functions, respectively, and  $f_{\text{filter}}(t)$  is the applied filter function. Multiplication of two functions in the

time-domain is equivalent to the convolution of the Fourier transforms in the frequency domain, i.e.:

$$F_{\text{filtered}}(\omega) = \text{FT}[f_{\text{filtered}}(t)] = \text{FT}[f_{\text{original}}(t) \times f_{\text{filter}}(t)] = F_{\text{original}}(\omega) * F_{\text{filter}}(\omega) \quad (1.66)$$

where  $*$  indicates the convolution between  $F_{\text{original}}(\omega)$  and  $F_{\text{filter}}(\omega)$ . Some commonly used filter functions are:

### 1. Exponential weighting

$$f_{\text{filter}}(t) = e^{-t/T_w} \quad (1.67)$$

A decreasing monoexponential apodization function improves the S/N of the frequency-domain spectrum, since the (noisy) data points at the end of the FID are attenuated, while the data points at the beginning of the FID are relatively unaffected. Another consequence of the exponential weighting function is an increase in the resonance linewidths, since the apparent  $T_{2w}^*$  becomes:

$$\frac{1}{T_{2w}^*} = \frac{1}{T_2^*} + \frac{1}{T_w} \quad (1.68)$$

If sufficient data have been recorded to minimize truncation artifacts ( $t_{\text{max}} > 3T_2^*$ ), then optimal sensitivity is obtained by using a so-called matched filter in which  $T_w = T_2^*$ . The improved S/N comes at the expense of a doubling of the spectral linewidth, i.e. spectral resolution has been traded for sensitivity. Besides improving the S/N, this apodization can also be used on FIDs where the last data points have been truncated resulting in artifacts in the frequency domain (Figure 1.10F/G). For  $T_w < 0$  the apodization leads to a resolution enhancement, since the apparent  $T_{2w}^*$  becomes longer, resulting in line narrowing. However, the S/N is decreased since the data points at the end of the FID with relative high noise contribution are becoming more pronounced.

### 2. Lorentz–Gaussian transformation

$$f_{\text{filter}}(t) = e^{+t/T_L} e^{-t^2/T_G^2} \quad (1.69)$$

The Lorentz–Gaussian filtering function converts a Lorentzian lineshape to a Gaussian lineshape. A Gaussian lineshape decays to the baseline in a narrower frequency range as would a Lorentzian lineshape with the same linewidth at half height, i.e. a Lorentzian lineshape produces longer ‘tails’ which are a disadvantage when accurate determination (by integration) of overlapping resonance lines is required (Figure 1.11E). It is therefore sometimes advantageous to convert the theoretically predicted Lorentzian NMR lineshape to a more narrow Gaussian lineshape. The principle of the Lorentz–Gaussian transformation is to cancel (or decrease) the Lorentzian part of the FID [by multiplying with  $\exp(+t/T_L)$ , where  $T_L = T_2^*$ , such that  $\exp(+t/T_L) \times \exp(-t/T_2^*) = 1$ ] while increasing the Gaussian character of the FID [by multiplying with  $\exp(-t^2/T_G^2)$ ]. Using a sufficiently long  $T_G$  value, a significant resolution enhancement can be achieved. Figure 1.11 shows the process of time-domain filtering on a  $^{31}\text{P}$  FID. Even though an increasing exponential filter (Figure 1.11C) and a Lorentz–Gaussian transformation can achieve the same resolution enhancement, the former is accompanied by a significant decrease in sensitivity, which can be minimal with a Lorentz–Gaussian transformation. In fact, the two adjustable parameters in Equation (1.69) can be used to improved the

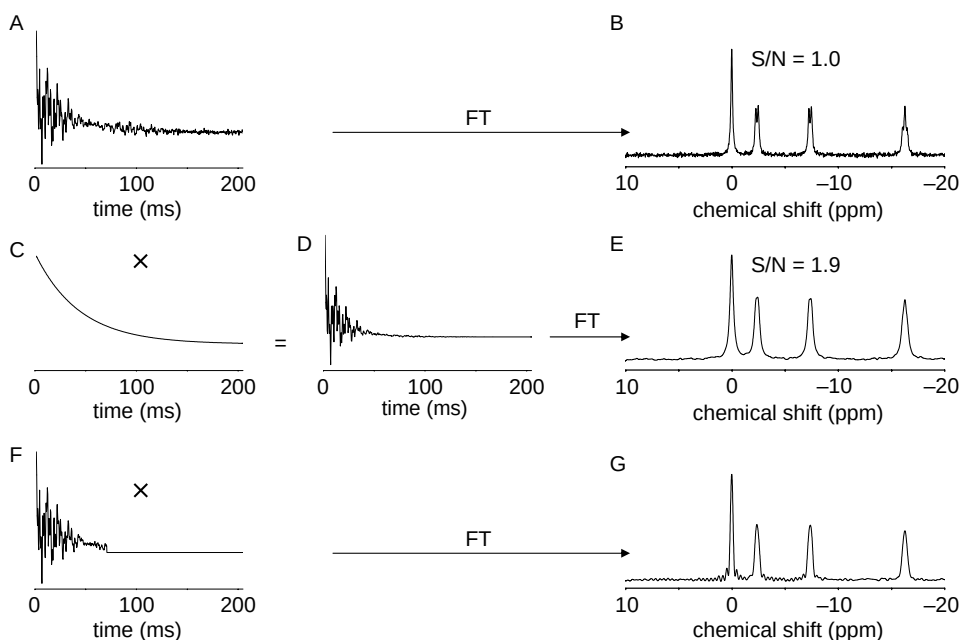


**Figure 1.10** The effects of time-domain apodization on the frequency-domain spectrum. (A) Fourier transformation of a  $^{31}\text{P}$  FID gives (B) a NMR spectrum composed of resonances from phosphocreatine and ATP. The spectral S/N, as defined as the peak height over the root mean square noise level, is typically not optimal. (C, D) Multiplication of the FID with a decaying exponential function will lead to (E) a significant increase in S/N, at the expense of a decrease in spectral resolution. (F) When time-domain acquisition has stopped before the NMR signal has decayed to zero, (G) the resulting NMR spectrum displays characteristic sinc-like wiggles. Apodization of the truncated time-domain signal can restore the Lorentzian lineshapes, giving a weighted FID and spectrum similar to those in (D) and (E).

S/N without a significant decrease in spectral resolution (Figure 1.11F/G) or to improve the spectral resolution without a significant decrease in sensitivity (Figure 1.11H/I). Besides the mentioned, most commonly used apodization functions, a wide range of other functions are available each with specific characteristics regarding sensitivity and resolution [21].

### 1.9.3 Analog-To-Digital Conversion

So far the NMR signal has been described as a continuous, analog signal. However, the relatively simple but tedious Fourier transformation (and many other mathematical operations, like phasing and time domain filtering) are most conveniently performed by digital computer algorithms. As a consequence the analog FID signal received in the coil must be converted to a digital signal. This is done with an analog-to-digital converter (ADC), which measures the instantaneous value of the FID at equal time intervals (Figure 1.12). The speed of the analog-to-digital conversion is prescribed by the sampling theory [17]. This theory

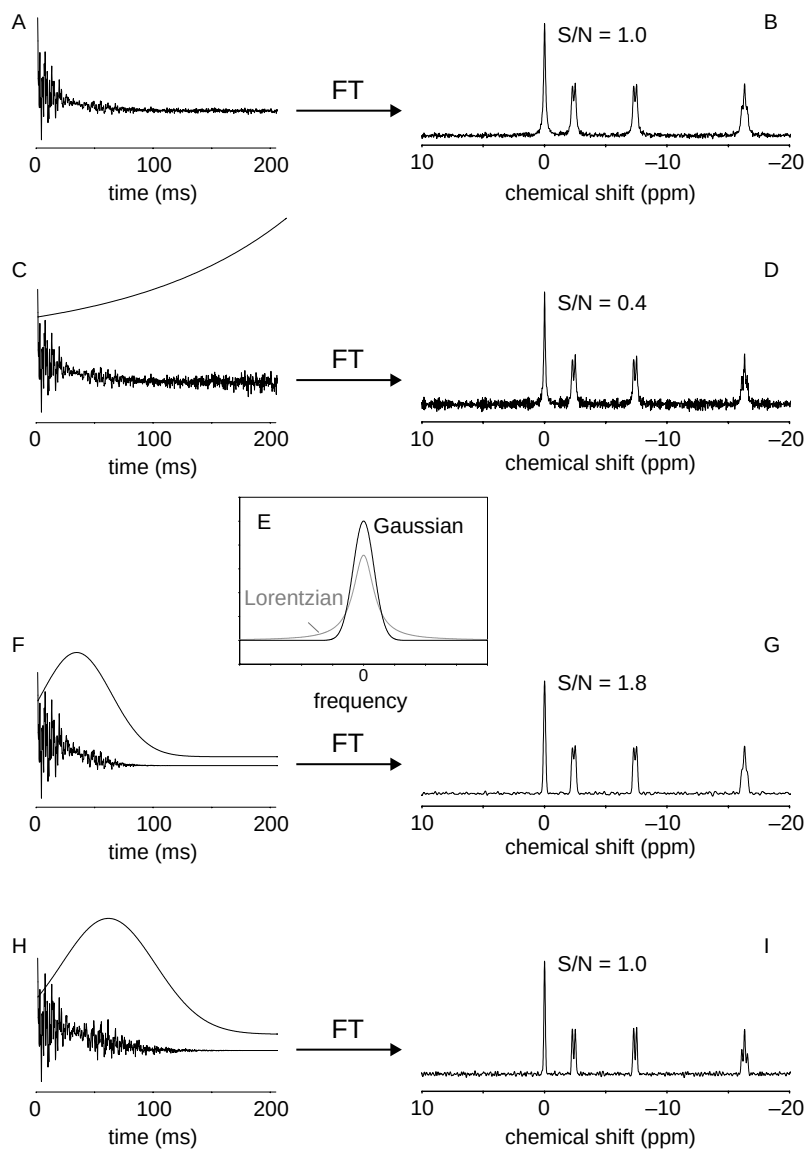


**Figure 1.11** The effects of time-domain apodization on the frequency-domain spectrum. (A, B) Time and frequency domain signals without apodization. The spectral S/N in (B) was assigned a relative value of 1.0. (C, D) Time-domain multiplication with an increasing exponential function improves the spectral resolution at the expense of a greatly reduced S/N. (E) Lorentzian and Gaussian resonance lines of equal FWHM and integrated amplitude. (F, G) Lorentz-to-Gauss transformation adjusted to give the same spectral resolution as in (B), results in an improved sensitivity. (H, I) When the Lorentz-to-Gauss transformation parameters are adjusted to provide the same spectral SNR as in (B), the spectral resolution is significantly improved.

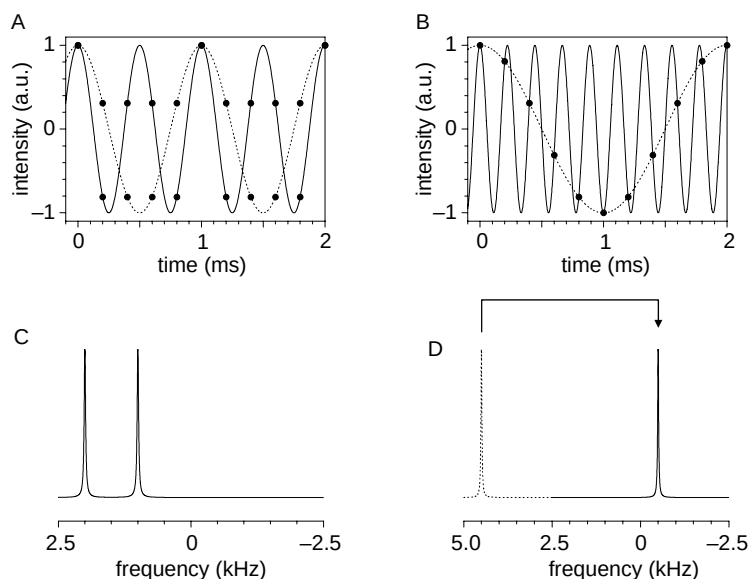


**Figure 1.12** Theory of analog-to-digital conversion. (A) A given time domain signal is sampled at 0.2 ms intervals, giving rise to a total spectral width of 5000 Hz and a Nyquist sampling frequency of 2500 Hz. The Larmor frequency of both resonances is smaller than the Nyquist sampling frequency, such that they can be adequately sampled. This gives a NMR spectrum (C) consisting of two resonances at the appropriate Larmor frequencies. (B) When the sample contains a resonance with a Larmor frequency  $\nu$  above the Nyquist sampling frequency  $F$ , the signal is still sampled, but now at an apparent frequency  $\nu_0 - F + \nu$ , resulting in a NMR spectrum with a resonance at the incorrect, apparent frequency (D).

states that any sinusoidal signal of frequency  $F$  can be accurately described when it is sampled at least twice per cycle. This minimum sampling rate is called the Nyquist frequency  $F_{\text{Nyquist}}$ . The spectral bandwidth  $SW$  equals  $2F_{\text{Nyquist}}$ , since frequencies between  $-F_{\text{Nyquist}}$  and  $+F_{\text{Nyquist}}$  are accurately sampled. The time between the data points is known as the dwell time and equals  $1/SW$ . If a signal is present with an absolute frequency greater than the Nyquist frequency, then this signal will still be digitized, but at an incorrect frequency (Figure 1.12). A resonance with frequency  $\nu_0 + F_{\text{Nyquist}} + \nu$ , where  $\nu_0$  is the center of the spectral bandwidth, will appear after Fourier transformation at a position with frequency  $\nu_0 - F_{\text{Nyquist}} + \nu$ . This so-called aliasing of resonances can be eliminated by increasing the spectral bandwidth, after which the minimum spectral bandwidth needed to unambiguously observe all the resonances can be determined. Aliasing of signal seems at first sight a large problem in FT NMR, since noise from outside the spectral region would be folded back into the spectrum thereby dramatically decreasing the obtainable S/N. However, high-frequency noise components can easily be filtered out before the ADC sampling by audio-filters (see Chapter 10). A cut-off filter, such as a Butterworth filter, does not affect signals within the spectral range, while suppressing (i.e. multiplying by zero) all signals (i.e. noise) outside the spectral range. To obtain optimal S/N without distortions from the cut-off points of

the filter, the filter bandwidth is normally set 10–25 % larger than the spectral width. More recent advances in digital electronics have led to the introduction of digital audio-filters with a much sharper cut-off profile.

### 1.9.4 Zero Filling

In general, the FID of a spectrum with spectral width  $SW = 2F_{\text{Nyquist}}$  is sampled by the ADC over  $N$  points in accordance with the Nyquist sampling frequency. Through a discrete Fourier transformation, the NMR spectrum will also contain  $N$  points. The spectral resolution  $\Delta\nu$  is therefore  $SW/N$ , which is equivalent to the reciprocal of the total acquisition (sampling) time  $T_{\text{acq}}$ , which is composed of  $N$  sampling periods of duration  $\Delta t$ :

$$\Delta\nu = \frac{1}{T_{\text{acq}}} = \frac{1}{N\Delta t} \quad (1.70)$$

For an experiment with 256 points sampled and  $T_{\text{acq}} = 102.4$  ms, leading to  $SW = 2F_{\text{Nyquist}} = 2500$  Hz, the spectral resolution is 9.77 Hz (2500 Hz/256). As can be seen from Figure 1.13, this spectral resolution is often too low to fully resolve the resonances present, i.e. it is desirable to have knowledge about the spectral amplitudes at intermediate frequencies. This can be achieved by decreasing the spectral width or by increasing the acquisition time. However, aliasing limits the increase in spectral resolution by decreasing the spectral width. Increasing the acquisition time will lead to increased data storage and an increase of the relative noise contribution as the signal intensity decreases with increasing acquisition time. Alternatively, the process of extending the acquisition time can be simulated by extending the acquired FID (which has decayed to zero amplitude) artificially by adding a string of points with zero amplitude to the FID prior to Fourier transformation. This process is known as zero filling. Figure 1.13 shows the effect of zero filling on the appearance of the  $\beta$ -ATP

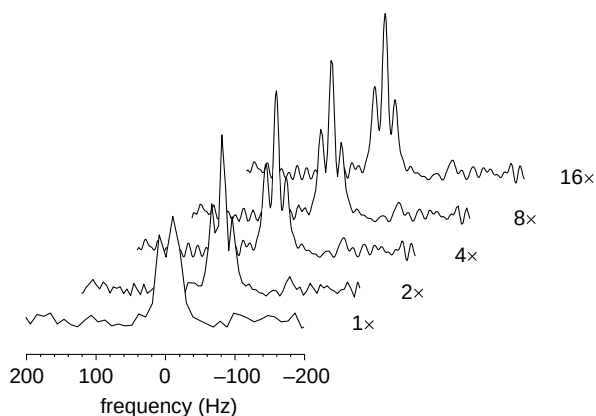


**Figure 1.13** Effect of zero filling on the spectral resolution. The triplet resonance of  $\beta$ -ATP is not well-resolved following a FT of the acquired data points (giving a spectral resolution of 9.77 Hz per point). Zero filling the original data (with a power of 2), completely resolves the triplet resonance (giving a spectral resolution of 0.61 Hz per point after 16 times zero filling). After four times zero filling no further improvement in spectral resolution is observed.



resonance from a  $^{31}\text{P}$  spectrum. The relatively coarse spectral resolution results in an apparent doublet signal following direct Fourier transformation of the measured time domain signal. While the signal at intermediate frequencies is accurately digitized in the time-domain (since the frequencies are smaller than the Nyquist frequency), it is not visualized in the frequency-domain simply because the discrete Fourier transformation only calculates the signal at a limited number of discrete frequencies. Increasing the number of time domain points by zero filling allows the calculation of additional frequency domain points at intermediate frequencies, revealing the expected triplet structure for  $\beta$ -ATP. While zero filling does not increase the information content of the data, it can greatly improve the spectral appearance.

## 1.10 Spin-Spin Coupling

The NMR resonance frequencies, or chemical shifts, give direct information about the chemical environment of nuclei, thereby greatly aiding in the unambiguous detection and assignment of compounds. The integrated resonance area is, in principle (see Exercise 1.5 and Chapter 9), directly proportional to the concentration of the compounds, thereby making NMR a quantitative technique. An additional feature that can be observed in high-resolution NMR spectra is the splitting of resonances into several smaller lines, a phenomenon often referred to as scalar coupling, J coupling or spin-spin coupling [22]. Scalar coupling originates from the fact that nuclei with magnetic moments can influence each other, besides directly through space (dipolar coupling) also through electrons in chemical bonds (scalar coupling). Even though dipolar interactions are the main mechanism for relaxation in a liquid, there is no net interaction between nuclei since rapid molecular tumbling averages the dipolar interactions to zero. However, interactions through chemical bonds do not average to zero and give rise to the phenomenon of scalar coupling.

In the following a qualitative description of scalar coupling is given. A more quantitative description can be found in Chapter 8. Consider an isolated proton and an isolated carbon-13 atom as depicted in Figure 1.14A. Electrons in s-orbitals have a finite probability of being at the nucleus, giving rise to a hyperfine interaction between nuclear and electronic spins. The Fermi contact governs the interaction between the nuclear and electron spins and (energetically) *favors* an antiparallel over a parallel arrangement. In terms of energy level diagrams, the two separate (two-level)  $^1\text{H}$  and  $^{13}\text{C}$  energy level diagrams can be combined into one diagram (Figure 1.15A) with four energy levels, corresponding to the four nuclear spin combinations. The four allowed energy level transitions (for which the spin quantum number  $m$  changes by  $\pm 1$ ) give rise to two resonance frequencies,  $\nu_{\text{H}}$  at the proton frequency and  $\nu_{\text{C}}$  at the carbon-13 frequency.

Now consider the situation where the proton and carbon-13 nuclei are covalently bound, as in  $[1-^{13}\text{C}]\text{glucose}$  (Figure 1.14B). The interaction between the two electrons inside a chemical bond is governed by the Pauli exclusion principle which *demands* that the electron spins are antiparallel. When both nuclear spins are antiparallel to the external magnetic field  $B_0$ , i.e. the high-energy  $\beta\beta$  state, the two bonding electrons can not both be antiparallel to the nuclear spins, leading to an energetically less favorable state (Figure 1.15B). The  $\beta\beta$  energy level increases by an amount proportional to  $^1J_{\text{HC}}/4$  where  $^1J_{\text{HC}}$  is the one-bond, heteronuclear scalar coupling constant. Similar arguments can be used

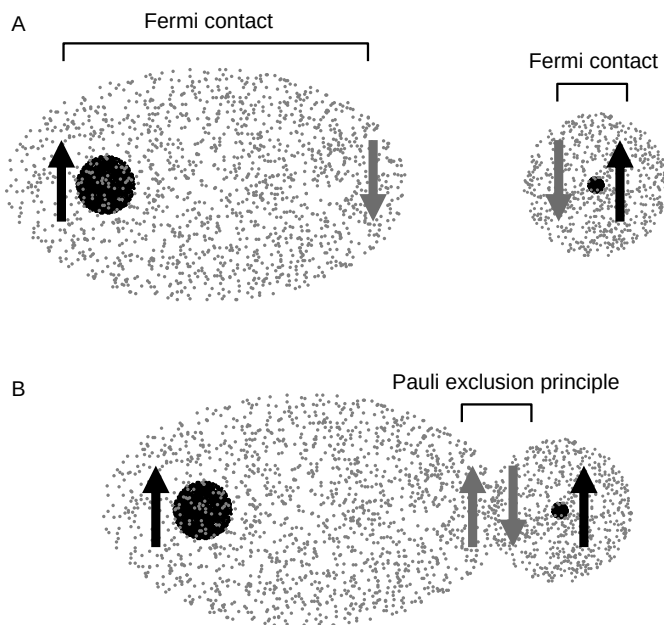


**Figure 1.14** Spin-spin interactions involved with scalar coupling. (A) In isolated atoms, the Fermi contact energetically favors an antiparallel orientation between nuclear and electronic spins. (B) In chemical bonds, the Pauli exclusion principle demands that the electron spins are in an antiparallel orientation thereby potentially forcing nuclear and electron spins in an energetically higher parallel orientation, depending on the nuclear spin state.

to describe the energy increase for the  $\alpha\alpha$  state. However, for the  $\alpha\beta$  and  $\beta\alpha$  states the electron spins can be antiparallel to the nuclear spins leading to an energetically more favorable situation. The energy level diagram for a scalar coupled two-spin system still only allows four transitions, but they now correspond to four different frequencies at  $\nu_H + {}^1J_{CH}/2$  and  $\nu_H - {}^1J_{CH}/2$  on the proton channel and at  $\nu_C + {}^1J_{CH}/2$  and  $\nu_C - {}^1J_{CH}/2$  on the carbon-13 channel. Each of the resonances has been divided into two new resonances of equal intensity separated by  ${}^1J_{CH}$ , giving rise to the NMR spectrum shown in Figure 1.16.

Similar arguments can be used to explain scalar coupling over two or three chemical bonds. While scalar coupling constants over one and three chemical bonds are typically positive, the scalar coupling constant over two chemical bonds is typically negative as can easily be deduced following arguments identical to those used for Figure 1.14. Because the basis of scalar coupling relies on magnetic interactions between electron spins and distant nuclear spins, the scalar coupling constant rapidly decreases with increasing number of chemical bonds and can typically be ignored for four or more bonds. The scalar coupling constant is independent of the applied external magnetic field, since it is based on the fundamental principle of spin-spin pairing and is therefore expressed in Hertz (Hz). Typical magnitudes of scalar coupling constants are:  ${}^1H$ - ${}^1H$ , 1–15 Hz;  ${}^1H$ - ${}^{13}C$ , 100–200 Hz;  ${}^1H$ - ${}^{15}N$ , 70–110 Hz;  ${}^1H$ - ${}^{31}P$ , 10–20 Hz;  ${}^{13}C$ - ${}^{13}C$ , 30–80 Hz; and  ${}^{31}P$ -O- ${}^{31}P$ , 15–20 Hz.

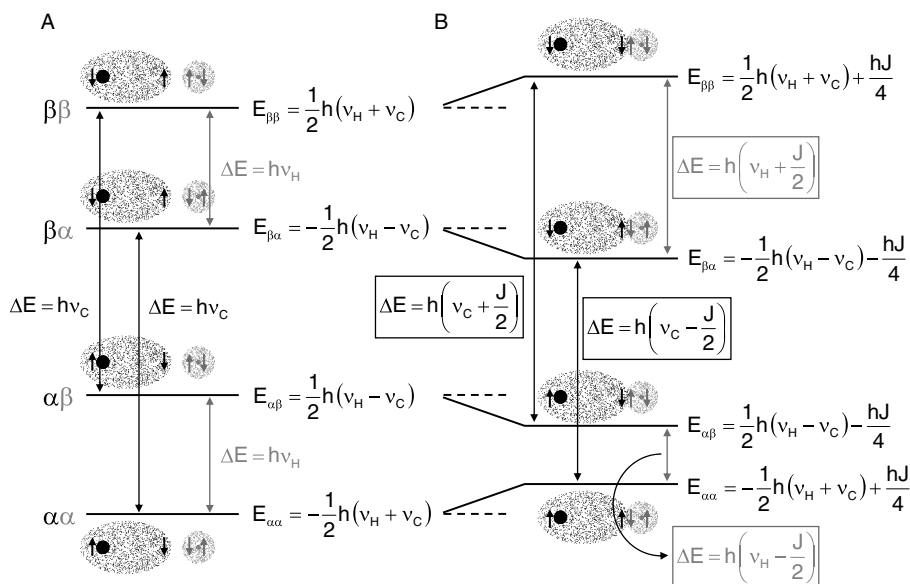


**Figure 1.15** Energy level diagram for (A) two isolated carbon-13 and proton nuclei and (B) a  $^{13}\text{C}$ - $^1\text{H}$  'molecule' with a covalent chemical bond between the carbon-13 and proton nuclei. The diagram in (A) is simply an extension of Figure 1.1B and allows two carbon-13 transitions with the same frequency  $\nu_C$  and two proton transitions with the same frequency  $\nu_H$  giving rise to singlet resonances in the carbon-13 and proton NMR spectra, respectively. When the carbon-13 and proton nuclei form a chemical bond, the nuclear spins affect each other through the bonding electrons. The  $\beta\beta$  spin state (i.e. the nuclear spin for both  $^{13}\text{C}$  and  $^1\text{H}$  is in the  $\beta$  state) becomes energetically less favorable as one of the two nuclear-electronic spin orientations is forced to be parallel. The same is true for the  $\alpha\alpha$  spin state, whereas in the  $\alpha\beta$  and  $\beta\alpha$  spin states all spin orientations can be antiparallel. The same energy-level perturbations now give rise to two carbon-13 transitions with different frequencies,  $\nu_C + J/2$  and  $\nu_C - J/2$  and two protons transitions with different frequencies,  $\nu_H + J/2$  and  $\nu_H - J/2$ , which will lead to the NMR spectra shown in Figure 1.16.

All scalar coupling constants are for one chemical bond, except for  $^1\text{H}$ - $^1\text{H}$  and  $^{31}\text{P}$ -O- $^{31}\text{P}$  interactions which stretch over three and two bonds, respectively.

The situation shown in Figure 1.16 is only valid when the frequency difference between the two scalar-coupled spins is much larger than the scalar coupling between them. For a heteronuclear interaction as shown in Figure 1.15 this requirement is certainly valid, as the frequency difference is typically several tens of MHz, while the heteronuclear scalar coupling is less than 200 Hz. When the condition  $|\nu_A - \nu_X| \gg J_{AX}$  holds, the two-spin AX spin system is referred to as a weakly coupled spin system and the corresponding NMR spectrum is often referred to as a first-order spectrum. However, for many homonuclear interactions the frequency difference  $|\nu_A - \nu_B|$  is of the same order of magnitude as the homonuclear scalar coupling constant  $J_{AB}$ , giving rise to so-called strongly coupled spin systems. In a strongly coupled two-spin system, the  $\alpha\beta$  and  $\beta\alpha$  spin states become mixed, as summarized in Table 1.2. As a result of this mixing of spin states, the simple

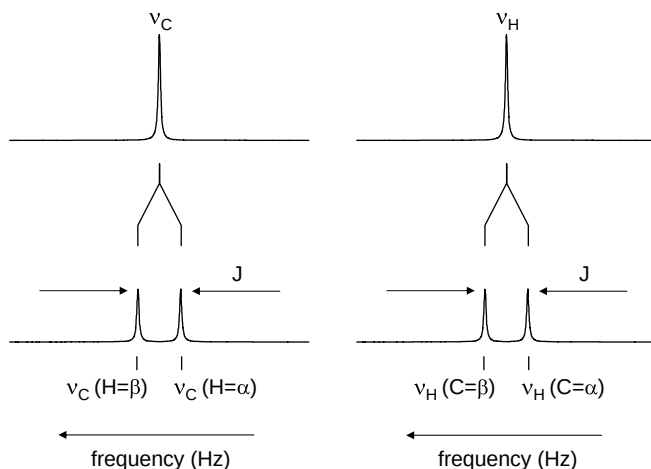


**Figure 1.16** Scalar coupling between carbon-13 and proton nuclei leads to a splitting of the singlet resonances into so-called doublet resonances. The resonances at the lower and higher frequencies are associated with energy level transitions in which the nuclear spin of the scalar-coupling partner is in the  $\alpha$  and  $\beta$  spin-state, respectively.

four-resonance-line spectrum (Figure 1.16) becomes more complicated as shown in Figure 1.17A and summarized in Table 1.3. The effects of strong coupling on the appearance of NMR spectra can not be understood in a classical sense, but requires full quantum-mechanical density matrix calculations [23]. Strongly coupled spin-systems produce so-called second-order spectra that are characterized by features not present in first-order spectra. Most noticeably from Figure 1.17A is the so-called ‘roof effect’ in which a line from the outer to the inner resonances forms an imaginary roof. This effect is another feature of NMR spectra that indicates that two multiplets belong to the same molecule and can therefore aid in the identification of compounds.

**Table 1.2** Energy characteristics for an AB spin system

| Energy level | Spin function <sup>a</sup>                          | Energy <sup>b</sup>                                 |
|--------------|-----------------------------------------------------|-----------------------------------------------------|
| 1            | $\beta\beta$                                        | $\frac{1}{2}h(\nu_A + \nu_B) + \frac{1}{4}hJ_{AB}$  |
| 2            | $\alpha\beta \cos \theta + \beta\alpha \sin \theta$ | $\frac{1}{2}hC - \frac{1}{4}hJ_{AB}$                |
| 3            | $\beta\alpha \cos \theta - \alpha\beta \sin \theta$ | $-\frac{1}{2}hC - \frac{1}{4}hJ_{AB}$               |
| 4            | $\alpha\alpha$                                      | $-\frac{1}{2}h(\nu_A + \nu_B) + \frac{1}{4}hJ_{AB}$ |

<sup>a</sup> $2\theta = \arcsin(J_{AB}/C)$ .

<sup>b</sup> $C = \sqrt{(\nu_A - \nu_B)^2 + J_{AB}^2}$ .

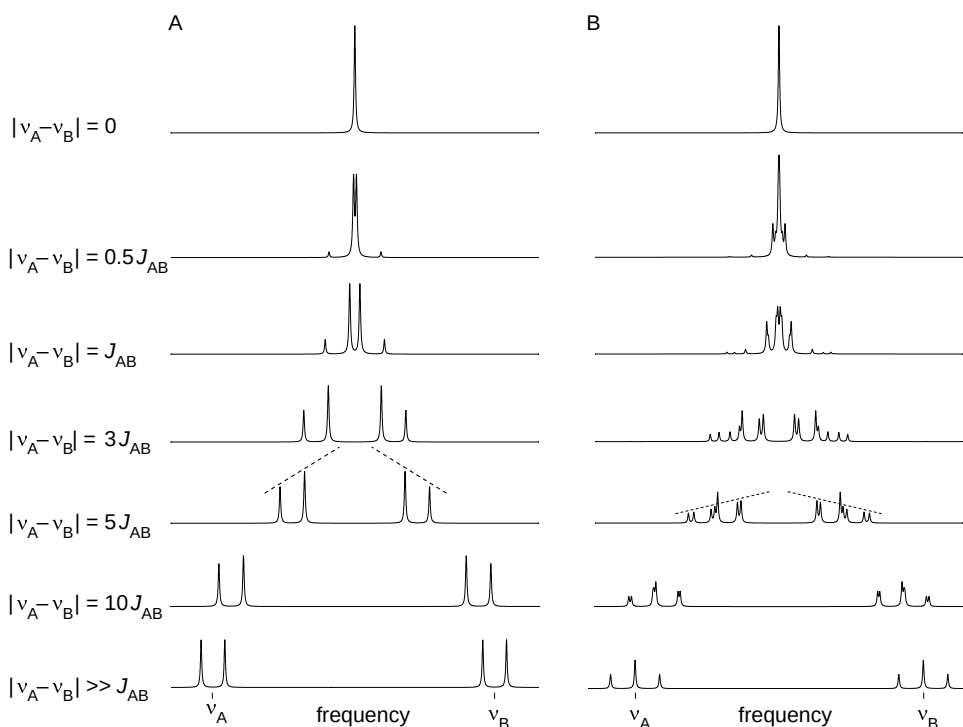


**Figure 1.17** Simulated (A) AB and (B)  $A_2B_2$  NMR spectra showing the effects of varying the ratio of the scalar coupling constant to the frequency difference between the A and B resonances. The lower NMR spectra are indicative of weakly coupled AX and  $A_2X_2$  spin systems, producing a first-order NMR spectrum, while the higher spectra are indicative of strongly coupled AB and  $A_2B_2$  spin systems, displaying strong second-order effects, like the appearance of additional resonances, as well as the so-called 'roof effect' (dotted lines).

### 1.10.1 Spectral Characteristics

To understand the splitting pattern encountered in NMR spectra of more complicated, but weakly coupled multi-spin systems, it is convenient to discriminate between nonequivalent, chemically equivalent and magnetically equivalent nuclei. For equivalent nuclei all physical and chemical properties (like reaction rates or exchange processes) are the same. The difference between chemically and magnetically equivalent nuclei is more subtle. Consider two nuclei with the same chemical shift, which are coupled to a third magnetic nucleus having a different chemical shift. When the scalar coupling constant of the two nuclei with the third nucleus is different, the nuclei are said to be chemically equivalent (since the chemical shift and therefore the chemical environment are identical) but not magnetically equivalent. For magnetically equivalent nuclei, the scalar coupling constant with a shared third nucleus must be identical.

**Table 1.3** Transition frequencies and relative intensities for an AB spin system

| Transition        | Frequency                                                       | Relative intensity |
|-------------------|-----------------------------------------------------------------|--------------------|
| 1 $\rightarrow$ 2 | $\frac{1}{2}(\nu_A + \nu_B) + \frac{1}{2}J_{AB} - \frac{1}{2}C$ | $1 - \sin 2\theta$ |
| 1 $\rightarrow$ 3 | $\frac{1}{2}(\nu_A + \nu_B) + \frac{1}{2}J_{AB} + \frac{1}{2}C$ | $1 + \sin 2\theta$ |
| 3 $\rightarrow$ 4 | $\frac{1}{2}(\nu_A + \nu_B) - \frac{1}{2}J_{AB} - \frac{1}{2}C$ | $1 + \sin 2\theta$ |
| 2 $\rightarrow$ 4 | $\frac{1}{2}(\nu_A + \nu_B) - \frac{1}{2}J_{AB} + \frac{1}{2}C$ | $1 - \sin 2\theta$ |

Magnetically equivalent nuclei are always chemically equivalent and chemically equivalent nuclei can never produce a first-order spectrum. Keeping the issues of equivalence in mind, the appearance of first-order spectra can be predicted by some simple rules:

1. Magnetically equivalent nuclei do not produce an observable splitting of the corresponding resonance lines. This is because quantum mechanical selection rules prohibit the appropriate transitions. Therefore, for instance, there is no scalar coupling between the protons within an isolated methyl group ( $\text{CH}_3$ ) even though they are only separated by two chemical bonds.
2. When there are more than two magnetic nuclei in a molecule, scalar coupling may occur between each pair of nuclei, resulting in a complex splitting pattern. The pattern for a given nucleus can be explained by the method of successive splitting. Consider three nonequivalent spins A, M and X (a so-called AMX spin system). The large difference in alphabetical order of the spins indicates a large difference in resonance frequency. An ABC spin system represents a scalar-coupled three-spin system with three nonequivalent spins which have similar chemical shifts (and do therefore not produce a first order spectrum). An  $\text{AX}_2$  spin system represents a three-spin system with two nonequivalent nuclei (A and X) and two magnetically equivalent nuclei ( $\text{X}_2$ ). The AMX spin system has spin-spin coupling between A and M (with  $J_{AM}$ ) and between M and X (with  $J_{MX}$ ). The splitting pattern of spin A is relatively simple, since it only experiences spin M. The resonance line of spin A will therefore be split in two lines separated by the scalar coupling constant  $J_{AM}$ . Similarly, the resonance for spin X is split once by the scalar coupling constant  $J_{MX}$ . However, the pattern for spin M is more complicated. First the coupling to spin A is considered resulting in two lines (a doublet), followed by the coupling to spin X, resulting in a splitting of each line in two more lines, giving a final 'doublet-of-doublets' (four lines of equal intensity).
3. The presence of magnetically equivalent nuclei in a group of interacting spins simplifies the appearance of the spectrum. The splitting pattern of spin A in an  $\text{AX}_n$  spin system (where n is the number of magnetically equivalent spins) is simply given by a binomial distribution in which the lines are separated by the scalar coupling constant (e.g.  $n = 2$  results in three lines with amplitudes in a 1:2:1 ratio).

Using these rules the appearance of all first-order spectra can be predicted. For example, Figure 1.18 shows the  $^1\text{H}$  spectrum of lactic acid (lactate). Lactate can be seen as a  $\text{AX}_3$  spin system, i.e. three magnetically equivalent methyl protons coupled to a single methine

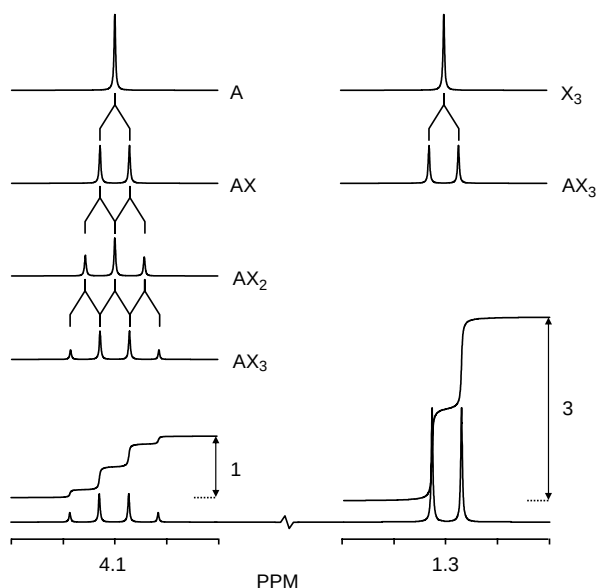


**Figure 1.18** The method of successive splitting for an  $AX_3$  spin system (e.g. lactate, with a methyl group resonating at 1.31 ppm and a methine proton resonating at 4.10 ppm). The A resonance splits successively in a doublet, a triplet and a quartet, while the X resonance splits into a doublet. Note that the binomial distribution (e.g. 1:3:3:1 for a quartet) only arises for magnetically equivalent nuclei which have an identical scalar coupling constant with a common coupling partner. Since the multiplet resonances at 4.10 and 1.31 ppm originate from one and three protons, respectively, the relative integrated areas of the two multiplets are therefore also one and three.

proton. Generally, the carbonyl and hydroxyl protons are invisible due to rapid exchange with water protons. Since the magnetically equivalent methyl protons do not produce any splitting among themselves, they only feel the methine proton, resulting in a doublet signal. The methine proton experiences three spins with an identical scalar coupling constant, resulting in four lines (a quartet) with a 1:3:3:1 binomial signal distribution. All the signals in the doublet and in the quartet are separated by the same scalar coupling constant. The relative *integrated* amplitude of the peaks at 1.31 ppm and 4.11 ppm is 3:1, respectively, since there are three methyl protons versus one methine proton.

In a two-spin system the effects of strong coupling changed the relative intensity and frequency of resonances (Figure 1.17A). However, in situations with more than two spins, like in a  $A_2B_2$  four-spin system, the effects of strong coupling can also lead to additional resonances (Figure 1.17B). This is because the mixed energy levels allow energy transitions ( $\Delta m = \pm 1$ ) that are simply not present when the energy levels are not mixed (i.e. when the spin-system is weakly coupled). It should be realized that while the behavior and spectral appearance of strongly coupled spin systems is no longer intuitive, it can still be quantitatively calculated through the use of the density matrix formalism, as will be discussed in Chapter 9. Note that the ‘roof effect’ is also visible for more complicated spin systems, as evident in Figure 1.17B.

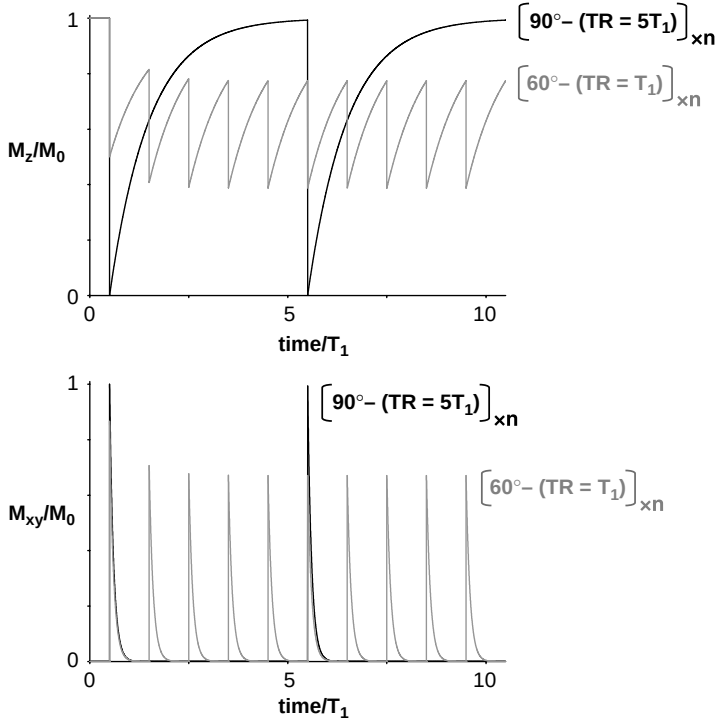


**Figure 1.19** The effects of multiple excitations and  $T_1$  relaxation on the establishment of a longitudinal steady-state condition. When the repetition time  $TR$  is five times the  $T_1$  relaxation time (black line) a steady-state situation is instantaneously achieved for which  $M_{xy}(0) = M_0$ . However, when  $TR < 5T_1$  (gray line),  $T_1$  relaxation is incomplete in between excitations and a steady-state situation is achieved only following a number of excitations.

### 1.11 $T_1$ Relaxation

In Section 1.6  $T_1$  and  $T_2$  relaxation were introduced as the return of longitudinal magnetization following a perturbation and the disappearance of transverse magnetization, respectively.  $T_1$  and  $T_2$  relaxation are so fundamental to NMR that they essentially affect any NMR experiment. Knowledge of  $T_1$  relaxation is required for signal quantification, the study of chemical exchange and the design of optimal timings for data acquisition. Consider a simple pulse-acquire experiment with an  $\alpha^\circ$  excitation pulse and a repetition time  $TR$ . Figure 1.19 shows the amount of longitudinal magnetization over time when the sequence is continuously repeated, as would be the situation in the case of signal averaging. In the case of a  $90^\circ$  pulse the longitudinal magnetization is reduced to zero after each  $90^\circ$  pulse, after which it is allowed to recover through  $T_1$  relaxation according to:

$$M_z(TR) = M_0(1 - e^{-TR/T_1}) \quad (1.71)$$

which reduces to  $M_z(TR) = M_0$  for  $TR > 5T_1$ . The amplitude of the transverse magnetization is given by  $M_{xy} = M_z \sin \alpha = M_0$ . However, even though full excitation is achieved



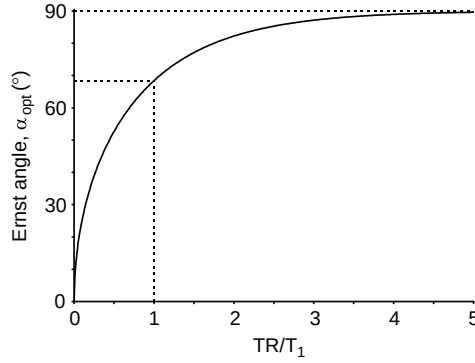


**Figure 1.20** Graphical representation of the relation between the optimal nutation angle (Ernst angle, in degrees) and the ratio of repetition time  $TR$  to the  $T_1$  relaxation time of a ( $\alpha^\circ$  - acquisition) experiment. The optimal nutation angle is the nutation angle which produces the highest  $S/N$  per unit of time for a given  $TR$  to  $T_1$  ratio.

during each scan, the experiment is not optimal in terms of signal per unit of time [=  $0.2(M_0/T_1)$ ] because the majority of scan time is used to wait for recovery of the longitudinal magnetization by  $T_1$  relaxation. Repeating the experiment with a  $60^\circ$  excitation angle  $TR = T_1$  results in the temporal  $M_z$  and  $M_{xy}$  modulations shown in Figure 1.19. During the first few scans the excitation pulse rotates more magnetization away from the longitudinal axis than can recover through  $T_1$  relaxation. However, after about three scans the amount of signal decrease by excitation and signal recovery by  $T_1$  relaxation are equal to each other, making the *steady-state* longitudinal magnetization prior to each excitation pulse equal to (see also Exercise 1.7):

$$M_z(\alpha, T_1) = \frac{M_0(1 - e^{-TR/T_1})}{(1 - \cos \alpha e^{-TR/T_1})} \quad (1.72)$$

Note that Equations (1.71) and (1.72) are both derived under the assumption that  $M_{xy} = 0$  immediately prior to excitation. Experimentally this can be achieved by using  $TR > 5T_2$  or by applying magnetic field gradient crushers to dephase any remaining transverse magnetization (see Chapter 4 for more details). For a  $60^\circ$  excitation pulse and  $TR = T_1$  the signal per unit of time increases to  $\sim 0.67(M_0/T_1)$ . Even though the signal acquired per excitation is smaller as compared with  $\alpha = 90^\circ$  and  $TR = 5T_1$ , the number of excitations per unit time has increased fivefold leading to a higher amount of acquired signal per unit time. It is straightforward to show (see also Exercise 1.7) that the optimal nutation angle  $\alpha_{\text{opt}}$  for maximum signal per unit time is given by:

$$\alpha_{\text{opt}} = \arccos(e^{-TR/T_1}) \quad (1.73)$$

which is known as the Ernst angle. When  $TR > 5T_1$ , the exponential term vanishes and the optimal nutation angle is  $90^\circ$ . For shorter  $TR$ , the nutation angle gets smaller in order to reduce the saturation of longitudinal magnetization and to maximize the acquired signal. Figure 1.20 shows the Ernst angle as a function of  $TR/T_1$ . Note that in general signal acquisition should not begin until the steady-state condition underlying Equation (1.73)

has been reached. Experimentally this is achieved with so-called dummy scans which are identical to the real scan with the only difference that no data are acquired. The dummy scans help to achieve the steady-state condition, after which data acquisition can commence. Certain  $\alpha$  and  $TR/T_1$  combinations require several dozen dummy scans before the steady-state situation is achieved.

Data acquisition with a short repetition time and corresponding Ernst angle is frequently used in fast MRI (see Chapter 4), as well as in MRS on low-sensitivity nuclei, like phosphorus-31. But while the more efficient data acquisition improves the spectral S/N, it also introduces significant  $T_1$  weighting which varies for metabolites with different  $T_1$  relaxation times. Therefore, quantitative interpretation of metabolite spectra acquired under saturating conditions requires knowledge of the  $T_1$  relaxation time.

The inversion recovery sequence is the classical 'gold-standard' for the determination of  $T_1$  relaxation times. The inversion recovery method consists of two pulses and two delays. After full signal recovery during a long repetition time  $TR$ , the longitudinal magnetization is inverted by a  $180^\circ$  inversion pulse. The magnetization partially recovers during an inversion recovery delay  $t$ , after which the longitudinal magnetization is excited onto the transverse plane by a  $90^\circ$  excitation pulse. Following data acquisition, the sequence can be repeated, starting with recovery of longitudinal magnetization during the repetition time  $TR$ .

The signal intensity  $M_z(t)$  during the recovery period  $t$  following the  $180^\circ$  inversion pulse can be described by:

$$M_z(t) = M_0 - (M_0 - M_z(0))e^{-t/T_1} \quad (1.74)$$

where  $M_z(0)$  is the longitudinal magnetization at  $t = 0$ , immediately following the inversion pulse. For a perfect inversion pulse,  $M_z(0) = -M_0$ . The  $T_1$  relaxation time constant can be obtained by acquiring NMR spectra (or images) at different recovery times  $t$  between 0 and  $5T_1$ . For  $t = 0$ , the inverted longitudinal magnetization has not yet recovered and is excited to the  $-y'$  axis by a  $90^\circ$  pulse along the  $-x'$  axis, resulting in a maximal negative resonance line after Fourier transformation. For  $t = 5T_1$ , the inverted magnetization has completely recovered to the  $+z'$  axis and is excited to  $+y'$ , resulting in a maximal positive resonance line. Figure 1.21A shows typical inversion recovery spectra as a function of the recovery time  $t$ . Fitting the integrated resonance areas to Equation (1.74) gives an estimate of the  $T_1$  relaxation time (Figure 1.21B). In general a three parameter fit [ $M_0$ ,  $M_z(0)$  and  $T_1$ ] is preferred over a two-parameter fit (only  $M_0$  and  $T_1$ ), since the additional parameter makes the estimation of  $T_1$  independent of the inversion accuracy or systematic offsets in the inversion recovery delays. A crude method of estimating the  $T_1$  relaxation time constant is to determine the time of zero-crossing,  $t_{\text{null}}$  in the recovery curve, after which the  $T_1$  relaxation can be calculated as  $T_1 = t_{\text{null}}/\ln 2$ . Inversion recovery is a very reliable technique for the measurement of  $T_1$ , with an inherent insensitivity toward  $B_0$  and  $B_1$  magnetic field inhomogeneity. However, the technique is rather time-inefficient, since the experimental duration is dictated by the return of the thermal equilibrium magnetization following excitation. The low temporal resolution of inversion recovery has led to the development of many fast alternatives. Saturation recovery is a simple modification, in which the  $180^\circ$  inversion pulse is replaced by a  $90^\circ$  excitation pulse. Since the  $90^\circ$  excitation pulse reduces the longitudinal magnetization to zero at time  $t = 0$ , *irrespective* of the signal recovery prior to excitation, saturation recovery does not require a long repetition time, thereby significantly increasing the temporal resolution. Many other methods are more than an

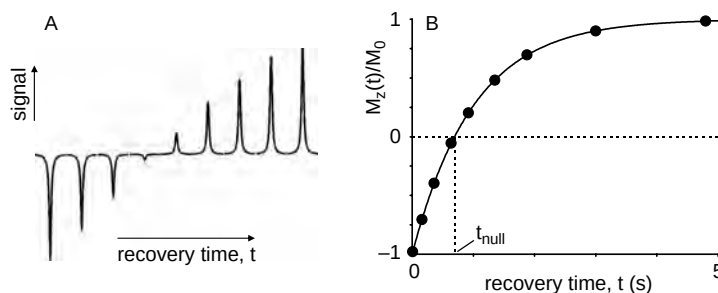


**Figure 1.21** Measurement of  $T_1$  relaxation through the use of an inversion recovery method. (A) Upon inversion of the longitudinal magnetization, the magnetization relaxes back to its thermal equilibrium value with a  $T_1$  relaxation time constant. Excitation at different inversion times results in spectra representing a discrete sampling of the  $T_1$  recovery curve. (B)  $T_1$  relaxation constants can be obtained by fitting the spectra in (A) with Equation (1.74). The time of zero-crossing ('nulling') of the longitudinal magnetization,  $t_{null}$ , is given by  $T_1 \ln 2$  and can provide a crude estimate of  $T_1$ .

order of magnitude faster than inversion recovery and some will be discussed in terms of fast  $T_1$  mapping by MRI (Chapter 4). However, it should be realized that the increased time resolution is often traded for increased sensitivity towards experimental imperfections, like  $B_1$  magnetic field inhomogeneity, decreased S/N or sufficient accuracy over only a limited range of  $T_1$  relaxation times.

## 1.12 $T_2$ Relaxation and Spin-echoes

The observation of NMR signal depends upon the generation of phase coherence. The existence of phase coherence is finite due to  $T_2^*$  relaxation. According to Equation (1.49),  $T_2^*$  relaxation is composed of intrinsic  $T_2$  relaxation and dephasing by macroscopic and microscopic magnetic field inhomogeneity. Following a  $90^\circ$  pulse, phase coherence is generated which disappears with a time constant  $T_2^*$ , thereby obscuring any information about  $T_2$ . However, through the generation of so-called spin-echoes [24] it is possible to separate the contribution of  $T_2$  and magnetic field inhomogeneity. The simplest experiment to generate spin echoes (and obtain information on  $T_2$ ) is the Hahn sequence [24] of two RF pulses shown in Figure 1.22A. An initial  $90^\circ$  RF pulse (irradiated along the  $-x$  axis of the rotating frame, i.e.  $90^\circ_{-x}$ ) creates transverse magnetization (phase coherence) along the  $y$  axis (Figure 1.22B). During the subsequent delay the magnetization starts losing coherence, since spins experience, besides the intrinsic  $T_2$  relaxation, a range of  $B_0$  magnetic fields and therefore precess about  $z$  with a variety of Larmor frequencies (Figure 1.22C). In other words, spins at different spatial positions acquire different phases due to variations in the main magnetic field. The phase  $\phi(\mathbf{r})$  acquired by spins at position  $\mathbf{r}$  is given by  $\phi(\mathbf{r}) = \gamma \Delta B_0(\mathbf{r})TE/2$ , where  $\Delta B_0(\mathbf{r})$  represents the magnetic field inhomogeneity, being the difference between the magnetic field at position  $\mathbf{r}$ ,  $B_0(\mathbf{r})$  and the nominal magnetic field across the entire sample,  $B_{nom}$ . After the delay  $TE/2$ , a  $180^\circ_y$  RF pulse is applied to the sample, which causes all magnetization vectors to rotate about  $y$  by  $180^\circ$ , leading to a

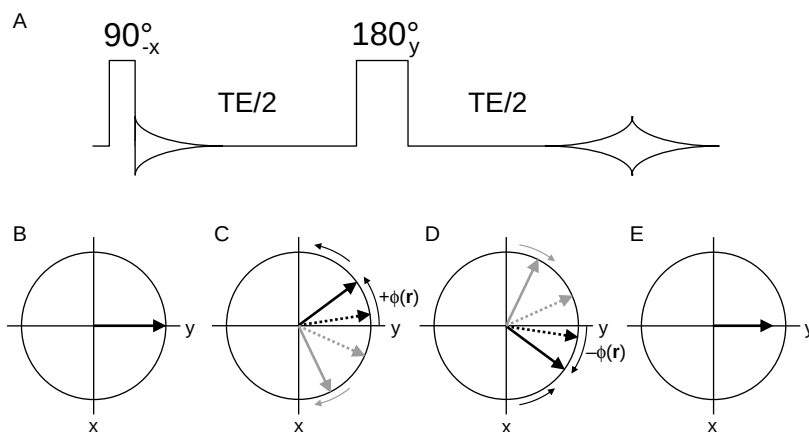


**Figure 1.22** Spin-echo formation for uncoupled spins. In a spin-echo experiment (A), the spins are excited (B) after which they dephase in the transverse plane during the first half of the echo time, due to  $B_0$  magnetic field inhomogeneity and frequency offsets (C). A  $180^\circ$  refocusing pulse mirrors all magnetization vectors along the  $y$  axis (D) after which the spins rephase during the second half of the echo time due to the same  $B_0$  magnetic field inhomogeneity and frequency offsets. At the echo time  $TE$ , the rephasing is complete and a spin-echo is formed (E). Obviously, the signal has decayed due to  $T_2$  relaxation.

resetting of the acquired phase from  $+\phi(\mathbf{r})$  to  $-\phi(\mathbf{r})$ . During a second delay  $TE/2$  the spins precess again at their local Larmor frequencies (Figure 1.22D) and because the phase was reset by the  $180^\circ$  pulse, the spins will be refocused along the  $y'$  axis at the end of the second delay to form a spin echo (Figure 1.22E). The time between the  $90^\circ$  pulse and the top of the spin-echo (i.e. where optimal refocusing occurs) is referred to as the echo time  $TE$ . At the top of the echo, the effects of  $B_0$  magnetic field inhomogeneity are refocused (i.e. their phase effect is eliminated) and the signal decrease is caused exclusively by inherent  $T_2$  relaxation (neglecting diffusion effects). The spin echo experiment is one of the most important elementary pulse sequences for *in vivo* NMR spectroscopy. Spin-echoes form the basis for spatial localization, water suppression, spectral editing and a wide range of additional delayed-acquisition methods. Spin echo techniques can also be used to filter out components with short  $T_2$  relaxation times and they allow the acquisition of an artifact-free FID (e.g. the second half of the echo). This is because in a simple  $90^\circ$  pulse-acquire experiment, the first points of the FID can be distorted due to the close proximity of a high power  $90^\circ$  pulse (i.e. breakthrough of RF power). Furthermore, the spin-echo sequence can be used to measure the  $T_2$  relaxation time by performing several experiments in which the echo time is varied (Figure 1.23). The corresponding spectra can be fitted to an exponential curve, according to:

$$M_{xy}(TE) = M_{xy}(0)e^{-TE/T_2} \quad (1.75)$$

to obtain the  $T_2$  relaxation time constant. An alternative method to measure  $T_2$  is the Carr–Purcell–Meiboom–Gill (CPMG) experiment [25,26], in which the single  $180^\circ$  refocusing pulse is replaced by a train of successive  $180^\circ$  pulses. The main advantage of the

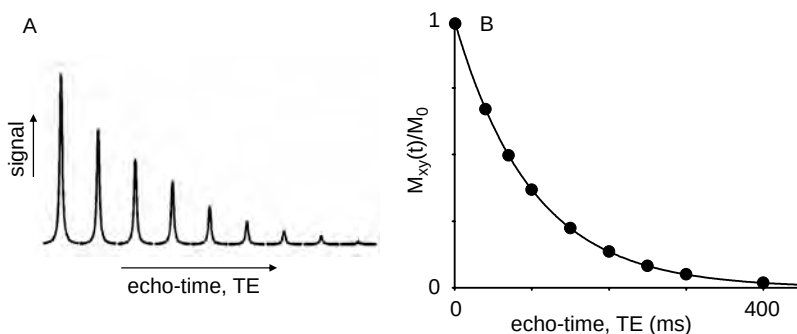


**Figure 1.23** Measurement of  $T_2$  relaxation through the use of a spin-echo method. (A) NMR spectra obtained at different echo times. (B) the  $T_2$  relaxation time constants can be obtained by fitting the data presented in (A) with Equation (1.75).

CPMG method is that signal loss during the echo time as a result of diffusion is greatly reduced, such that the measured  $T_2$  relaxation time constant is closer to the intrinsic, dipolar  $T_2$  relaxation time constant. The effects of diffusion are detailed in Chapter 3.

### 1.13 Exercises

**1.1** A 2 L water-filled sphere ( $T = 298.15$  K) is placed inside a 3.0 T MR magnet.

**A** Calculate the net access of proton spins in the low-energy  $\alpha$ -state (hint: water density =  $1.00 \text{ g mL}^{-1}$  and Avogadro constant =  $6.02214 \times 10^{23} \text{ mol}^{-1}$ ).

**B** Calculate the error that is made by ignoring all higher order terms in the Taylor expansion of Equation (1.23) for  $T = 298.15$  K, 4.0 K and 0.01 K.

**1.2** Derive the Bloch equations in the laboratory frame in the absence of relaxation [Equations (1.33)–(1.35)] from Equation (1.28).

**1.3** Show that free precession of the transverse magnetization according to:

$$M_x(t) = M_x(0) \cos \omega t + M_y(0) \sin \omega t$$

and

$$M_y(t) = M_y(0) \cos \omega t - M_x(0) \sin \omega t$$

is a solution of the Bloch equations in the laboratory frame [Equations (1.33)–(1.35)] in the absence of a perturbing magnetic RF field.

**1.4 A** Derive the Bloch equations in the rotating frame [Equations (1.45)–(1.47)] from the Bloch equations in the laboratory frame [Equations (1.39)–(1.41)].

**B** Show that Equations (1.50) and (1.51) are solutions of the Bloch equations in the rotating frame (assume that  $T_2 = T_2^*$ ).

**1.5 A** Derive the expression for the full line width at half maximum (FWHM) for the absorption component of a Lorentzian line [e.g. Equation (1.56)].

**B** Derive the expression for the FWHM for the magnitude component of a Lorentzian line.

- C Derive the expression for the absorption and dispersion parts of a resonance line originating from a full spin-echo (as opposed to a FID).
- D Determine the peak heights and integrals of (the absorption component of) Lorentzian lines originating from a FID and a full spin-echo.
- 1.6** Longitudinal magnetization can be ‘excited’ into the transverse plane by a  $90^\circ$  (or  $\pi/2$ ) pulse.
- A Starting from the Bloch equations in the rotating frame, derive an expression for the conversion of longitudinal magnetization  $M_z$  into transverse magnetization  $M_y$  by a RF pulse of length  $T$  and amplitude  $B_1$  applied on-resonance along the  $x'$  axis. Ignore  $T_1$  and  $T_2$  relaxation. Show how the nutation angle depends on the pulse amplitude and length.
- B If the pulse length of the  $90^\circ$  pulse is 1.0 ms, what is the required  $B_1$  magnitude in  $\mu\text{T}$  to achieve excitation?
- C How many Larmor precession cycles will occur in the laboratory frame at  $B_0 = 3.0\text{ T}$  during the  $90^\circ$  excitation pulse?
- 1.7** Consider a pulse-acquire experiment consisting of a RF pulse generating a nutation angle  $\alpha$  followed by a recovery time  $TR$ .
- A Starting with the Bloch equation for  $T_1$  relaxation [i.e. Equation (1.38)] derive an expression for the recovery of the longitudinal magnetization following a perturbation.
- B Derive the expression for the steady-state longitudinal magnetization [i.e. Equation (1.72)] for the pulse-acquire sequence.
- C Calculate after how many experiments the longitudinal magnetization is within 1 % of the steady-state magnetization when  $\alpha = 40^\circ$  and  $TR = T_1$ .
- D Suppose that 10 blocks of four averages are acquired sequentially in one experiment with  $\alpha = 40^\circ$  and  $TR = T_1$  starting from an initial thermal equilibrium situation. Calculate the difference between the acquired signal in the first and the last block due to incomplete  $T_1$  saturation during the first block.
- E Derive the Ernst angle expression from Equation (1.72).
- F Calculate the Ernst angle for the excitation pulse of a spin-echo sequence with  $TR = 0.5T_1$ . Assume negligible  $T_1$  relaxation during the echo time  $TE$ .
- 1.8** In a properly executed spin-echo sequence, the resonances of all (uncoupled) spins appear with the same relative phase. The absolute phase of all resonances can be made zero by a simple zero-order phase correction.
- A Calculate the phase difference between the creatine methyl (3.03 ppm) and NAA methyl (2.01 ppm) proton resonances at 7.05 T in the presence of a 500  $\mu\text{s}$  timing error.
- B In a proton spectrum acquired at 4.0 T (water is on-resonance at 4.7 ppm), the choline methyl (3.22 ppm) and NAA methyl (2.01 ppm) resonances appear with relative phases of  $30^\circ$  and  $210^\circ$ , respectively. Calculate the required zero- and first-order phase corrections to properly phase the spectrum to pure absorption lines.
- 1.9** Given the Gaussian line shape:

$$F_G(\omega) = \sqrt{\frac{\pi}{4}} M_0 T_{2G} e^{-\frac{(\omega_0 - \omega)^2 T_{2G}^2}{4}}$$

- A** Find the expression for the FWHM.
- B** For single Lorentzian and Gaussian resonance lines of equal line width and area, calculate the signal height-to-noise advantage of a Gaussian line (assuming equal noise levels).
- C** For single Lorentzian and Gaussian resonance lines of equal line width and area, calculate the line width advantage of a Gaussian line at 10 % of the respective peak heights.

**1.10** Consider a (hypothetical)  $^1\text{H}$  NMR spectrum with the following five resonances:

- Resonance 1: triplet resonance ( $^3J_{\text{HH}} = 7$  Hz) at 1.1 ppm with relative intensity (as determined by numerical integration) of 307.
- Resonance 2: quartet resonance ( $^3J_{\text{HH}} = 7$  Hz) at 3.9 ppm with relative intensity 198.
- Resonance 3: doublet-of-doublets ( $^3J_{\text{HH}} = 11$  and 8 Hz) at 7.2 ppm with relative intensity 102.
- Resonance 4: doublet resonance ( $^3J_{\text{HH}} = 8$  Hz) at 8.5 ppm with relative intensity 105.
- Resonance 5: doublet resonance ( $^3J_{\text{HH}} = 11$  Hz) at 10.0 ppm with relative intensity 96.

With the knowledge that the  $^1\text{H}$  NMR spectrum originates from an organic molecule  $\text{C}_5\text{H}_8\text{O}_2$ , determine the complete chemical structure of the compound.

**1.11** Consider a weakly coupled four-spin system  $\text{AMX}_2$  with chemical shift positions given by  $\delta_{\text{A}} = 5.0$  ppm,  $\delta_{\text{M}} = 1.5$  ppm and  $\delta_{\text{X}} = 3.0$  ppm relative to a carrier frequency of 200 MHz.

- A** Sketch the NMR spectrum for this compound when  $J_{\text{AM}} = 20$  Hz,  $J_{\text{MX}} = 10$  Hz and  $J_{\text{AX}} = 0$  Hz. Assume equal  $T_1$  and  $T_2$  characteristics for all resonances.
- B** Sketch the NMR spectrum for this compound when  $J_{\text{AM}} = 20$  Hz,  $J_{\text{MX}} = 10$  Hz and  $J_{\text{AX}} = 5$  Hz. Assume equal  $T_1$  and  $T_2$  characteristics for all resonances.
- C** When the NMR spectrum is acquired with a pulse-acquire sequence ( $\alpha = 90^\circ$ ,  $\text{TR} = 0.5$  s, number of averages 8 192) sketch the NMR spectrum for this compound when  $J_{\text{AM}} = 0$  Hz,  $J_{\text{MX}} = 10$  Hz and  $J_{\text{AX}} = 0$  Hz and  $T_{1\text{A}} = 5.0$  s,  $T_{1\text{M}} = 1.0$  s,  $T_{1\text{X}} = 2.0$  s. Assume equal  $T_2$  characteristics for all resonances.

**1.12** A proton NMR signal is acquired as 512 complex points during an acquisition time of 102.4 ms.

- A** Determine the spectral width of the experiment.
- B** Determine the apparent spectral frequency position of a signal with a frequency of +3800 Hz.
- C** Determine the apparent spectral frequency position of a signal with a frequency of -16 000 Hz.

**1.13** In a proton NMR spectrum the resonance from 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) is detected at 170.345213 MHz. Two other resonances occur at 170.345453 MHz and 170.345668 MHz, respectively.

- A** Calculate the chemical shifts of the two resonances in PPM.
- B** Calculate the frequencies of the two compounds at 7.05 T when DSS appears at a frequency of 300.176544 MHz.

- 1.14** The time domain data from a sample consists of three sinusoidal functions ( $M_0 = 150, 300$  and  $200$ ) oscillating at different frequencies ( $250, 300$  and  $500$  Hz) and decaying at different rates ( $T_2 = 50, 50$  and  $100$  ms).
- A** Sketch the Fourier transform spectrum acquired from the sample when the initial phase is zero for all resonances. Indicate linewidths (in Hz) and relative peaks heights.
- B** Sketch the Fourier transform spectrum acquired from the sample when the initial phases are  $0^\circ, 90^\circ$  and  $135^\circ$ , respectively.
- 1.15** Show that the real and imaginary frequency domain signals given by Equations (1.54) and (1.55) reduce to pure absorption and dispersion signals following a phase correction according to Equations (1.58) and (1.59) with  $\phi_c = \phi$ .
- 1.16** Hund's rule states that if two or more empty orbitals are available, electrons occupy each with spins parallel until all orbitals have one electron. When chemical bonds in  $sp^3$  hybridized structures are considered, describe the signs of  $^2J_{HH}$  and  $^3J_{HH}$  relative to  $^1J_{CH}$  using similar arguments as used for Figures 1.14 and 1.15.

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# 2

## ***In Vivo* NMR Spectroscopy – Static Aspects**

### **2.1 Introduction**

Magnetic resonance spectroscopy (MRS) is feasible on any nucleus possessing a magnetic moment. For *in vivo* MRS applications the metabolically most interesting nuclei which have this property are proton ( $^1\text{H}$ ), carbon-13 ( $^{13}\text{C}$ ), phosphorus ( $^{31}\text{P}$ ) and sodium ( $^{23}\text{Na}$ ). Even though the number of relevant nuclei is limited, each nucleus provides a wealth of information, since a large number of metabolites can be detected simultaneously.  $^1\text{H}$  MRS allows the detection of a number of important neurotransmitters, such as glutamate, GABA and aspartate and related compounds, like glutamine, as well as the end product of glycolysis, lactate.  $^{13}\text{C}$  MRS offers the possibility to study noninvasively the fluxes through important metabolic pathways, like the tricarboxylic acid cycle, *in vivo*.  $^{31}\text{P}$  MRS provides information about energetically important metabolites, intracellular pH, magnesium concentration and reaction fluxes. Besides the mentioned nuclei, *in vivo* MRS is in special cases also relevant on other nuclei, like helium ( $^3\text{He}$ ), lithium ( $^7\text{Li}$ ), nitrogen ( $^{15}\text{N}$ ), oxygen ( $^{17}\text{O}$ ), fluorine ( $^{19}\text{F}$ ), silicium or silicon ( $^{29}\text{Si}$ ) and potassium ( $^{39}\text{K}$ ).

This chapter will review the static aspects of MRS, like chemical shifts, scalar coupling constants and concentrations. The next chapter will focus more on the dynamic processes underlying MRS, like  $T_1$  and  $T_2$  relaxation, diffusion and chemical exchange.

### **2.2 Proton NMR Spectroscopy**

The proton nucleus is, besides the low abundance hydrogen isotope tritium, the most sensitive nucleus for NMR (see Table 1.1), both in terms of intrinsic NMR sensitivity (high gyromagnetic ratio) and high natural abundance (>99.9 %). Since nearly all metabolites contain protons, *in vivo*  $^1\text{H}$  NMR spectroscopy is in principle a powerful technique to

observe, identify and quantify a large number of biologically important compounds in intact tissue.

However, the application of  $^1\text{H}$  MRS to intact tissues *in vivo* is challenging for a number of reasons. Firstly, the water resonance is several orders of magnitude larger than the low concentration metabolites, making metabolite detection difficult and ambiguous (see Chapter 6). Secondly, other large signals like extracranial lipids can also overwhelm small metabolite signals and thirdly, heterogeneous magnetic field distributions significantly decrease the spectral resolution. Therefore, water suppression and spatial localization are prerequisites for meaningful *in vivo*  $^1\text{H}$  MRS studies and will be discussed in detail in Chapter 6. Chapter 10 will deal with methods to optimize the magnetic field homogeneity. An inherent limitation of  $^1\text{H}$  MRS is the narrow chemical shift range of only 5 ppm for nonexchangeable protons. This causes a large number of metabolite resonances to overlap, making their separation and quantification difficult. Separation of metabolites by spectral editing is discussed in Chapter 8, while the quantification of spectra is detailed in Chapter 9. Finally, even though the proton nucleus is the most sensitive for NMR studies, (*in vivo*) NMR is in general a very insensitive technique, making the detection of low concentration metabolites a compromise between time resolution and signal-to-noise ratio (S/N).

Despite the challenges, *in vivo*  $^1\text{H}$  MRS is a powerful technique to observe a large number of biologically relevant metabolites. On a state-of-the-art MR system, up to circa 15–20 different metabolites can be extracted simultaneously from short-TE  $^1\text{H}$  NMR spectra of normal brain [1, 2]. While the next chapters deal with the acquisition and processing of NMR spectra, this chapter will discuss the information content of  $^1\text{H}$  NMR spectra. In particular, the structure, NMR characteristics (scalar coupling constants/patterns and chemical shifts) and metabolic functions of the major resonances appearing in *in vivo*  $^1\text{H}$  NMR spectra will be discussed.

Figure 2.1 shows a typical short-TE  $^1\text{H}$  NMR spectrum acquired from rat brain *in vivo* at 11.75 T. The excellent spectral resolution achievable at high magnetic fields allows the detection of over 15 different metabolites. The identification of the various metabolites is based on a variety of methods, including prior biochemical knowledge, systemic

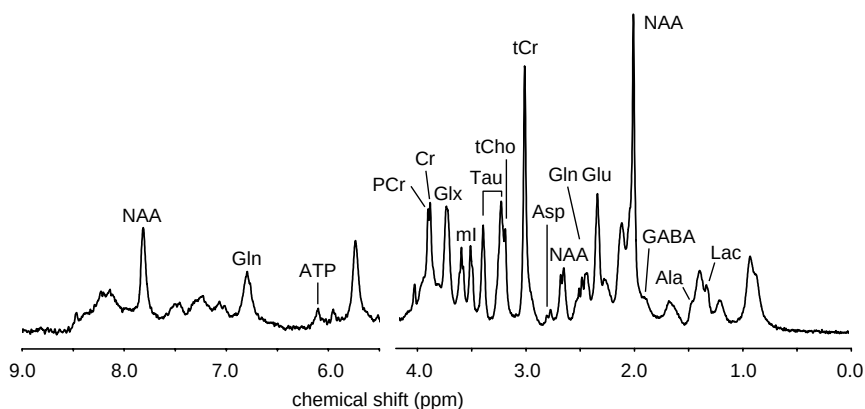


**Figure 2.1**  $^1\text{H}$  NMR spectrum obtained from rat brain *in vivo* at 11.75 T ( $TR/TE = 4000/12$  ms,  $100\ \mu\text{l}$ ) without (direct) water suppression (see Chapter 6). The excellent spectral resolution obtainable at high magnetic field strengths allows the visual separation of > 15 metabolites.

perturbations, physiological alterations (i.e. diseased states) and information obtained from *in vitro* biophysical techniques like gas chromatography-mass spectrometry, high-resolution liquid-state NMR and biochemical assays. In the next sections the NMR characteristics of the most commonly encountered metabolites will be discussed.

All chemical shifts and scalar coupling constants (Table 2.1) mentioned throughout this chapter are taken from literature [2] or are measured on 25 mM solutions at 500 MHz, 310 K and pH = 7.0. The chemical shifts are referenced against nine equivalent protons of 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) at 0.00 ppm [3]. Since the majority of metabolites detected by *in vivo* NMR are present in the central nervous system (CNS), the following discussion will focus on these compounds after which metabolites detected outside the CNS will be discussed. All simulated  $^1\text{H}$  NMR spectra are calculated as pulse-acquire spectra at 300 MHz with a line width of 1 Hz.

### 2.2.1 Acetate (Ace)

Acetate ( $\text{CH}_3\text{COO}^-$ ) is a small molecule containing a single methyl group that provides a singlet resonance at 1.90 ppm, directly overlapping with a multiplet of GABA-H3 at 1.89 ppm. Acetate is under normal conditions not observed in *in vivo*  $^1\text{H}$  NMR spectra of brain. However, acetate is readily taken up by the brain and observed by  $^1\text{H}$  NMR when the plasma acetate levels are raised by intravenous infusion. This is of particular importance for studies on energy and neurotransmitter metabolism by  $^{13}\text{C}$  NMR, since the absence of neuronal acetate transporters leads to a selective brain uptake by the astroglia [4]. This has been used to confirm and extend earlier [ $1\text{-}^{13}\text{C}$ ]glucose studies on brain energy metabolism and allows for a more sensitive detection of astroglial metabolism [5–7]. More discussion on acetate metabolism can be found in Chapter 3.

### 2.2.2 N-Acetyl Aspartate (NAA)

In  $^1\text{H}$  NMR spectra of normal brain tissue the most prominent resonance originates from the methyl group of NAA at 2.01 ppm (Figure 2.2). Smaller resonances appear as doublet-of-doublets at 2.49 ppm, 2.67 ppm and 4.38 ppm corresponding to the protons of the aspartate  $\text{CH}_2$  and  $\text{CH}$  groups. The amide  $\text{NH}$  proton is exchangeable with water and gives a broad, temperature-sensitive resonance at 7.82 ppm.

NAA is exclusively localized in the central and peripheral nervous systems. Its concentration varies in different parts of the brain [8–12] and undergoes large developmental changes [13–15], increasing in rat brain from <1 mM at birth to >5 mM in adulthood [16, 17]. Even though there is a substantial amount of literature on the synthesis, distribution and possible function, reviewed by Birken and Oldendorf [18] and others [19, 20], the exact function of NAA remains largely unknown. Suggested functions for NAA include osmoregulation [21] and a breakdown product of the neurotransmitter NAAG [22]. Others have suggested the involvement of NAA in fatty acid and myelin synthesis [23, 24] by acting as a storage form for acetyl groups.  $^{13}\text{C}$  NMR spectroscopy offers the possibility of measuring NAA turnover directly in human [25] and rat [26] brain *in vivo*. Using [ $1\text{-}^{13}\text{C}$ ]glucose as the substrate, NAA turnover is extremely slow with a time constant of circa 14 h [26], thereby supporting the notion that NAA is not significantly involved in glucose brain energy metabolism at rest.

For *in vivo*  $^1\text{H}$  MRS applications, NAA has played two roles. Firstly, the NAA resonance has been used as a marker of neuronal density. This is supported by the observation of a

**Table 2.1** Chemical shifts, multiplicities, connectivities and scalar coupling constants<sup>a</sup> for <sup>1</sup>H-containing cerebral metabolites

| Compound                                     | Group                        | Chemical shift (ppm) | Multiplicity <sup>b</sup> | Interaction | Scalar coupling (Hz) |
|----------------------------------------------|------------------------------|----------------------|---------------------------|-------------|----------------------|
| Acetate                                      | <sup>2</sup> CH <sub>3</sub> | 1.904                | s                         | —           | —                    |
| <i>N</i> -Acetyl aspartate (NAA)             |                              |                      |                           |             |                      |
| Acetyl moiety                                | <sup>2</sup> CH <sub>3</sub> | 2.008                | s                         | —           | —                    |
| Aspartate moiety                             | <sup>2</sup> CH              | 4.382                | m                         | 2–3         | 3.86                 |
|                                              | <sup>3</sup> CH <sub>2</sub> | 2.673                | dd                        | 2–3'        | 9.82                 |
|                                              |                              | 2.486                | dd                        | 3–3'        | –15.59               |
|                                              | NH                           | 7.820                | d                         | NH–2        | 7.90                 |
| <i>N</i> -Acetyl aspartatyl glutamate (NAAG) |                              |                      |                           |             |                      |
| Acetyl moiety                                | <sup>2</sup> CH <sub>3</sub> | 2.042                | s                         | —           | —                    |
| Aspartate moiety                             | <sup>2</sup> CH              | 4.607                | dd                        | 2–3         | 4.41                 |
|                                              | <sup>3</sup> CH <sub>2</sub> | 2.721                | dd                        | 2–3'        | 9.52                 |
|                                              |                              | 2.519                | dd                        | 3–3'        | –15.91               |
| Glutamate moiety                             | <sup>2</sup> CH              | 4.128                | dd                        | 2–3/2–3'    | n.m. <sup>c</sup>    |
|                                              | <sup>3</sup> CH <sub>2</sub> | 1.881                | m                         | 3–3'        | n.m.                 |
|                                              |                              | 2.049                | m                         | 3–4/3–4'    | n.m.                 |
|                                              | <sup>4</sup> CH <sub>2</sub> | 2.190                | m                         | 3'–4/3'–4'  | n.m.                 |
|                                              |                              | 2.180                | m                         | 4–4'        | n.m.                 |
| Adenosine triphosphate (ATP)                 |                              |                      |                           |             |                      |
| Ribose moiety                                | <sup>1</sup> CH              | 6.127                | d                         | 1–2         | 5.7                  |
|                                              | <sup>2</sup> CH              | 4.796                | dd                        | 2–3         | 5.3                  |
|                                              | <sup>3</sup> CH              | 4.616                | dd                        | 3–4         | 3.8                  |
|                                              | <sup>4</sup> CH              | 4.396                | dd                        | 4–5         | 3.0                  |
|                                              | <sup>5</sup> CH <sub>2</sub> | 4.295                | m                         | 4–5'        | 3.1                  |
|                                              |                              | 4.206                | m                         | 5–5'        | –11.8                |
|                                              |                              |                      |                           | 4–P         | 1.9                  |
|                                              |                              |                      |                           | 5–P         | 6.5                  |
|                                              |                              |                      |                           | 5'–P        | 4.9                  |
| Adenosine moiety                             | <sup>2</sup> CH              | 8.224                | s                         |             |                      |
|                                              | <sup>8</sup> CH              | 8.514                | s                         |             |                      |
|                                              | NH <sub>2</sub>              | 6.755                | s                         |             |                      |
| Alanine                                      | <sup>2</sup> CH              | 3.775                | q                         | 2–3         | 7.23                 |
|                                              | <sup>3</sup> CH <sub>3</sub> | 1.467                | d                         |             |                      |
| $\gamma$ -Aminobutyric acid (GABA)           | <sup>2</sup> CH <sub>2</sub> | 2.283                | t                         | 2–3         | 7.30                 |
|                                              |                              |                      |                           | 3–4         | 7.30                 |
|                                              | <sup>3</sup> CH <sub>2</sub> | 1.889                | m                         |             |                      |
|                                              | <sup>4</sup> CH <sub>2</sub> | 3.012                | t                         |             |                      |
| Ascorbic acid (vitamin C)                    | <sup>4</sup> CH              | 4.492                | d                         | 4–5         | 2.07                 |
|                                              | <sup>5</sup> CH              | 4.002                | m                         | 5–6         | 6.00                 |
|                                              | <sup>6</sup> CH <sub>2</sub> | 3.743                | dd                        | 5–6'        | 7.60                 |
|                                              |                              | 3.716                | dd                        | 6–6'        | –11.50               |
| Aspartate                                    | <sup>2</sup> CH              | 3.891                | dd                        | 2–3         | 3.65                 |
|                                              | <sup>3</sup> CH <sub>2</sub> | 2.801                | dd                        | 2–3'        | 9.11                 |
|                                              |                              | 2.653                | dd                        | 3–3'        | –17.43               |

**Table 2.1** *Continued*

| Compound          | Group                           | Chemical shift (ppm) | Multiplicity <sup>b</sup> | Interaction | Scalar coupling (Hz) |
|-------------------|---------------------------------|----------------------|---------------------------|-------------|----------------------|
| Choline           | (CH <sub>3</sub> ) <sub>3</sub> | 3.185                | s                         |             |                      |
|                   | <sup>1</sup> CH <sub>2</sub>    | 4.054                | m                         | 1–2/1'–2'   | 3.15                 |
|                   | <sup>2</sup> CH <sub>2</sub>    | 3.501                | m                         | 1'–2/1–2'   | 6.99                 |
| Creatine          | CH <sub>3</sub>                 | 3.027                | s                         |             |                      |
|                   | CH <sub>2</sub>                 | 3.913                | s                         |             |                      |
|                   | NH                              | 6.650                | s                         |             |                      |
| Ethanolamine      | <sup>1</sup> CH <sub>2</sub>    | 3.818                | m                         | 1–2/1'–2'   | 3.85                 |
|                   | <sup>2</sup> CH <sub>2</sub>    | 3.147                | m                         | 1'–2/1'–2'  | 6.75                 |
| Glucose, α-anomer | <sup>1</sup> CH                 | 5.216                | d                         | 1–2         | 3.8                  |
|                   | <sup>2</sup> CH                 | 3.519                | dd                        | 2–3         | 9.6                  |
|                   | <sup>3</sup> CH                 | 3.698                | dd                        | 3–4         | 9.4                  |
|                   | <sup>4</sup> CH                 | 3.395                | dd                        | 4–5         | 9.9                  |
|                   | <sup>5</sup> CH                 | 3.822                | m                         | 5–6         | 1.5                  |
|                   | <sup>6</sup> CH                 | 3.826                | dd                        | 5–6'        | 6.0                  |
|                   | <sup>6'</sup> CH                | 3.749                | dd                        | 6–6'        | –12.1                |
|                   | <sup>1</sup> CH                 | 4.630                | d                         | 1–2         | 8.0                  |
| Glucose, β-anomer | <sup>2</sup> CH                 | 3.230                | dd                        | 2–3         | 9.1                  |
|                   | <sup>3</sup> CH                 | 3.473                | dd                        | 3–4         | 9.4                  |
|                   | <sup>4</sup> CH                 | 3.387                | dd                        | 4–5         | 8.9                  |
|                   | <sup>5</sup> CH                 | 3.450                | m                         | 5–6         | 1.6                  |
|                   | <sup>6</sup> CH                 | 3.882                | dd                        | 5–6'        | 5.4                  |
|                   | <sup>6'</sup> CH                | 3.707                | dd                        | 6–6'        | –12.3                |
|                   | <sup>2</sup> CH                 | 3.746                | dd                        | 2–3/2–3'    | 7.33/4.65            |
|                   | <sup>3</sup> CH <sub>2</sub>    | 2.042                | m                         | 3–3'        | –14.85               |
| Glutamate         |                                 | 2.120                |                           | 3–4/3–4'    | 6.41/8.41            |
|                   | <sup>4</sup> CH <sub>2</sub>    | 2.336                | m                         | 3'–4/3'–4'  | 8.48/6.88            |
|                   |                                 | 2.352                |                           | 4–4'        | –15.92               |
|                   | <sup>2</sup> CH                 | 3.757                | dd                        | 2–3/2–3'    | 5.84 / 6.53          |
| Glutamine         | <sup>3</sup> CH <sub>2</sub>    | 2.135                | m                         | 3–3'        | –14.45               |
|                   |                                 | 2.115                |                           | 3'–4/3–4'   | 9.16/6.35            |
|                   | <sup>4</sup> CH <sub>2</sub>    | 2.434                | m                         | 3'–4/3'–4'  | 8.48/6.88            |
|                   |                                 | 2.456                |                           | 4–4'        | –15.92               |
| Glutathione       |                                 |                      |                           |             |                      |
| Glycine moiety    | <sup>10</sup> CH <sub>2</sub>   | 3.769                | s                         |             |                      |
|                   | <sup>9</sup> NH                 | 7.154                | s                         |             |                      |
| Cysteine moiety   | <sup>7</sup> CH                 | 4.561                | dd                        | 7–7'        | 7.09                 |
|                   | <sup>7'</sup> CH <sub>2</sub>   | 2.926                | dd                        | 7–7''       | 4.71                 |
|                   |                                 | 2.975                | dd                        | 7'–7''      | –14.06               |
| Glutamate moiety  | <sup>6</sup> NH                 | 8.177                | s                         |             |                      |
|                   | <sup>2</sup> CH                 | 3.769                | dd                        | 2–3/2–3'    | 6.34/6.36            |
|                   | <sup>3</sup> CH <sub>2</sub>    | 2.159                | m                         | 3–3'        | –15.48               |
|                   |                                 | 2.146                |                           | 3–4/3–4'    | 6.7/7.6              |
|                   | <sup>4</sup> CH <sub>2</sub>    | 2.510                | m                         | 3'–4/3'–4'  | 7.6/6.7              |
|                   |                                 | 2.560                |                           | 4–4'        | –15.92               |
| Glycerol          | <sup>1</sup> CH <sub>2</sub>    | 3.552                | dd                        | 1–2/2–3     | 4.43                 |
|                   |                                 | 3.640                | dd                        | 1'–2/2–3'   | 6.49                 |
|                   | <sup>2</sup> CH                 | 3.770                | m                         | 1–1'/3–3'   | –11.72               |
|                   | <sup>3</sup> CH <sub>2</sub>    | 3.640                | dd                        |             |                      |
|                   |                                 | 3.552                | dd                        |             |                      |

*Continued*

**Table 2.1** Continued

| Compound                                 | Group                           | Chemical shift (ppm)         | Multiplicity <sup>b</sup> | Interaction                                | Scalar coupling (Hz) |
|------------------------------------------|---------------------------------|------------------------------|---------------------------|--------------------------------------------|----------------------|
| Glycerophosphocholine<br>Glycerol moiety | <sup>1</sup> CH <sub>2</sub>    | 3.605                        | dd                        | 1–2/2–3                                    | 5.77                 |
|                                          |                                 | 3.672                        | dd                        | 1'–2/2–3'                                  | 4.53                 |
|                                          | <sup>2</sup> CH                 | 3.903                        | m                         |                                            |                      |
|                                          | <sup>3</sup> CH <sub>2</sub>    | 3.871                        | m                         |                                            |                      |
|                                          |                                 | 3.946                        | m                         |                                            |                      |
| Choline moiety                           | (CH <sub>3</sub> ) <sub>3</sub> | 3.212                        | s                         |                                            |                      |
|                                          | <sup>7</sup> CH <sub>2</sub>    | 4.312                        | m                         | 7–8/7'–8'                                  | 3.10                 |
|                                          | <sup>8</sup> CH <sub>2</sub>    | 3.659                        | m                         | 7'–8/7–8'                                  | 5.90                 |
| Glycine<br>Histamine                     | <sup>2</sup> CH <sub>2</sub>    | 3.547                        | s                         |                                            |                      |
|                                          | $\alpha$ CH <sub>2</sub>        | 2.981                        |                           | $\alpha$ – $\beta$ / $\alpha$ – $\beta'$   | 6.87/8.15            |
|                                          |                                 | 2.990                        |                           | $\alpha'$ – $\beta$ / $\alpha'$ – $\beta'$ | 7.00/6.27            |
|                                          | $\beta$ CH <sub>2</sub>         | 3.292                        |                           | $\alpha$ – $\alpha'$                       | –16.12               |
|                                          |                                 |                              |                           | $\beta$ – $\beta'$                         | –14.15               |
| Imidazole moiety                         | <sup>2</sup> CH                 | 7.85 <sup>d</sup>            |                           |                                            |                      |
|                                          | <sup>5</sup> CH                 | 7.09 <sup>d</sup>            |                           |                                            |                      |
| Histidine                                | $\alpha$ CH                     | 3.975                        |                           | $\alpha$ – $\beta$                         | 7.92                 |
|                                          | $\beta$ CH <sub>2</sub>         | 3.120                        |                           | $\alpha$ – $\beta'$                        | 4.81                 |
|                                          |                                 | 3.221                        |                           | $\beta$ – $\beta'$                         | –15.50               |
| Imidazole moiety                         | <sup>2</sup> CH                 | 7.79 <sup>d</sup>            |                           |                                            |                      |
|                                          | <sup>5</sup> CH                 | 7.06 <sup>d</sup>            |                           |                                            |                      |
| Homocarnosine                            | $\alpha$ CH                     | 4.467                        | dd                        | $\alpha$ – $\beta$                         | 5.02                 |
|                                          | $\beta$ CH <sub>2</sub>         | 3.191                        | dd                        | $\alpha$ – $\beta'$                        | 8.64                 |
| Imidazole moiety                         |                                 | 3.013                        | dd                        | $\beta$ – $\beta'$                         | –15.30               |
|                                          |                                 | <sup>2</sup> CH              | 7.08 <sup>d</sup>         |                                            |                      |
| <sup>5</sup> CH                          |                                 | 8.08 <sup>d</sup>            |                           |                                            |                      |
| GABA moiety                              | <sup>2</sup> CH <sub>2</sub>    | 2.969                        | m                         | 2–2'                                       | –12.5                |
|                                          |                                 | 2.944                        | m                         | 2–3/2–3'                                   | 7.5                  |
|                                          | <sup>3</sup> CH <sub>2</sub>    | 1.896                        | m                         | 2–3'/2'–3                                  | 8.0                  |
|                                          |                                 | 1.881                        | m                         | 3–3'                                       | –13.9                |
|                                          | <sup>4</sup> CH <sub>2</sub>    | 2.378                        | m                         | 3–4/3'–4'                                  | 7.5                  |
|                                          |                                 | 2.348                        | m                         | 3–4'/3'–4                                  | 7.5                  |
|                                          |                                 |                              |                           | 4–4'                                       | –15.2                |
|                                          | $\beta$ -Hydroxybutyrate        | <sup>2</sup> CH <sub>2</sub> | 2.388                     | dd                                         | 2–2'                 |
| 2.294                                    |                                 |                              | dd                        | 2–3                                        | 7.3                  |
| Myo-inositol                             | <sup>3</sup> CH                 | 4.133                        | m                         | 2'–3                                       | 6.3                  |
|                                          | <sup>4</sup> CH <sub>3</sub>    | 1.186                        | d                         | 3–4                                        | 6.3                  |
|                                          | <sup>1</sup> CH                 | 3.522                        | dd                        | 1–2                                        | 2.89                 |
|                                          | <sup>2</sup> CH                 | 4.054                        | dd                        | 2–3                                        | 3.01                 |
|                                          | <sup>3</sup> CH                 | 3.522                        | dd                        | 3–4                                        | 10.00                |
|                                          | <sup>4</sup> CH                 | 3.614                        | dd                        | 4–5                                        | 9.49                 |
|                                          | <sup>5</sup> CH                 | 3.269                        | dd                        | 5–6                                        | 9.48                 |
|                                          | <sup>6</sup> CH                 | 3.614                        | dd                        | 1–6                                        | 10.00                |
| Scyllo-inositol                          | <sup>1–6</sup> CH               | 3.340                        | s                         |                                            |                      |
| Lactate                                  | <sup>2</sup> CH                 | 4.097                        | q                         | 2–3                                        | 6.93                 |
|                                          | <sup>3</sup> CH <sub>3</sub>    | 1.313                        | d                         |                                            |                      |

**Table 2.1** *Continued*

| Compound               | Group                           | Chemical shift (ppm) | Multiplicity <sup>b</sup> | Interaction         | Scalar coupling (Hz) |
|------------------------|---------------------------------|----------------------|---------------------------|---------------------|----------------------|
| Phenylalanine          | $\alpha$ CH                     | 3.975                | dd                        | $\alpha$ - $\beta$  | 5.21                 |
|                        | $\beta$ CH <sub>2</sub>         | 3.273                | dd                        | $\alpha$ - $\beta'$ | 8.01                 |
|                        |                                 | 3.105                | dd                        | $\beta$ - $\beta'$  | -14.57               |
|                        | $^2$ CH                         | 7.322                | m                         | 2-3/2-4             | 7.9/1.6              |
|                        | $^3$ CH                         | 7.420                | m                         | 2-5/2-6             | 0.5/1.4              |
|                        | $^4$ CH                         | 7.369                | m                         | 3-4/3-5             | 7.2/1.0              |
|                        | $^5$ CH                         | 7.420                | m                         | 3-6/4-5             | 0.5/7.5              |
|                        | $^6$ CH                         | 7.322                | m                         | 4-6/5-6             | 1.0/7.4              |
| Phosphocreatine        | CH <sub>3</sub>                 | 3.029                | s                         |                     |                      |
|                        | $^2$ CH <sub>2</sub>            | 3.930                | s                         |                     |                      |
|                        | NH                              | 6.58 <sup>d</sup>    | s                         |                     |                      |
|                        | NH                              | 7.30 <sup>d</sup>    | s                         |                     |                      |
| Phosphorylcholine      | (CH <sub>3</sub> ) <sub>3</sub> | 3.209                | s                         |                     |                      |
|                        | $^1$ CH <sub>2</sub>            | 4.282                | m                         | 1-2                 | 2.28                 |
|                        |                                 |                      |                           | 1-2'                | 7.23                 |
|                        | $^2$ CH <sub>2</sub>            | 3.643                | m                         | 1'-2                | 7.33                 |
|                        |                                 |                      |                           | 1'-2'               | 2.24                 |
| Phosphorylethanolamine | $^1$ CH <sub>2</sub>            | 3.977                | m                         | 1-2                 | 3.18                 |
|                        |                                 |                      |                           | 1-2'                | 6.72                 |
|                        | $^2$ CH <sub>2</sub>            | 3.216                | m                         | 1'-2                | 7.20                 |
|                        |                                 |                      |                           | 1'-2'               | 2.98                 |
| Pyruvate               | $^3$ CH <sub>3</sub>            | 2.358                | s                         |                     |                      |
| Serine                 | CH                              | 3.835                | dd                        | 2-3                 | 5.98                 |
|                        | $^3$ CH <sub>2</sub>            | 3.937                | dd                        | 2-3'                | 3.56                 |
|                        |                                 | 3.976                | dd                        | 3-3'                | -12.25               |
| Succinate              | CH <sub>2</sub>                 | 2.394                | s                         |                     |                      |
| Taurine                | $^1$ CH <sub>2</sub>            | 3.420                | dd                        | 1-2                 | 6.74                 |
|                        |                                 |                      |                           | 1-2'                | 6.46                 |
|                        | $^2$ CH <sub>2</sub>            | 3.246                | dd                        | 1'-2                | 6.40                 |
|                        |                                 |                      |                           | 1'-2'               | 6.79                 |
| Threonine              | $^2$ CH                         | 3.578                | d                         | 2-3                 | 4.92                 |
|                        | $^3$ CH                         | 4.246                | m                         | 3-4                 | 6.35                 |
|                        | $^4$ CH <sub>3</sub>            | 1.316                | d                         |                     |                      |
| Tryptophan             | $\alpha$ CH                     | 4.047                | dd                        | $\alpha$ - $\beta$  | 4.85                 |
|                        | $\beta$ CH <sub>2</sub>         | 3.475                | dd                        | $\alpha$ - $\beta'$ | 8.15                 |
|                        |                                 | 3.290                | dd                        | $\alpha$ - $\beta'$ | -15.37               |
|                        | $^2$ CH                         | 7.312                | s                         |                     |                      |
|                        | $^4$ CH                         | 7.726                | m                         | 4-5                 | 7.60                 |
|                        | $^5$ CH                         | 7.278                | m                         | 4-6                 | 1.00                 |
|                        | $^6$ CH                         | 7.197                | m                         | 4-7                 | 0.95                 |
|                        | $^7$ CH                         | 7.536                | m                         | 5-6                 | 7.51                 |
|                        |                                 |                      |                           | 5-7                 | 1.20                 |
|                        |                                 |                      |                           | 6-7                 | 7.68                 |

*Continued*



**Table 2.1** Continued

| Compound | Group                   | Chemical shift (ppm) | Multiplicity <sup>b</sup> | Interaction         | Scalar coupling (Hz) |
|----------|-------------------------|----------------------|---------------------------|---------------------|----------------------|
| Tyrosine | $\alpha$ CH             | 3.928                | dd                        | $\alpha$ - $\beta$  | 5.15                 |
|          | $\beta$ CH <sub>2</sub> | 3.192                | dd                        | $\alpha$ - $\beta'$ | 7.88                 |
|          |                         | 3.037                | dd                        | $\beta$ - $\beta'$  | -14.73               |
|          | $^2$ CH                 | 7.186                | m                         | 2-3                 | 7.98                 |
|          | $^3$ CH                 | 6.890                | m                         | 2-5                 | 0.31                 |
|          | $^5$ CH                 | 6.890                | m                         | 2-6                 | 2.54                 |
|          | $^6$ CH                 | 7.186                | m                         | 3-5                 | 2.45                 |
|          |                         |                      |                           | 3-6                 | 0.46                 |
|          |                         |                      |                           | 5-6                 | 8.65                 |
| Valine   | $^2$ CH                 | 3.595                |                           | 2-3                 | 4.41                 |
|          | $^3$ CH                 | 2.259                |                           | 3-4                 | 6.97                 |
|          | $^4$ CH <sub>3</sub>    | 1.028                |                           | 3-4'                | 7.07                 |
|          | $^{4'}$ CH <sub>3</sub> | 0.977                |                           |                     |                      |

<sup>a</sup>Only homonuclear  $^1\text{H}$ - $^1\text{H}$  scalar coupling constants are included. Heteronuclear  $^1\text{H}$ - $^{31}\text{P}$  and  $^1\text{H}$ - $^{14}\text{N}$  scalar couplings are summarized in the text.

<sup>b</sup>Multiplicities are defined as: singlet (s), doublet (d), triplet (t), quartet (q), quintet (qu), multiplet (m) and double doublet (dd).

<sup>c</sup>n.m. = not measured.

Limited measurement accuracy due to broadened line widths and/or a strong pH/temperature dependency.

decrease in NAA intensity in disorders which are accompanied with neuronal loss, such as the chronic stages of stroke [27, 28], tumors [29–32] and multiple sclerosis [33, 34]. However, care is required when using NAA as a neuronal density marker since NAA concentrations differ among neuron types [35] and it has also been found in other cells, like immature oligodendrocytes [36]. Dynamic changes of neuronal NAA concentrations have

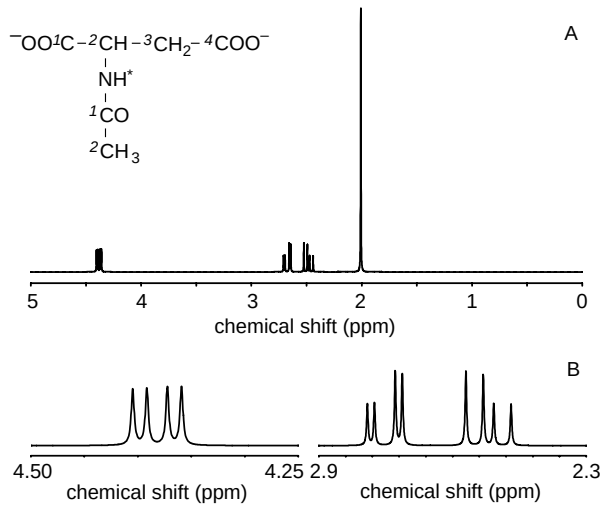


**Figure 2.2** (A) Chemical structure and simulated  $^1\text{H}$  NMR spectrum of NAA. (B) Expanded spectrum of (A). The exchangeable amide proton gives a broad resonance at 7.82 ppm (not shown).

**Table 2.2** Concentration ranges for NMR-observable metabolites in normal adult human and rat brain<sup>a</sup>

|                          | Human (average, range) (mmol L <sup>-1</sup> ) <sup>b</sup> | Rat (average, range) (mmol L <sup>-1</sup> ) <sup>b</sup> |
|--------------------------|-------------------------------------------------------------|-----------------------------------------------------------|
| Acetate                  | 0.0–0.5                                                     | 0.0–0.2                                                   |
| NAA                      | 7.5–17.0                                                    | 4.5–9.0                                                   |
| NAAG                     | 0.5–2.5                                                     | 0.2–0.5                                                   |
| ATP                      | 2.0–4.0                                                     | 2.0–4.0                                                   |
| Alanine                  | 0.1–1.5                                                     | 0.1–1.0                                                   |
| GABA                     | 1.0–2.0                                                     | 0.5–2.5                                                   |
| Ascorbic acid            | 0.5–1.5                                                     | 1.5–3.0                                                   |
| Aspartate                | 1.0–2.0                                                     | 1.0–3.0                                                   |
| Choline (total)          | 0.5–2.5                                                     | 0.2–1.5                                                   |
| Creatine                 | 4.5–10.5                                                    | 4.0–5.5                                                   |
| Ethanolamine             | 0.0–1.5                                                     | 0.0–3.5                                                   |
| Glucose                  | 1.0–2.0                                                     | 1.0–3.0                                                   |
| Glutamate                | 6.0–12.5                                                    | 7.0–12.5                                                  |
| Glutamine                | 3.0–6.0                                                     | 2.0–5.5                                                   |
| Glutathione              | 1.5–3.0                                                     | 0.5–3.5                                                   |
| Glycerophosphorylcholine | 0.5–1.5                                                     | 0.0–0.5                                                   |
| Glycine                  | 0.2–1.0                                                     | 0.0–1.0                                                   |
| Glycogen                 | 3.0–6.0                                                     | 3.0–6.0                                                   |
| Homocarnosine            | 0.1–0.4                                                     | <0.1                                                      |
| Myo-inositol             | 4.0–9.0                                                     | 4.0–10.0                                                  |
| Scyllo-inositol          | 0.2–0.5                                                     | 0.0–0.2                                                   |
| Lactate                  | 0.2–1.0                                                     | 0.5–3.0                                                   |
| Phosphocreatine          | 3.0–5.5                                                     | 3.0–5.0                                                   |
| Phosphorylcholine        | 0.2–1.0                                                     | 0.2–1.0                                                   |
| Phosphorylethanolamine   | 1.0–2.0                                                     | 1.0–2.0                                                   |
| Pyruvate                 | 0.0–0.5                                                     | 0.0–0.5                                                   |
| Serine                   | 0.2–2.0                                                     | 0.2–2.0                                                   |
| Succinate                | 0.0–0.5                                                     | 0.0–0.5                                                   |
| Taurine                  | 2.0–6.0                                                     | 2.0–6.0                                                   |
| Threonine                | 0.0–0.5                                                     | 0.0–0.5                                                   |

<sup>a</sup> Only compounds with a concentration >0.1 mmol L<sup>-1</sup> are tabulated.<sup>b</sup> Brain tissue density = 1.05 g mL<sup>-1</sup>.

been observed, indicating that NAA levels may reflect neuronal dysfunction rather than neuronal loss. This is substantiated by recovery of NAA levels during incomplete reversible ischemia [37] and brain injury [38]. Furthermore, reduced NAA levels have been observed in multiple sclerosis in the absence of neuronal loss [39]. The sole disease known to date which is accompanied by a permanent increase in NAA intensity is Canavan's syndrome in newborn infants and young children [39–41]. Secondly, NAA has been used as a concentration marker, since its concentration is relatively immune to acute metabolic disturbances such as ischemia or hypoxia. A disadvantage of using NAA as an internal reference is that the concentration is not uniform over the entire brain, with higher concentrations in gray matter (~8–11 mM) as compared with white matter (~6–9 mM) [9–12, 42]. Furthermore, at shorter echo times NAA is overlapping with glutamate and macromolecules. In addition, resonances from other *N*-acetyl containing metabolites, such as *N*-acetyl aspartyl glutamate (NAAG) will essentially coincide with the NAA methyl signal, thereby further

complicating the NAA quantification [43]. Table 2.2 gives the cerebral concentration of NAA in human and rat brain, together with those of other metabolites. The values tabulated in Table 2.2 represent the concentration ranges found in the literature [1, 2, 8, 44–47] and should be used with caution, as they include a range of different techniques, conditions and brain areas (e.g. gray and white matter concentrations are not separated) performed by researchers in different laboratories. As such, Table 2.2 should be used as a rough guideline for the expected metabolite concentrations, rather than indicating the exact values.

### 2.2.3 N-Acetyl Aspartyl Glutamate (NAAG)

NAAG is a dipeptide of N-substituted aspartate and glutamate with a nonuniform distribution within the brain across a concentration range of 0.6–3.0 mM [48–52]. NAAG is suggested to be involved in excitatory neurotransmission as well as being a source of glutamate. However, its exact role remains unclear.

NMR detection of NAAG was first reported in the human brain by Frahm *et al.* [43] and confirmed on rat brain extracts by Holowenko *et al.* [53], after which regional variations across the human brain were established [52]. The largest resonance of NAAG at 2.04 ppm resonates very close to the larger methyl resonance from NAA at 2.01 ppm. This requires excellent magnetic field homogeneity to unambiguously detect NAAG separately from NAA. In cases where this separation can not be achieved, the sum of NAA and NAAG provides a reliable estimate of NAA-containing molecules. Besides the three methyl protons, NAAG has eight other nonexchangeable protons, as well as three water-exchangeable protons. However, these other resonances are typically not observed directly due to the lower intensity and spectral overlap with more concentrated metabolites.

### 2.2.4 Adenosine Triphosphate (ATP)

Together with phosphocreatine (PCr), ATP is the principal donor of free energy in biological systems. It is a nucleotide, consisting of an adenine group, a ribose ring and a triphosphate unit (Figure 2.3). When one phosphate group is donated, for example to creatine in the creatine-kinase-catalyzed reaction to form phosphocreatine, ATP is converted to adenosine diphosphate (ADP). While the normal ATP concentration in the human brain is circa 3 mM [54], the ADP concentration is typically well below 100  $\mu$ M. In contrast to the PCr concentration which is relatively stable across the brain at circa 3.5 mM, the ATP concentration shows significant variation between gray matter (2 mM) and white matter (3.5 mM) [55].

ATP is normally detected with  $^{31}\text{P}$  NMR spectroscopy, where it produces three well-separated resonances. ATP detection by  $^1\text{H}$  NMR spectroscopy is more difficult because (1) the coupling patterns for most protons are complex and (2) most resonances appear close to the water resonance or are otherwise in exchange with the water. In the ATP adenine group, the  $^2\text{CH}$  and  $^8\text{CH}$  protons resonate as singlets at 8.22 and 8.51 ppm. However, observation of these (pH-dependent) resonances *in vivo* is difficult since the lines are broadened due to slow exchange with water and possibly cross-relaxation with exchangeable protons in adjacent amide groups. While two separate resonances can be seen *in vitro*, only one broadened resonance is observed at 8.22 ppm *in vivo*. The water-exchangeable  $\text{NH}_2$  protons give rise to a broad resonance at 6.75 ppm while the ribose- $^{1'}\text{CH}$  proton resonates as a

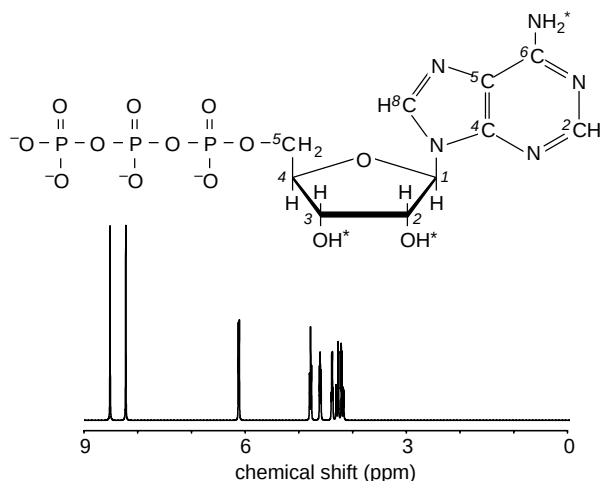


**Figure 2.3** Chemical structure and simulated  $^1\text{H}$  NMR spectrum of ATP. While detectable in  $^1\text{H}$  NMR spectra in vivo (e.g. see Figure 2.1), ATP detection is commonly performed with  $^{31}\text{P}$  NMR, as detailed in Section 2.3.

doublet at 6.13 ppm. The other ribose-ring protons resonate as multiplets between 4.2 ppm and 4.8 ppm. The structure and  $^1\text{H}$  NMR spectrum of ATP are shown in Figure 2.3.

## 2.2.5 Alanine (Ala)

Ala is a nonessential amino acid that is present in mammalian brain at a concentration below 0.5 mM. Increased Ala has been observed in meningiomas [56] and following ischemia [37]. The three methyl protons couple to a single methine proton to form a weakly coupled  $\text{AX}_3$  spin system, closely resembling lactate, with a doublet resonance at 1.47 ppm and a quartet at 3.78 ppm. In high-field NMR spectra from rat brain, as well as in high-resolution spectra from brain slices, the doublet of Ala is often observable as a shoulder on the macromolecular resonance at 1.4 ppm [1]. In lower-field  $^1\text{H}$  NMR spectra and in the presence of spectral overlap from lipids, Ala can be observed through the use of spectral editing [57] or at longer echo times. Figure 2.4 shows the structure and  $^1\text{H}$  NMR spectrum of Ala.

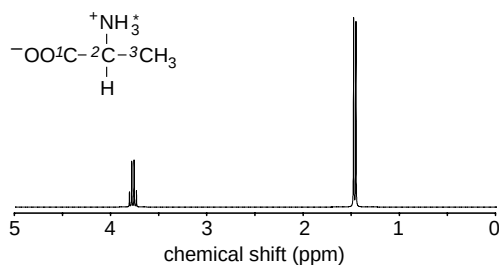


**Figure 2.4** Chemical structure and simulated  $^1\text{H}$  NMR spectrum of Ala.

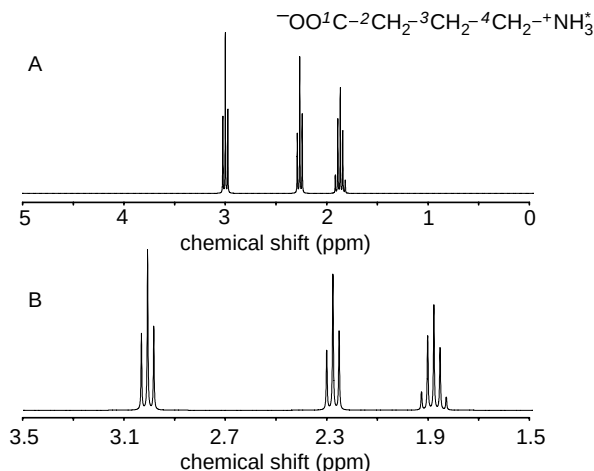


**Figure 2.5** (A) Chemical structure and simulated  $^1\text{H}$  NMR spectrum of GABA. (B) Expanded spectrum of (A). At most magnetic fields, all three resonances of GABA are overlapping with more intense resonances, necessitating the use of spectral editing methods for unambiguous GABA detection (see Chapter 8).

## 2.2.6 $\gamma$ -Aminobutyric Acid (GABA)

GABA is an inhibitory neurotransmitter that has a brain concentration of circa 1 mM, although altered concentrations are associated with the menstrual cycle [58], acute deaf-ferentation [59], visual light–dark adaptation [60], alcohol and substance abuse [61, 62], as well as several neurological and psychiatric disorders [63], including epilepsy [64], depression [65, 66] and panic disorder [67]. Several anti-epileptic drugs, like vigabatrin, have been designed to raise cerebral GABA levels, which has been extensively studied with (edited)  $^1\text{H}$  MRS [68, 69].

GABA has six NMR observable protons (Figure 2.5) in three methylene groups, forming an  $\text{A}_2\text{M}_2\text{X}_2$  spin system at higher magnetic fields ( $>4$  T). At lower magnetic fields, significant strong coupling effects can be observed for GABA-H2 and H3 [70]. The triplet resonances for GABA-H4 and H2 appear at 3.01 ppm and 2.28 ppm, while the GABA-H3 quintet is centered at 1.89 ppm. All three resonances of GABA overlap with other, more intense resonances, such that GABA detection is usually achieved with spectral editing methods (see Chapter 8). More recently it has been demonstrated that under conditions of elevated GABA levels, direct detection becomes feasible with the combination of spectral fitting and high magnetic fields [71].

## 2.2.7 Ascorbic Acid (Asc)

Asc (vitamin C) occurs physiologically as the ascorbate anion, a water-soluble antioxidant that is found throughout the body with the highest concentration and retention capacities in the brain, spinal cord and adrenal glands [72]. While brain ascorbate levels are closely regulated, the exact role of Asc remains elusive. Accumulative data over 25 years indicate

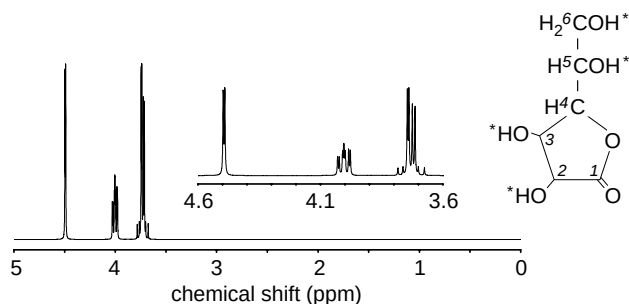


**Figure 2.6** Chemical structure and simulated  $^1\text{H}$  NMR spectrum of Asc (vitamin C).

ascorbate as part of the intracellular antioxidant network with neuroprotective properties. Other functions may include neuromodulation and a specific extracellular antioxidant. The average brain concentration of ascorbate is around 1.0 mM with local neuronal and astroglial concentrations of 10 mM and 1 mM. Ascorbate is heterogeneously distributed throughout the brain with higher concentrations in the cortex and hippocampus as compared with brain stem and spinal cord [72].

Ascorbate is composed of four NMR-observable protons (Figure 2.6) distributed over one methylene and two methine groups. The  $^4\text{CH}$  proton gives rise to a doublet at 4.49 ppm, while the  $^5\text{CH}$  and  $^6\text{CH}_2$  protons give multiplet resonances at 4.00 ppm and 3.73 ppm, respectively. Terpstra and Gruetter [73] were able to observe ascorbate noninvasively in human brain by edited  $^1\text{H}$  MRS. By selectively perturbing ascorbate-H5, the protons of ascorbate-H6 could be selectively observed and were quantified at a concentration of  $1.3 \pm 0.3 \mu\text{mol g}^{-1}$ . In rat brain, the Asc concentration shows a strong age dependence with levels dropping from  $4.0 \mu\text{mol g}^{-1}$  at P7 to  $2.0 \mu\text{mol g}^{-1}$  at adulthood [74].

## 2.2.8 Aspartate (Asp)

Asp is a nonessential amino acid that acts as an excitatory neurotransmitter. It does not cross the blood–brain barrier, but is instead synthesized from glucose and possibly other precursors. The formation of  $^{13}\text{C}$ -labeled aspartate from  $^{13}\text{C}$ -labeled glucose is readily observed in  $^{13}\text{C}$  and  $^1\text{H}$ - $^{13}\text{C}$ -NMR experiments, as will be discussed in Chapter 3. Asp has an approximate brain concentration of 1–2 mM [1, 2]. The  $^3\text{CH}_2$  and  $^2\text{CH}$  groups give a typical ABX spectral pattern consisting of a doublet-of-doublets for the CH group at 3.89 ppm and a pair of doublet-of-doublets from the methylene protons at 2.65 ppm and 2.80 ppm. The structure and  $^1\text{H}$  NMR spectrum of aspartic acid are shown in Figure 2.7.

## 2.2.9 Choline-containing Compounds (tCho)

Besides resonances from NAA and total creatine, the most prominent resonance in  $^1\text{H}$  NMR spectra from brain arises from the methyl protons of choline-containing compounds at 3.2 ppm (Figure 2.8). Since the resonance contains contributions from free choline, glycerophosphorylcholine (GPC) and phosphorylcholine (PC) it is often referred to as ‘total choline’ (tCho). At short echo times overlapping resonances from ethanolamine,

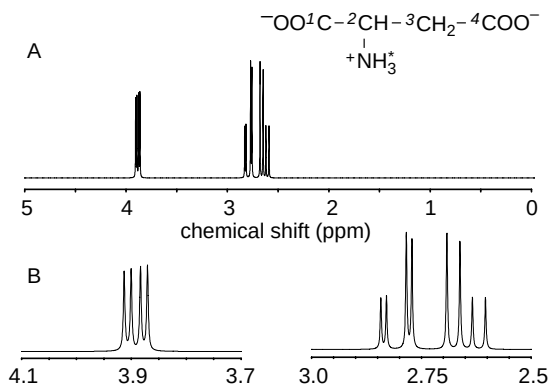


**Figure 2.7** (A) Chemical structure and simulated  $^1\text{H}$  NMR spectrum of Asp. (B) Expanded spectrum of (A).

*myo*-inositol, glucose and especially taurine should be taken into account. In liver and kidney the resonance at 3.26 ppm is almost entirely composed of betaine. The total choline concentration in human brain is approximately 1–2 mM with a nonuniform distribution [11, 12, 75]. Free choline is a minor contributor to the ‘total choline’ resonance, since the concentration is well below the NMR detection limit. The two main contributors, PC and GPC can not be separated based on the methyl protons due to the small chemical shift difference relative to the spectroscopic line widths. However, separation can in principle be achieved through the smaller resonances from the methylene protons. Furthermore, with  $^{31}\text{P}$  MRS the relative contributions of PC and GPC can be readily established [75, 76].

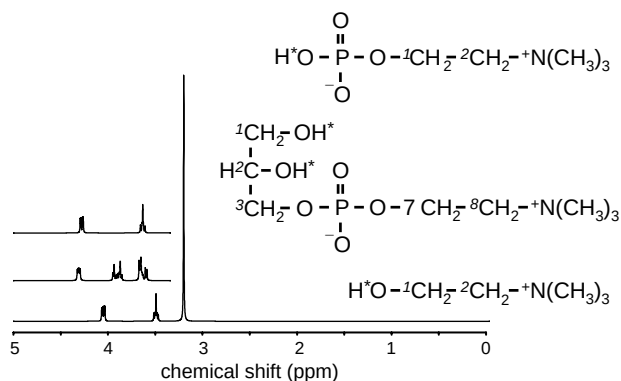


**Figure 2.8** Chemical structures and simulated  $^1\text{H}$  NMR spectra of choline-containing compounds. Structures shown are for phosphorylcholine (PC, top), glycerophosphorylcholine (GPC, middle) and choline (bottom). All NMR spectra exhibit a strong singlet resonance at 3.22 ppm originating from nine equivalent protons, whereby small differences can be observed between 3.5 ppm and 4.3 ppm.

GPC has a total of 18 nonexchangeable protons from the glycerol (5 protons) and choline moieties (13 protons of which 9 protons are in three magnetically equivalent methyl groups). The trimethyl protons resonate at 3.21 ppm as a singlet. The  $^7\text{CH}_2$  and  $^8\text{CH}_2$  protons of the choline moiety resonate at 4.31 ppm and 3.66 ppm, respectively. The five glycerol protons resonate as a strongly coupled spin system between 3.6 ppm and 4.0 ppm. Besides the homonuclear scalar coupling interactions, GPC also displays a heteronuclear interaction between the  $^7\text{CH}_2$  protons and the adjacent nitrogen-14 head group ( $^1J_{\text{HN}} = 2.7$  Hz), as well as an interaction between the  $^8\text{CH}_2$  protons and the nearby  $^{31}\text{P}$  nucleus ( $^2J_{\text{HP}} = 6.0$  Hz).

Choline and phosphorylcholine both give rise to 13 nonexchangeable protons. The trimethyl protons appear as a singlet at 3.21 ppm, whereas the remaining  $^1\text{CH}_2$  and  $^2\text{CH}_2$  protons appear as multiplets between 3.5 ppm and 4.3 ppm.

Choline-containing compounds are involved in pathways of phospholipid synthesis and degradation, thereby reflecting membrane turnover. Increased choline signal is observed in cancer [77], Alzheimer's disease [78] and multiple sclerosis [79, 80], while decreased choline levels are associated with liver disease and stroke [81]. However, the exact interpretation of changes in total choline signal is complicated due to the multiple contributions to the observed total choline resonance. Furthermore, in breast cancer an additional contribution can be observed due to artifacts arising from vibration-induced sidebands of large signals (e.g. lipids), thereby further complicating interpretation if the data acquisition is not properly modified [82]. In tissues outside the CNS, the resonance at circa 3.2 ppm contains a dominant contribution from betaine (trimethylglycine).

## 2.2.10 Creatine (Cr) and Phosphocreatine (PCr)

In  $^1\text{H}$  NMR spectra of normal tissue which contains creatine kinase, the singlet resonances at 3.03 ppm and 3.93 ppm arise from the methyl and methylene protons of Cr and phosphorylated creatine, i.e. PCr. Together they are often referred to as 'total creatine' (tCr). In the brain, Cr and PCr are present in both neuronal and glial cells. Cr and PCr, together with ATP play a crucial role in the energy metabolism of tissues [83]. Although some controversy remains about the exact role, it has been suggested that phosphocreatine (in combination with creatine kinase) serves as (1) an energy buffer, retaining constant ATP levels through the creatine kinase reaction and as (2) an energy shuttle, diffusing from energy producing (i.e. mitochondria) to energy utilizing sites (i.e. myofibrilles in muscle or nerve terminals in brain). The concentrations in human brain for PCr and Cr are circa 4.0–5.5 mM and 4.5–6.0 mM, respectively, with higher levels in gray matter (6.4–9.7 mM) than in white matter (5.2–5.7 mM). The concentration of total creatine is relatively constant, with no changes reported with age [84] or a variety of diseases. As such the resonance is frequently used as an internal concentration reference. While convenient and under most conditions reliable, the use of any internal concentration reference should be used with caution. Decreased Cr levels have been observed in the chronic phases of many pathologies, including tumors and stroke. Furthermore, it has been shown that part of the 'total creatine' resonance is NMR invisible due to restricted rotational mobility [85, 86].

The  $^1\text{H}$  NMR spectra of Cr and PCr are very similar. The difference between the Cr (3.027 ppm) and PCr (3.029 ppm) methyl resonances is too small to allow a reliable separation *in vivo*. However, the difference between the Cr (3.913 ppm) and PCr (3.930 ppm) methylene resonances is large enough to separate the two compounds at magnetic



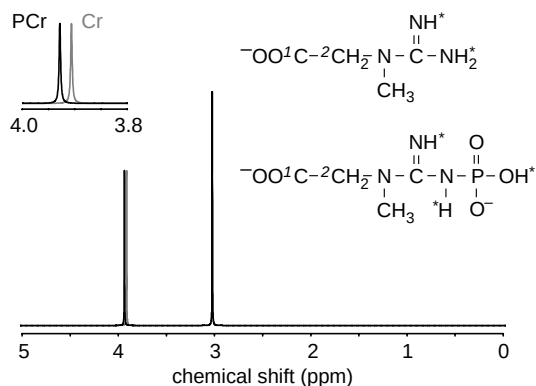


**Figure 2.9** Chemical structures and simulated  $^1\text{H}$  NMR spectra for Cr and PCr. The methyl resonances are indistinguishable at any magnetic field, but the methylene resonances are separated enough ( $\sim 0.02$  ppm) to allow routine detection at magnetic fields higher than circa 7 T. Both resonances have broad resonances downfield from water that are typically not observed.

fields of circa 7.0 T and higher. The structures and  $^1\text{H}$  NMR spectra of Cr and PCr are shown in Figure 2.9.

### 2.2.11 Ethanolamine and Phosphorylethanolamine (PE)

Ethanolamine is a common alcohol moiety of phosphoglycerides and a precursor of PE. Figure 2.10 shows their chemical structures and  $^1\text{H}$  NMR spectra. Both compounds have four NMR-observable protons in two methylene groups which give rise to two multiplet resonances around 3.1–3.2 and 3.8–4.0 ppm. Ethanolamine has been detected in high-resolution NMR spectra of brain extracts [87] with an estimated concentration of several mM. However, the *in vivo* detection of ethanolamine is not straightforward, as the two resonances overlap with peaks from more concentrated compounds. PE can be detected with  $^{31}\text{P}$  NMR spectroscopy and was measured at a concentration of 0.5–1.0 mM in human brain [76]. Increased levels of ethanolamine and PE have been observed during ischemia

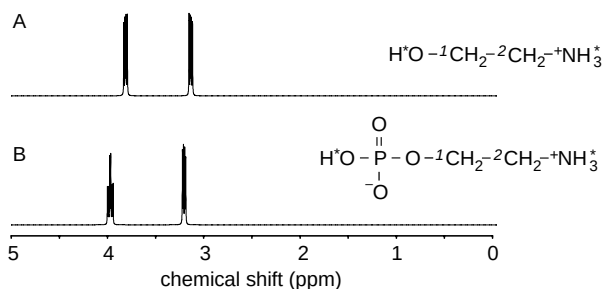


**Figure 2.10** Chemical structures and simulated  $^1\text{H}$  NMR spectra for (A) ethanolamine and (B) PE.

[87] and seizures [88], respectively. PE can be observed with enhanced sensitivity through polarization transfer [89–91] utilizing the heteronuclear  $^1\text{H}$ - $^{31}\text{P}$  scalar coupling between the  $^{31}\text{P}$  nucleus and the  $^1\text{CH}_2$  protons ( $^3J_{\text{HP}} = 7.1$  Hz and  $^3J_{\text{HP}} = 7.3$  Hz).

### 2.2.12 Glucose (Glc)

Glc contains seven nonexchangeable protons, as well as five water-exchangeable protons in hydroxyl groups. Glc exists in two configurations, in which the  $^1\text{CH}$  proton resides in an axial or equatorial orientation relative to the ring, giving rise to the  $\alpha$  and  $\beta$  anomers. These anomers coexist in aqueous solutions, with an equilibrium concentration of 36 % for the  $\alpha$  anomer and 64 % for the  $\beta$  anomer. Unfortunately, since the  $\beta$ - $^1\text{CH}$  proton resonance appears at 4.63 ppm it is typically eliminated during water suppression. The other six nonexchangeable protons resonate as a strongly coupled spin system between 3.2 ppm and 3.9 ppm, which somewhat simplifies *in vivo* to two broad resonances at 3.43 and 3.80 ppm due to the larger line widths. Glc was initially detected in rat brain through these two inner resonances [92], although severe spectral overlap limited the detection reliability. Gruetter *et al.* [93] were able to detect changes in Glc levels and study cerebral Glc transport kinetics by performing difference spectroscopy following the intravenous infusion of Glc. With the increased availability of higher magnetic fields, Glc is readily detected from the  $\alpha$ - $^1\text{CH}$  resonance at 5.22 ppm [94,95]. Alternatively, Glc can be unambiguously detected through spectral editing methods [96,97].

Glc is an essential substrate for the brain and functions both as a source of energy and a precursor for a large number of compounds. The resting cerebral Glc concentration is circa 1.0 mM but can be increased to >5.0 mM by intravenous Glc infusion. More details on the measurement of Glc transport kinetics as well as the role of Glc in energy and neurotransmitter metabolism will be given in Chapter 3. Figure 2.11 shows the structure and  $^1\text{H}$  NMR spectrum of Glc.

### 2.2.13 Glutamate (Glu)

Glu is a nonessential amino acid with multiple roles *in vivo*. First and foremost, glutamate is the major excitatory neurotransmitter in mammalian brain [98]. Secondly it is the direct precursor for the major inhibitory neurotransmitter, GABA. Besides these roles, Glu is also an important component in the synthesis of other small metabolites (e.g. glutathione), as well as larger peptides and proteins [98]. Glu is present at an average concentration of 6–12.5 mM with significant differences between gray and white matter [99–102]. Glu is present in all cell types with the largest pool in glutamatergic neurons and smaller pools in GABAergic neurons and astroglia. The central role of Glu in the Glu–glutamine (Gln) neurotransmitter cycle will be discussed in more detail in Chapter 3.

Glu has two methylene groups and a methine group that are strongly coupled, forming an AMNPQ spin system (Figure 2.12A). As a result of the extensive and strong scalar coupling interactions Glu has a complex NMR spectrum with signal spread out over many low intensity resonances. Signal from the single  $^2\text{CH}$  methine proton appears as a doublet-of-doublets at 3.75 ppm, while the resonances from the other four protons appear as multiplets between 2.04 ppm and 2.35 ppm. Glu and Gln are virtually indistinguishable at lower magnetic fields, whereas the Glu-H4 and Gln-H4 protons become separate resonances at magnetic field strengths of 7 T and higher [103] (see Figure 2.13).

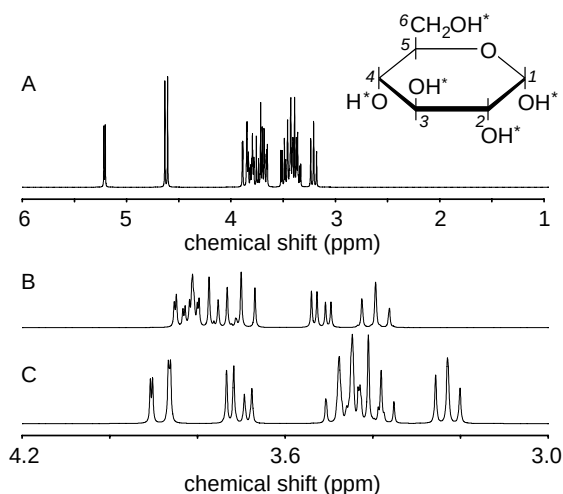


**Figure 2.11** (A) Simulated  $^1\text{H}$  NMR spectrum of  $\alpha$ -D-glucose and chemical structure of  $\alpha$ -D-glucose. The chemical structure of  $\beta$ -D-glucose is identical to that shown for  $\alpha$ -D-glucose, with the exception that the  $\beta\text{H}$  and  $\text{OH}$  protons are reversed. Under normal physiological conditions, glucose represents a mixture of 36 %  $\alpha$ - and 64 %  $\beta$ -glucose. Partial  $^1\text{H}$  NMR spectra for  $\alpha$ - and  $\beta$ -glucose are shown in (B) and (C), respectively.

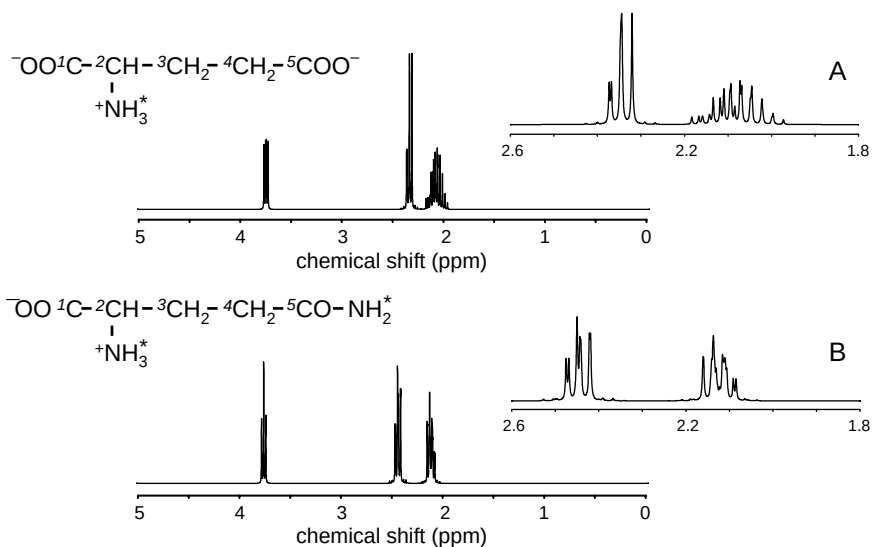


**Figure 2.12** Chemical structure and simulated  $^1\text{H}$  NMR spectrum of (A) Glu and (B) Gln. Gln also has broad resonances downfield from water (not shown).

### 2.2.14 Glutamine (Gln)

The amino acid Gln is an important component of intermediary metabolism and is primarily located in astroglia at a concentration of 2–4 mM [1, 2, 12]. Gln is synthesized from Glu by Gln synthetase in the astroglia and it is broken down to Glu by phosphate-activated glutaminase in neurons. Besides its role in the Glu–Gln neurotransmitter cycle (see Chapter 3), a main function of Gln is in ammonia detoxification. Gln markedly increases during hyperammonemia and under those conditions brain Gln is a good indicator of the liver disease hepatic encephalopathy [104, 105].

Gln is structurally similar to glutamate with a single methine group and two methylene groups (Figure 2.12B). As a result, the chemical shifts and scalar coupling interactions are also similar. The  $^2\text{CH}$  methine proton resonates as a triplet at 3.76 ppm, while the multiplets of the four methylene protons are closely grouped between 2.12 ppm and 2.46 ppm. In addition, Gln has two NMR detectable amide protons at 6.82 and 7.73 ppm. The relative intensity of the 6.82 ppm down-field resonance is typically much higher than the 7.53 ppm resonance because the water–amide exchange rate is different.

In order to understand the important roles of Glu and Gln in intermediary metabolism, the separate detection of these two compounds is essential. Unfortunately, the similar chemical structures lead to very similar  $^1\text{H}$  NMR spectra. The increase in magnetic field strength has greatly benefited the separation between the H4-resonances of Glu and Gln (Figure 2.13). At magnetic fields of 7 T or higher, the Glu and Gln H4 resonances are visually separated, leading to a greatly enhanced quantification accuracy. At lower fields,

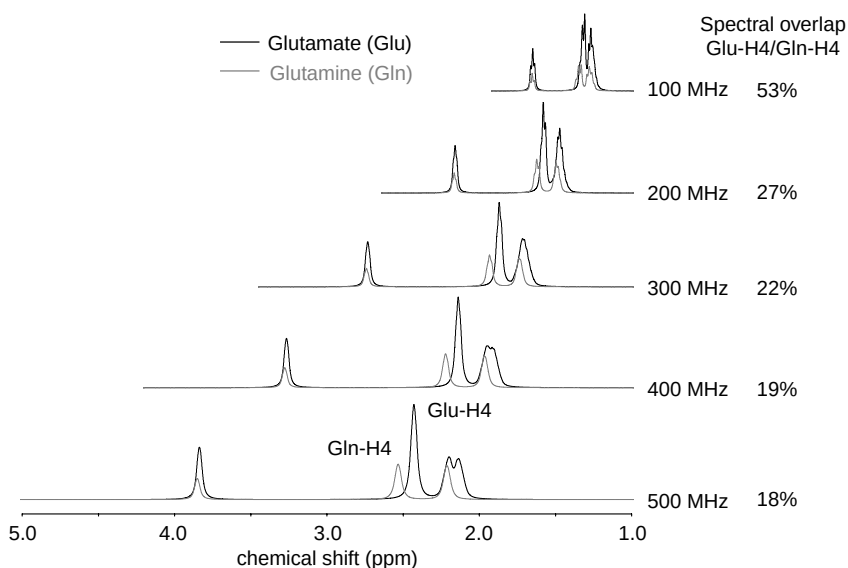


**Figure 2.13** Magnetic field dependence of the spectral features for Glu and Gln. At low magnetic fields all H3 and H4 protons from Glu and Gln are strongly coupled leading to severe spectral overlap. However, with increasing magnetic field strength the spectral patterns simplify substantially, thereby allowing a reliable separation of Glu-H4 and Gln-H4.

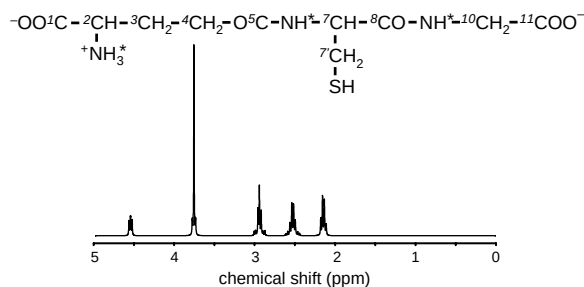


**Figure 2.14** Chemical structure and simulated  $^1\text{H}$  NMR spectrum of the reduced form of glutathione (GSH). The oxidized form of glutathione (GSSH) is present at negligible levels under normal physiological conditions.

the separation between Glu and Gln becomes unreliable, although the sum (often referred to as Glx) can be quantified with high accuracy.

### 2.2.15 Glutathione (GSH)

GHS is a tripeptide consisting of glycine, cysteine and glutamate (Figure 2.14). It can exist in reduced (GSH) or oxidized (GSSH) forms, although it is present in the living brain almost entirely as GSH at concentrations of 1–3 mM [106–108]. It is primarily located in astrocytes [108]. GSH is an antioxidant, essential for maintaining normal red blood cell structure and keeping hemoglobin in the ferrous state. Other functions include that of an amino acid transport system, as well as a storage form of cysteine [108, 109]. Altered GSH levels have been reported in Parkinson's disease and other neurodegenerative diseases affecting the basal ganglia [110].

The methylene protons of the glycine moiety resonate as a singlet at 3.77 ppm, overlapping with the methine proton of the Glu moiety which appears as a doublet-of-doublets at the same chemical shift position. The four Glu methylene protons give rise to two separate multiplets at circa 2.15 and 2.55 ppm. The  $^7\text{CH}_2$  and  $^7\text{CH}$  protons of the cysteine moiety form an ABX spin system with three doublet-of-doublets at 2.93, 2.98 and 4.56 ppm. All resonances of GSH are overlapping with more intense signals from other metabolites, most noticeably Glu, Gln, Cr and NAA. *In vivo* detection of GSH has therefore mainly been achieved through spectral editing methods [106, 111, 112], although it has been shown that GSH can be detected directly when combining short-TE spectra with spectral fitting at high magnetic fields [1, 107].

### 2.2.16 Glycerol

Glycerol is a major constituent of phospholipids and free glycerol is imbedded into membrane phospholipids (Figure 2.15). NMR resonances from glycerol are not observed in  $^1\text{H}$  NMR spectra from normal brain, most likely due to line broadening caused by limited rotational mobility. Free glycerol obtained as an end product of membrane phospholipids breakdown, however, may become observable. NMR observation of glycerol has been

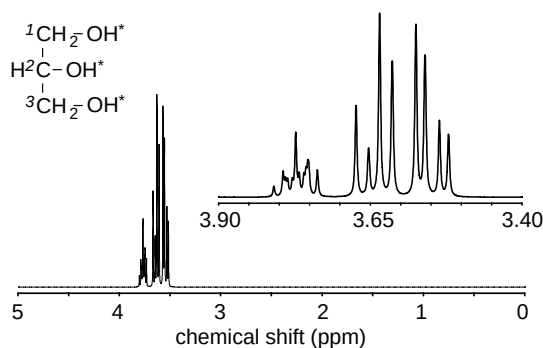


**Figure 2.15** Chemical structure and simulated  $^1\text{H}$  NMR spectrum of glycerol.

described for  $^1\text{H}$  NMR spectra of human brain homogenates following autopsy, where the glycerol concentration increases with postmortem sample preparation time [113]. The  $^1\text{H}$  NMR spectrum originates from five spins in two equivalent methylene groups and a single CH-proton. At the magnetic field strengths employed for *in vivo* NMR, glycerol is characterized as a strongly coupled spin system giving resonances centered at 3.55 and 3.64 ppm (methylene protons) and 3.77 ppm (CH proton multiplet). Unambiguous detection of glycerol is not straightforward due to significant spectral overlap with *myo*-inositol in the 3.50–3.65 ppm range.

### 2.2.17 Glycine

Glycine ( $\text{H}_3\text{N}^+\text{-CH}_2\text{-COO}^-$ ) is an inhibitory neurotransmitter and possible antioxidant, and is distributed throughout the CNS. Glycine is primarily being synthesized from Glc through serine with a concentration of approximately 1 mM in human brain. Glycine is also readily converted into Cr.

Glycine has two methylene protons that co-resonate as a singlet peak at 3.55 ppm. For *in vivo* NMR measurement, the glycine resonance overlaps with those of *myo*-inositol, making unambiguous observation of glycine impossible in (nonedited)  $^1\text{H}$  NMR spectra.

### 2.2.18 Glycogen

Carbohydrate reserves are mainly stored as glycogen in animals and humans. It is particularly abundant in muscle and liver, reaching concentrations up to 30–100 mmol  $\text{kg}^{-1}$  and 100–500 mmol  $\text{kg}^{-1}$ , respectively (see [114] for review). Glycogen is also present in the brain, residing in astroglia at a concentration of circa 5 mmol  $\text{kg}^{-1}$  [115–117]. The regulation of glycogen synthesis and breakdown plays an important role in systemic Glc metabolism and is crucial in the understanding of diseases such as diabetes mellitus.

The primary structure of glycogen consists of  $\alpha$ -[1,4]-linked Glc chains containing 12 to 13 Glc residues. The chains are linked together through  $\alpha$ -[1,6] branch points to form larger, spherical units referred to as  $\beta$ -particles. The  $\beta$ -particles can be further arranged into larger rosette-type structures termed  $\alpha$ -particles. Despite its high molecular weight

( $10^7$ – $10^9$  Da), glycogen gives rise to relatively narrow  $^1\text{H}$  and  $^{13}\text{C}$  NMR resonances both *in vitro* and *in vivo*, indicating a high degree of internal mobility which was confirmed by Shulman and coworkers [118–120]. Natural abundance  $^{13}\text{C}$  NMR glycogen detection is now a routine method to observe glycogen *in vivo* [121, 122]. Combined with the infusion of [ $1\text{-}^{13}\text{C}$ ]glucose, the formation of [ $1\text{-}^{13}\text{C}$ ]glycogen can be detected in muscle [123], liver [124] and more recently in brain [115]. While  $^{13}\text{C}$  NMR detection of glycogen is relatively straightforward,  $^1\text{H}$  detection of glycogen remains largely elusive. Only a single report [125] described the proton detection of glycogen in the liver. The  $^1\text{H}$  detection of glycogen is likely to be complicated by cross-relaxation and exchange pathways between glycogen and water.

### 2.2.19 Histamine

Histamine is a nonessential amino acid that is synthesized in the brain through the decarboxylation of histidine. Its proposed functions include neurotransmission and neuro-modulation involved with the regulation of sleep. Histamine is known to be distributed nonuniformly throughout the brain with the highest concentrations in the hypothalamus and the lowest in the cerebellum [126]. At normal concentrations of  $<0.1$  mM histamine is not observable by *in vivo* NMR. However, the concentration can be increased by histidine administration [126].

Histamine has six NMR-observable protons, two in the imidazole ring and four in the aliphatic side chain. Like many down-field signals the resonances of the imidazole ring protons are pH sensitive. At pH 7.0 they appear at 7.09 ppm and 7.85 ppm. The aliphatic  $^\alpha\text{CH}_2$  and  $^\beta\text{CH}_2$  protons appear as a multiplet and a triplet at 2.98 ppm and 3.29 ppm, respectively. Figure 2.16A shows the structure and  $^1\text{H}$  NMR spectrum of histamine.

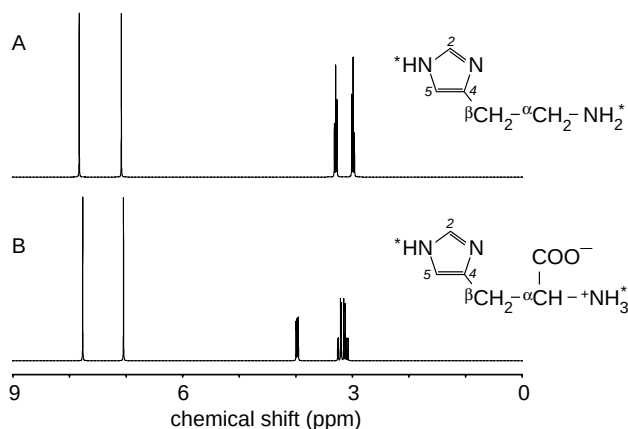


**Figure 2.16** Chemical structures and simulated  $^1\text{H}$  NMR spectra for (A) histamine and (B) histidine. The proton resonances downfield from water are pH-sensitive in the physiological pH range.

### 2.2.20 Histidine

Histidine (Figure 2.16B) is a neutral amino acid that is essential for the synthesis of proteins and for the production of histamine. While normal brain concentrations are circa 0.1 mM, histidine freely passes the blood–brain barrier such that its concentration can be increased by increasing plasma levels [127]. Increased histidine and histamine have been observed in hepatic encephalopathy [128, 129] and histidinemia [130], a defect of amino acid metabolism.

Structurally closely related to histamine, histidine has five nonexchangeable protons of which two reside on the imidazole ring and three in the aliphatic side chain. The two imidazole protons at circa 7.8 ppm and 7.1 ppm are pH-sensitive and have been used with *in vivo*  $^1\text{H}$  NMR to establish intracellular pH [127].

### 2.2.21 Homocarnosine

Homocarnosine is a dipeptide consisting of histidine and GABA. In the human brain it has a concentration range of 0.3–0.6 mM [131, 132], while the concentration in rodent brain is negligible. Homocarnosine has been associated with epileptic seizure control, showing a better correlation with the frequency of seizures than the inhibitory neurotransmitter GABA [64]. Homocarnosine levels can be raised by anti-epileptic drugs, like gabapentin [133]. Elevated brain and CSF levels are typically associated with homocarnosinosis, a metabolic disorder caused by a deficiency in the homocarnosine-metabolizing enzyme, homocarnosinase [131, 132].

Homocarnosine has 11 nonexchangeable protons of which two reside in the histidine-imidazole ring, three in the histidine side chain and six in the GABA moiety (Figure 2.17). The two imidazole protons resonate at circa 8.1 and 7.1 ppm and are pH sensitive [134]. The other histidine protons give rise to multiplets between 3.0 ppm and 4.5 ppm, whereas the resonance positions of the GABA moiety protons are very similar to those of free

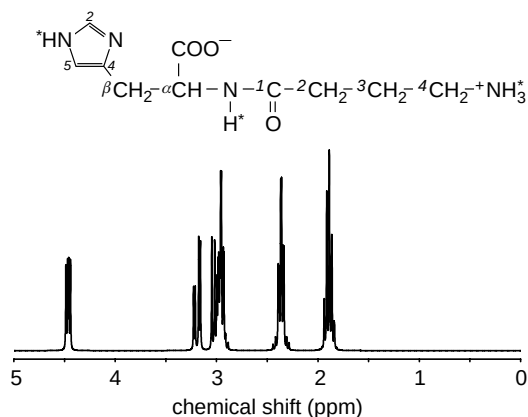


**Figure 2.17** Chemical structure and simulated  $^1\text{H}$  NMR spectrum of homocarnosine. The chemical shift of the two down-field resonances of homocarnosine (not shown) are sensitive to pH in the physiological range.



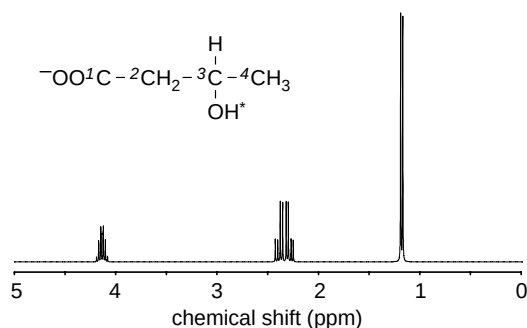


**Figure 2.18** Chemical structure and simulated  $^1\text{H}$  NMR spectrum of BHB.

GABA. As a result, the GABA-H4 resonance at 3.01 ppm as observed in spectral editing experiments should typically be referred to as ‘total GABA’ as it represents the sum of free GABA and homocarnosine. The homocarnosine fraction can be estimated from the down-field imidazole proton resonances which can be observed with short-TE  $^1\text{H}$  MRS [134].

### 2.2.22 $\beta$ -Hydroxybutyrate (BHB)

BHB (or 3-hydroxybutyrate) is, together with acetoacetate, often referred to as a ketone body. A ketone body can function as an alternative substrate for metabolism, typically under conditions of long fasting or high fat diets [135]. Under normal (fed) conditions the plasma ketone bodies are below 0.1 mM but can rise to 2–3 mM after 72 h of fasting [135].  $^1\text{H}$  MRS has shown that brain BHB levels increase to >1.5 mM under those conditions [136].  $^{13}\text{C}$  NMR studies with  $^{13}\text{C}$ -labeled BHB have shown that BHB is readily consumed by the brain [137]. Increased plasma ketone levels, achieved through a ketogenic diet, have shown great potential for seizure control in childhood epilepsy [138].

BHB has six nonexchangeable protons of which the three methyl protons resonate as a doublet at 1.19 ppm and are the common target for NMR observation. The methine proton resonates as a complex pattern at 4.13 ppm, while the two nonequivalent methylene protons resonate as doublet-of-doublets at 2.29 and 2.39 ppm. Figure 2.18 shows the structure and  $^1\text{H}$  NMR spectrum of BHB.

### 2.2.23 Myo-Inositol (mI) and scyllo-Inositol (sI)

mI is a cyclic sugar alcohol with six NMR detectable protons that give rise to four groups of resonances. A doublet-of-doublets centered at 3.52 ppm originates from the  $^1\text{CH}$  and  $^3\text{CH}$  protons, while the  $^4\text{CH}$  and  $^6\text{CH}$  protons give rise to a triplet at 3.61 ppm. A smaller triplet from the  $^5\text{CH}$  protons resides at 3.27 ppm and is typically obscured by the larger total choline resonances. Finally, the  $^2\text{CH}$  proton is observed as a triplet at 4.05 ppm. The structure and  $^1\text{H}$  NMR spectrum of mI are shown in Figure 2.19A.

Inositol has nine distinct isomers, of which mI represents the predominate form found in tissues. mI is readily detected in short-TE  $^1\text{H}$  NMR spectra of the brain due to its high concentration of 4–8 mM [12, 139]. The exact function of mI is not known [105],

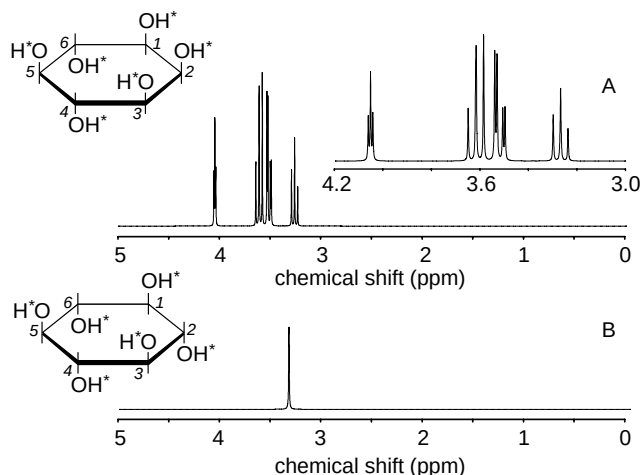


**Figure 2.19** Chemical structures and simulated  $^1\text{H}$  NMR spectra of (A) mI and (B) sI.

although it plays an important function in osmotic regulation in the kidney [140] and has a biochemical relationship to messenger-inositol polyphosphates [141]. It has been proposed as a glial marker [142], although that characteristic has been challenged by the detection of mI in various neuronal cell types [143, 144]. Altered levels have been encountered in patients with mild cognitive impairment and Alzheimer's disease [145–148], and brain injury [149].

Similar to mI, sI is a cyclic sugar alcohol with six NMR detectable protons (Figure 2.19B). However, due to molecular symmetry all six protons are magnetically equivalent giving rise to a singlet resonance at 3.34 ppm. sI is the second most abundant isomer of inositol and has been detected in a wide range of species [150]. sI levels have been detected by  $^1\text{H}$  MRS in normal brain [150, 151] and elevated levels during chronic alcoholism [152]. During the identification of sI in perchloric acid extracts, care should be taking to avoid misidentification due to methanol, which may be a contaminant of the extraction procedure [153].

## 2.2.24 Lactate (Lac)

Lac (or lactic acid) is the end-product of anaerobic glycolysis. Even though Lac is normally present at a low concentration of circa 0.5 mM, it is readily observable in short-TE  $^1\text{H}$  NMR spectra (see Figure 2.1) due to three equivalent protons resonating in a spectral area devoid of significant overlapping resonances. Increased Lac concentrations have been observed under a wide variety of conditions in which blood flow (and hence oxygen supply) is restricted such as ischemic stroke [27, 154], hypoxia [155, 156] and tumors [29, 30]. Transient increases in Lac levels have also been observed in human brain during and following functional activation [157–160] and hyperventilation [161, 162]. It has been proposed that Lac links astroglial glucose uptake and metabolism to neuronal neurotransmitter cycling and metabolism in the so-called astroglial-neuronal lactate shuttle (ANLS)

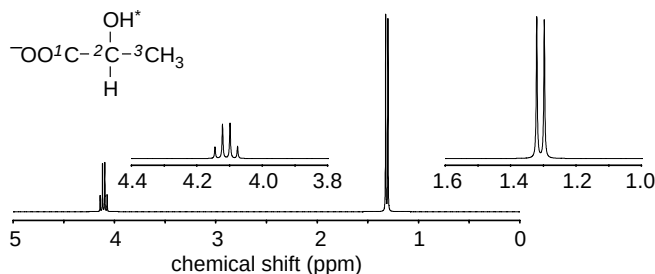


**Figure 2.20** Chemical structure and simulated  $^1\text{H}$  NMR spectrum of Lac.

hypothesis [163, 164]. However, this hypothesis is not uniformly accepted and the definite experimental proof remains to be established [165].

Lac has four nonexchangeable protons in methine and methyl groups (Figure 2.20). The three equivalent methyl protons give rise to a doublet resonance at 1.31 ppm, while the single methine proton resonates as a quartet at 4.10 ppm. In normal brain tissue, Lac only overlaps with macromolecular resonances. However, in tumors, stroke or with inadequate localization, Lac can be overlapping with large lipid resonances. In these cases, Lac is best observed with spectral editing techniques, as will be discussed in Chapter 8. Lac is one of the few scalar-coupled spin systems that can be considered weakly coupled, even at low magnetic fields.

### 2.2.25 Macromolecules

In short-TE  $^1\text{H}$  NMR spectra a significant fraction of the observed signal must be attributed to macromolecular resonances underlying those of the metabolites. Behar *et al.* [166, 167] and Kauppinen *et al.* [168, 169] have extensively studied macromolecular resonances in human and animal brain. Based on dialysis and fractionation studies and more recent *in vivo* detection [1, 170] a minimum of 10 macromolecular resonances can be observed (Figure 2.21B) at 0.93 ppm (M1), 1.24 ppm (M2), 1.43 ppm (M3), 1.72 ppm (M4), 2.05 ppm (M5), 2.29 ppm (M6), 3.00 ppm (M7), 3.20 ppm (M8), 3.8–4.0 ppm (M9) and 4.3 ppm (M10). While assignment to specific proteins is virtually impossible, the individual resonances can be tentatively assigned [166, 167] to methyl and methylene resonances of protein amino acids, such as leucine, isoleucine and valine (M1), threonine and alanine (M2 and M3), lysine and arginine (M4 and M7), glutamate and glutamine (M5 and M6) and the less well-defined band of resonances between 3.0 ppm and 4.5 ppm (M8 – M10) to  $\alpha$ -methine protons. The macromolecular resonances are further characterized by extensive scalar coupling patterns among the resonances, in particular between  $J_{\text{M1-M5}} = 7.3$  Hz (doublet) and  $J_{\text{M4-M7}} = 7.8$  Hz (triplet). The scalar coupling between M4 and M7 is of importance for the detection of GABA, as detailed in Chapter 8. The  $T_1$  and  $T_2$  relaxation times of macromolecular resonances are significantly shorter than those of the metabolites (see Chapter 3). The short  $T_2$  relaxation time constants effectively eliminate macromolecular resonances from long-TE  $^1\text{H}$  NMR spectra, leading to significant spectral simplification. The large difference between macromolecular and metabolite  $T_1$ s offers an opportunity for

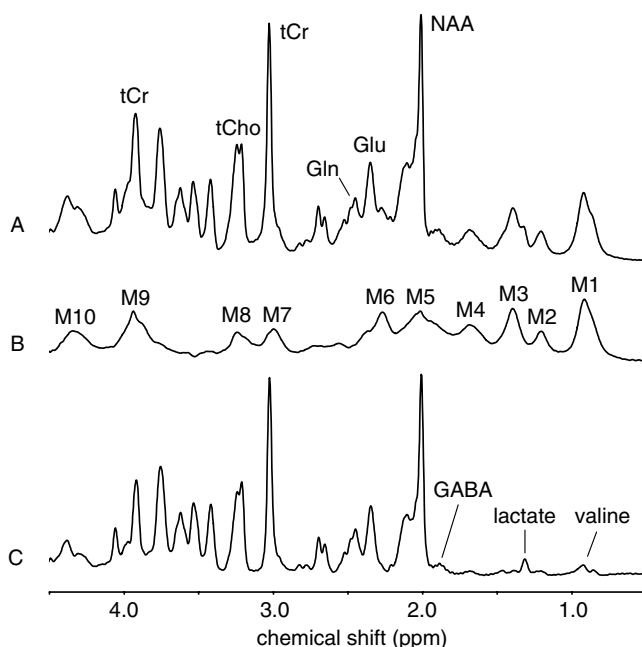


**Figure 2.21** Detection of macromolecular resonances in rat brain in vivo at 9.4 T. (A) Short-TE  $^1\text{H}$  NMR spectrum acquired with LASER localization ( $TR/TE = 4000/12$  ms,  $180\ \mu\text{L}$ ). The sharp resonances from metabolites are superimposed on a baseline of broader resonances from macromolecules that are only visible between 0.5 ppm and 2.0 ppm. (B) Same experiment as (A) with the extension of two nonselective inversion pulses ( $TR/TI1/TI2 = 3250/2100/630$  ms). The double inversion methods ‘nulls’ signals over a wide range of  $T_1$  relaxation times (1100–1900 ms), while retaining signal from compounds with shorter  $T_1$  relaxation times, like macromolecules. Ten upfield macromolecular resonances (M1–M10) are consistently detected from normal brain. (C) Subtracting (B) from (A) results in a virtually macromolecule-free spectrum, allowing direct observation of Lac, Ala and possibly GABA and Val.

the selective detection of metabolites or macromolecules [1, 167–170]. Figure 2.21 shows the selective detection of macromolecules in rat brain at 9.4 T. Using a double-inversion-recovery sequence ( $90^\circ - TR - 180^\circ - TI1 - 180^\circ - TI2 - 90^\circ - \text{acquisition}$ ) with  $TR = 3250$  ms,  $TI1 = 2100$  ms and  $TI2 = 630$  ms [171], the longitudinal magnetization of the metabolites is close to zero, while the shorter  $T_1$  of macromolecules leads to greater signal recovery. Therefore, this method ‘nulls’ the metabolite signals, allowing selective observation of the macromolecular resonances. While a similar effect can be achieved with a single-inversion-recovery method, the suppression of metabolites would be incomplete due to significant variation in metabolite  $T_1$ s. A double-inversion-recovery method is relatively insensitive to metabolite  $T_1$ s. Macromolecular  $^1\text{H}$  NMR spectra as shown in Figure 2.21B can be used as prior knowledge in spectral fitting algorithms to increase the accuracy and reliability of spectral quantification (see Chapter 9). Alterations in the macromolecular spectrum have been observed in stroke [172–174] and tumors [175, 176].

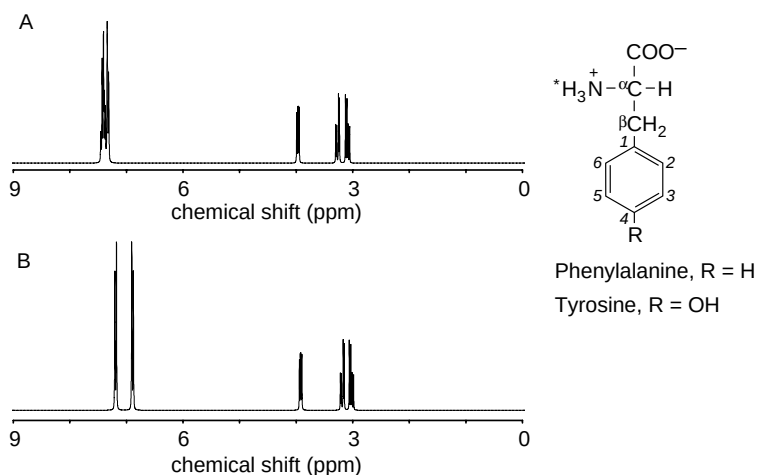


**Figure 2.22** Chemical structures and simulated  $^1\text{H}$  NMR spectra of (A) phenylalanine and (B) tyrosine.

### 2.2.26 Phenylalanine

Phenylalanine is an essential aromatic amino acid composed of a phenyl aromatic ring connected to the methyl group of alanine. It is a known precursor for catecholamine synthesis and is normally present in the human brain at circa 0.2 mM, following an age-dependent decrease.  $^1\text{H}$  MRS has been used to detect elevated cerebral phenylalanine levels in human [177] and animal [178] brain. At lower magnetic field, the vascular contribution of phenylalanine is NMR visible and should be accounted for during signal quantification [179]. The concentration is elevated up to 5.0 mM in phenylketonuria (PKU), an inborn error of phenylalanine metabolism. In PKU patients, the hydroxylation of phenylalanine to tyrosine is disturbed due to a deficiency of the liver enzyme phenylalanine hydroxylase or of the tetrahydrobiopterin cofactor. Proton  $^1\text{H}$  MRS has been used to detect and quantify phenylalanine in PKU patients [180–182]. PKU is treatable by restricted dietary phenylalanine intake.

Phenylalanine has eight NMR observable protons, five of which reside in the phenyl ring and three in the aliphatic side chain. The five nonequivalent phenyl ring protons give a complex multiplet between 7.30 ppm and 7.45 ppm and represent the typically observed resonance *in vivo*. The  $\alpha$ -proton resonates as a doublet-of-doublets at 3.98 ppm, while the  $\beta$ -protons give rise to two doublet-of-doublets centered at 3.11 ppm and 3.27 ppm. Figure 2.22A shows the structure and  $^1\text{H}$  NMR spectrum of phenylalanine.

### 2.2.27 Pyruvate

Pyruvic acid is an  $\alpha$ -keto acid and plays an important intermediate role in glucose metabolism. The carboxyate anion of pyruvic acid is known as pyruvate. One molecule of glucose breaks down into two molecules of pyruvate during glycolysis. Provided that sufficient oxygen is available, pyruvate is converted into acetyl-coenzyme A (CoA), which

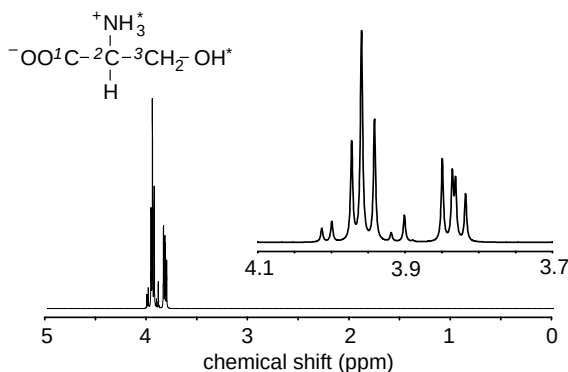


**Figure 2.23** Chemical structure and simulated  $^1\text{H}$  NMR spectrum of serine.

is the main input for a series of reactions known as the tricarboxylic acid (TCA) cycle, also known as the Krebs cycle or the citric acid cycle. Pyruvate is also broken down to oxaloacetate by a so-called anaplerotic reaction with the purpose of replenishing TCA cycle intermediates. If insufficient oxygen is available, the acid is broken down anaerobically by the enzyme lactate dehydrogenase and the coenzyme NADH, creating lactic acid (lactate). Pyruvate is readily transported from the blood and may have neuroprotective properties in stroke [183].

The singlet resonance of pyruvate at 2.36 ppm is readily observable in high-resolution  $^1\text{H}$  NMR spectra of brain extracts. However, the typical *in vivo* concentration is below 0.2 mM, such that detection has been limited to pathologies, like cystic lesions [184] and neonatal pyruvate dehydrogenase deficiency [185].

## 2.2.28 Serine

Serine is a nonessential amino acid that is commonly found in mammalian proteins. Serine can be synthesized in the body from other metabolites, including glycine. Serine participates in the biosynthesis of purines and pyrimidines, cysteine and a large number of other metabolites. The  $^1\text{H}$  NMR spectrum (Figure 2.23) is characterized by a cluster of resonances between 3.8 ppm and 4.0 ppm originating from three protons in methine and methylene groups that form a strongly-coupled ABC spin system. Serine is present throughout the brain at a concentration of 0.5 – 1.5 mM. However, since serine resonates in a complicated region of the  $^1\text{H}$  NMR spectrum with significant spectral overlap, the detection of serine is difficult, even at high magnetic fields [1].

## 2.2.29 Succinate

Succinic acid (or succinate for the anion form) is part of the TCA cycle, where it is formed from succinyl CoA by succinyl CoA synthetase. Alternatively, succinate can be formed from succinate semialdehyde by succinate semialdehyde dehydrogenase (SSADH) as part of the GABA shunt. Succinate is converted to the TCA cycle intermediate fumarate by

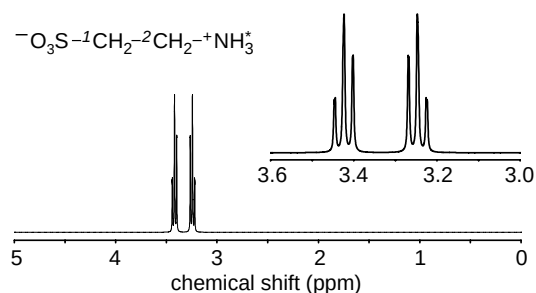


**Figure 2.24** Chemical structure and simulated  $^1\text{H}$  NMR spectrum of taurine.

succinate dehydrogenase, whereby FAD is converted to FADH<sub>2</sub>. Succinate is typically present at concentrations well below 0.5 mM.

The four protons in succinate are magnetically equivalent, giving rise to a singlet resonance at 2.39 ppm. While this peak is readily observed in high-resolution  $^1\text{H}$  NMR spectra of extracts, the *in vivo* observation is complicated by overlapping resonances from glutamate, glutamine and pyruvate, leading to ambiguous resonance assignment [186,187].

### 2.2.30 Taurine (Tau)

Taurine (2-aminoethanesulfonic acid) has two methylene groups with nonequivalent protons, thereby forming a AA'XX' spin system (Figure 2.24). At higher magnetic fields taurine can be considered an A<sub>2</sub>X<sub>2</sub> spin system giving two triplets centered at 3.25 and 3.42 ppm. When quantifying the total choline methyl resonance at 3.21 ppm, the upfield taurine multiplet should be taken into account as it forms a significant fraction of the observed 'choline' resonance. Especially at lower magnetic fields, taurine is essentially overlapping with mI and choline, such that unambiguous detection requires the use of spectral editing methods [188–190].

The exact function of taurine is not known, but it has been proposed as an osmoregulator and a modulator of neurotransmitter action. Taurine is present in all cells of the CNS, but is spatially heterogeneous with higher levels in the olfactory bulb, retina and cerebellum [191]. The concentration of taurine has a strong age dependence, decreasing from circa 12 mM at birth to 6–8 mM in adult rat brain [192]. The concentration in human brain is significantly lower at circa 1.5 mM [2]. Taurine is largely obtained through food, but it is a nonessential amino acid as it can be synthesized from other sulfur-containing amino acids.

### 2.2.31 Threonine (Thr)

Thr is obtained from food and is therefore an essential amino acid. While mammals can not synthesize Thr, plants and microorganisms are capable of synthesizing Thr from aspartate. Thr is an important amino acid for the nervous system, with relatively high levels of circa 0.3 mM. It has been used as a supplement to help alleviate anxiety and some cases of depression. Thr can be converted to glycine and serine.

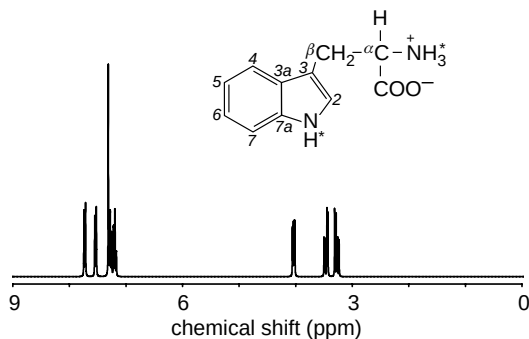


**Figure 2.25** Chemical structure and simulated  $^1\text{H}$  NMR spectrum of Trp.

Threonine has five nonexchangeable protons in a methyl and two methine groups. The  $^2\text{CH}$  and  $^3\text{CH}$  protons resonate as a doublet and multiplet at 3.58 ppm and 4.25 ppm, respectively. The most intense resonance of the three methyl protons appears as a doublet at 1.32 ppm and is therefore essentially overlapping with the methyl protons of lactate. Since the scalar coupling patterns of threonine and lactate are very similar, they can typically not be separated by spectral editing. It is therefore advised to refer to the 1.3 ppm resonance as total lactate, being the sum of lactate and threonine [193].

### 2.2.32 Tryptophan (Trp)

Trp is an essential amino acid that is necessary for the production of the neurotransmitter serotonin, the neurohormone melatonin and vitamin B3 (niacin). Normally present at low concentrations of circa 0.03 mM, the brain concentration can be increased by Trp consumption. Higher Trp levels lead to a twofold increase in serotonin synthesis, which has led to investigations for use in the treatment of mild insomnia as well as an antidepressant. Increased brain Trp levels are also associated with hepatic encephalopathy [194].

Tryptophan has eight NMR detectable protons. The  $^2\text{CH}$  proton gives a singlet at 7.31 ppm, whereas the four phenyl ring protons give two multiplets centered at 7.20 and 7.28 ppm. The three aliphatic side chain protons give three doublet-of-doublets 3.29, 3.48 and 4.05 ppm. Although Trp is normally present at low concentration, the indole ring resonances may combine sufficiently to give an identifiable signal even at low magnetic fields. Figure 2.25 shows the structure and  $^1\text{H}$  NMR spectrum of Trp.

### 2.2.33 Tyrosine (Tyr)

Tyrosine (4-hydroxyphenylalanine) is a nonessential amino acid that can be synthesized from phenylalanine. It is a precursor of the neurotransmitters epinephrine, norepinephrine and dopamine and of the thyroid hormones thyroxine and triiodothyronine. Tyrosine is converted to DOPA by tyrosine dehydroxylase. It plays a key role in signal transduction, since it can be phosphorylated by protein kinases to alter the functionality and activity of certain enzymes. (In its phosphorylated state, it is sometimes referred to as phosphotyrosine). Normal brain concentrations decrease from circa 0.25 mM after birth to



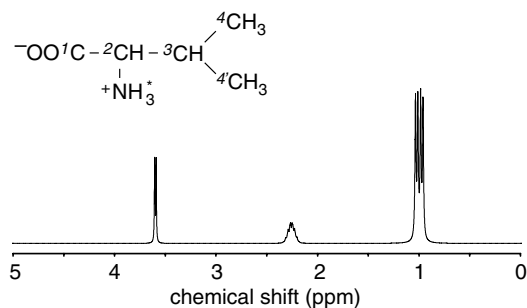


**Figure 2.26** Chemical structure and simulated  $^1\text{H}$  NMR spectrum of Val.

0.05 mM in adulthood, although it can be increased following oral consumption or hepatic encephalopathy.

Tyrosine has seven NMR observable protons, distributed over a phenyl ring and an aliphatic side chain. The four phenyl protons give a multiplet between 6.89 ppm and 7.19 ppm. The CH and CH<sub>2</sub> protons in the aliphatic side chain form an ABX spin system, giving rise to three doublet-of-doublets centered at 3.04 ppm, 3.19 ppm and 3.93 ppm. Figure 2.22B shows the  $^1\text{H}$  NMR spectrum and structure of tyrosine.

### 2.2.34 Valine (Val)

Val is an essential amino acid required for protein synthesis. Normal brain concentration is on the order of 0.1 mM. Hypervalinemia, branched-chain ketonuria and brain abscesses are three of several diseases in which Val concentrations are increased. In sickle-cell disease, it substitutes for the amino acid glutamic acid in hemoglobin leading to an incorrect folding of the hemoglobin molecule.

The NMR spectrum of Val (Figure 2.26) can be traced back to the six protons in two methyl groups resonating at 0.98 ppm and 1.03 ppm and two protons in two methine groups resonating at 2.26 ppm and 3.60 ppm. The *in vivo* detection of Val is not trivial as the most intense resonances (methyl protons) are broad multiplets overlapping with broad resonances from macromolecules (see also Figure 2.21).

### 2.2.35 Water

Water is the most abundant compound in the human and animal body with water contents ranging from 73 % for cerebral white matter, 82 % for cerebral gray matter, >95 % for cerebrospinal fluid (CSF) and 78 % for skeletal muscle. Cerebral water content has a strong age dependence with 92 % in 20–22 week fetuses, 90 % in newborns and 77 % (average) in adults. Since the water content only changes moderately among different pathologies, water is a reasonable candidate for internal concentration referencing (see Chapter 9). The T<sub>1</sub> and T<sub>2</sub> relaxation times, as well as magnetization transfer characteristics of water vary greatly among tissues, leading to excellent image contrast in MRI applications (see Chapters 3 and 4).

Besides many other unique applications that will be discussed in forthcoming chapters, the water chemical shift can be used to detect temperature changes noninvasively *in vivo*. The temperature dependence of the proton resonance frequency was first described by

Hindman [195]. In pure water the proton resonance frequency changes by approximately  $-0.01 \text{ ppm } ^\circ\text{C}^{-1}$  and can be modeled as [196, 197]:

$$\omega_{\text{water}}(T) = \gamma \left( 1 - \frac{2}{3} \chi(T) - \sigma(T) \right) B_0 \quad (2.1)$$

where the temperature dependencies arise from the volume/bulk magnetic susceptibility ( $\chi$ ) and the electronic screening constant ( $\sigma$ ) of the proton nucleus (see also Section 1.8).

The dominant source of the water temperature dependence is based on a change in the electronic shielding constant of the proton nuclei. Quantitative descriptions of this phenomenon are given by Hindman [195] and others [196]. A qualitative, general understanding of this effect can be gained by noting that the shielding of the proton nuclei by the surrounding electrons in an isolated water molecule is better than for a water molecule in solution. This is because in solution, adjacent water molecules form hydrogen bonds/bridges between hydrogen and oxygen nuclei. The electronegative oxygen atom partially ‘pulls’ the electron cloud from the hydrogen nucleus, leading to a reduced electronic shielding and hence a higher Larmor resonance frequency.

When the temperature increases, the hydrogen bonds stretch, bend and break more frequently such that the hydrogen nuclei spend less time on average in a hydrogen bonded state to another water molecule. As a consequence, as the temperature rises the electronic shielding of the proton nucleus increases, leading to a lower local magnetic field and hence a lower Larmor resonance frequency.

Change in the volume/bulk magnetic susceptibility is a relatively minor contribution to the temperature dependence of water. Pure water is diamagnetic and has a bulk magnetic susceptibility of  $-9.05 \text{ ppm}$  at room temperature. Water becomes less diamagnetic with increasing temperature by approximately  $+0.001 \text{ ppm } ^\circ\text{C}^{-1}$  mainly due to a decrease in water density [198].

Temperature changes can simply be obtained by monitoring the resonance frequency of the water resonance. However, many other effects, like drift of the magnetic field and motion can also lead to a shift in the water frequency. For NMR spectroscopy applications, the method can be made more reliable by measuring the chemical shift *difference* between water and a reference compound that does not have a temperature-dependent shift. The methyl group of NAA has been used for this purpose [199–201]. For MRI applications, the shift in water resonance frequency is typically detected as a phase change [202, 203]. Temperature mapping using the water resonance has a typical accuracy of circa  $0.1 ^\circ\text{C}$ , which is mainly due to the relatively small temperature dependence upon the chemical shift. Other compounds have been proposed and used that can have frequency shifts of  $>1.0 \text{ ppm } ^\circ\text{C}^{-1}$  with the possible option of simultaneous pH determination [204–206]. However, the use of exogenous compounds makes the measurement invasive, while the lower concentration may not necessarily improve the accuracy of the temperature measurement.

### 2.2.36 Intra- and Extramyocellular Lipids (IMCL and EMCL)

A typical *in vivo*  $^1\text{H}$  NMR spectrum from human skeletal muscle (Figure 2.27) is dominated by resonances from lipids resonating around  $1.5 \text{ ppm}$ . Lipids are either present as subcutaneous or interstitial adipose tissue, also referred to as EMCL, or as IMCL in the form of liquid droplets in the cytoplasm of muscle cells. Free (or protein-bound) lipids in the cytoplasm are typically of much lower concentration. While EMCL is metabolically

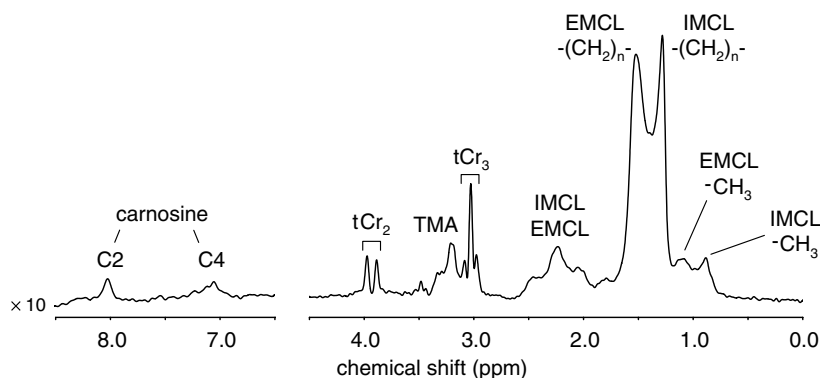


**Figure 2.27**  $^1\text{H}$  NMR spectrum acquired from human skeletal muscle at 4 T ( $TR/TE = 4000/15$  ms, 6 mL). Residual dipolar interactions lead to a splitting of the Cr methyl and methylene groups. Magnetic susceptibility differences between IMCL and EMCL lead to an orientation dependent separation in chemical shift, which reaches a maximum of  $\sim 0.2$  ppm when the muscle fibers are parallel to the main magnetic field. The two downfield resonances of carnosine are sensitive to pH in the physiological range. TMA-tetramethyl ammonium.

relatively inert, there is substantial evidence that IMCL can be mobilized and utilized for energy metabolism during strenuous exercise, particularly as they are primarily located immediately adjacent to mitochondria. Furthermore, using  $^1\text{H}$  NMR spectroscopy it has been found that IMCL levels are extremely sensitive to physical exercise [207–209] and diet [210,211]. In diabetes several groups have observed an inverse correlation between IMCL levels and insulin sensitivity [212–214]. A review of the role of  $^1\text{H}$  MRS in muscle lipid metabolism is given by Boesch *et al.* [215].

$^1\text{H}$  NMR spectroscopy is capable of separately detecting IMCL from EMCL due to differences in bulk magnetic susceptibility (BMS) shift [216–218]. IMCL, residing in spherical droplets, experience a total magnetic field independent of the angle relative to the magnetic field (see also Chapter 10). EMCL on the other hand, being present as macroscopic plates or bundles running parallel to the main axis of the muscle, experience an orientation-dependent local magnetic field. For EMCL parallel to the magnetic field  $B_0$ , the shift between IMCL and EMCL amounts to circa 0.2 ppm. Similar effects have been observed for Cr [219–221] and carnosine [219,222].

The distribution of IMCL is heterogeneous across different muscles [223]. Regional differences in IMCL in humans have been observed by *in vivo*  $^1\text{H}$  MR spectroscopic imaging and show the highest and lowest levels in soleus muscles and tibialis and gastrocnemius muscle, respectively [223–226].

### 2.2.37 Deoxymyoglobin (DMb)

Myoglobin (Mb), a 16.7 kDa protein, plays an important role in muscle physiology as an oxygen storage compound and a facilitator of oxygen diffusion. It has been shown by several groups that oxygen saturation in human skeletal and cardiac muscle can be determined by detecting the DMb signal by  $^1\text{H}$  NMR spectroscopy at  $\sim 79$  ppm [227–230].

Despite its low concentration ( $\sim 300 \mu\text{M}$  during muscle ischemia) the detection of DMb by  $^1\text{H}$  NMR spectroscopy is possible because the resonance position of the N- $\delta$  proton in the proximal F8 histidine of DMb is sufficiently shifted downfield, away from the more intense resonances of water and lipids. The short  $T_1$  relaxation time of  $\sim 10$  ms allows substantial signal averaging and hence an improved sensitivity. The oxygenated form of Mb does not have any resonances with paramagnetic shifts and therefore is unobservable. Under normoxic conditions Mb is completely oxygenated, such that no signal from DMb can be detected at rest. However, under ischemic conditions, as achieved by using a pressure cuff or during heavy exercise, a large DMb signal can be readily observed [231].

### 2.2.38 Citric Acid

Citric acid (or the anion form known as citrate) is best known as an intermediate of the TCA, Krebs or citric acid cycle, where it is formed when acetyl-CoA donates a carbonyl group to oxaloacetate. In brain tissue the concentration of citrate, as well as that of all other intermediates of the citric acid cycle is below the NMR detection limit. However, it is well known [232], that healthy prostate epithelial cells synthesize and secrete citrate in relatively large amounts ( $\sim 60$  mM), due to the fact that high levels of zinc inhibit the oxidation of citrate in the Krebs cycle. In cancerous epithelial cells, zinc levels are drastically reduced, resulting in greatly reduced citrate levels. As a result, monitoring citrate levels in the prostate has been used to obtain direct biochemical information to aid in the diagnosis of malignant adenocarcinoma and benign prostatic hyperplasia [233–237].

Citrate was first detected in human prostate *in vivo* by natural abundance  $^{13}\text{C}$  NMR spectroscopy as described by Sillerud *et al.* [238]. Current detection methods of prostate citrate rely almost exclusively on  $^1\text{H}$  NMR spectroscopy. The proton NMR spectrum of citrate arises from two magnetically equivalent  $\text{CH}_2$  moieties (see Figure 2.28). While the methylene groups are magnetically equivalent due to the molecular symmetry, the two protons in each methylene group are magnetically inequivalent. As a result, citrate is a classical example of a strongly coupled AB spin system. The exact chemical shift and

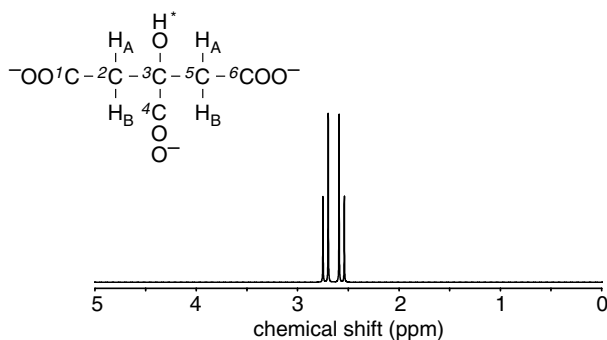


**Figure 2.28** Chemical structure and simulated  $^1\text{H}$  NMR spectrum of citrate. While the two  $H_A$  and the two  $H_B$  protons are equivalent due to molecular symmetry, the  $H_A$  and  $H_B$  protons within one methylene group are not equivalent leading to the characteristic NMR spectrum of a strongly coupled AB spin-system (compare with Figure 1.17A).

scalar coupling constants are dependent on the pH [239], as well as the cation concentration [240]. Average chemical shift and scalar coupling values for the physiological range are 2.57 ppm, 2.72 ppm and 15.5 Hz. The exact appearance of the four citrate resonances is also a function of magnetic field strength, echo time as well as other timing parameters (like TM in STEAM) and RF pulses, all of which require careful optimization to achieve high sensitivity citrate detection [241–243]. Citrate detection is often achieved through direct  $^1\text{H}$  MRS, due to the absence of significant spectral overlap. However, some authors have argued that detection of reduced citrate levels in cancerous prostate can benefit from spectral editing methods which remove contributions of partially overlapping resonances from total creatine and Gln [244].

### 2.2.39 Carnosine

Carnosine ( $\beta$ -alanyl-L-histidine), a dipeptide between alanine and histidine, is part of a series of compounds referred to as aminoacyl-histidine dipeptides. Other members include homocarnosine ( $\gamma$ -aminobutyl-L-histidine) and anserine ( $\beta$ -alanyl-L-1-methyl-histidine). This group of naturally occurring histidine-containing molecules is particularly abundant in excitable tissues, such as muscle and nervous tissue. The biological functions of the aminoacyl-histidine dipeptides remain enigmatic, although roles such as pH buffer and antioxidant have been proposed. Carnosine was first observed over a century ago and has subsequently been found by a wide range of techniques, including  $^1\text{H}$  NMR spectroscopy (see Figure 2.27). The pK of the C-2 and C-4 protons on the imidazole ring of histidine are in the physiological pH range ( $\text{pK} = 6.7$ ), providing a noninvasive method to measure intracellular pH with  $^1\text{H}$  NMR spectroscopy *in vivo*. The physical principle underlying the pH-dependent chemical shifts is discussed in Section 2.3 for  $^{31}\text{P}$  NMR spectroscopy. Yoshizaki *et al.* [245] initially used carnosine to determine pH in excised frog muscle, which has subsequently been followed by studies *in vivo*, including human muscle. Several studies [246, 247] have shown an excellent correlation between the determination of intracellular pH by  $^1\text{H}$  (carnosine) and  $^{31}\text{P}$  (inorganic phosphate) NMR.

Using carnosine for intracellular pH determination has several advantages over the more traditional methods using the chemical shift of inorganic phosphate in  $^{31}\text{P}$  NMR spectra. First, the sensitivity of carnosine detection is very high due to the inherently high sensitivity of  $^1\text{H}$  NMR, the high concentration of carnosine in skeletal muscle (up to 20 mM for human muscle) and the relatively short  $T_1$  relaxation times. This is especially important in studies in which the concentration of inorganic phosphate significantly decreases (e.g. the recovery stage in muscle exercise studies). The high sensitivity of carnosine detection would allow a substantially improved time resolution in exercise studies. Second, the measurement of intracellular pH using carnosine is relatively insensitive to the presence of divalent cations, like free magnesium ( $\text{Mg}^{2+}$ ). This is especially important in exercise studies where intracellular free  $\text{Mg}^{2+}$  concentrations increase.

## 2.3 Phosphorus-31 NMR Spectroscopy

The success of *in vivo* proton NMR spectroscopy in routine (clinical) applications is only matched by phosphorus NMR. The relatively high sensitivity of phosphorus NMR (circa

7 % of protons), together with a 100 % natural abundance allows the acquisition of high-quality spectra within minutes. Furthermore, the chemical shift dispersion of the phosphates found *in vivo* is relatively large ( $\sim 30$  ppm), resulting in excellent spectral resolution even at low (clinical) magnetic field strengths. Phosphorus NMR is very useful because with simple NMR methods it is capable of detecting all metabolites that play key roles in tissue energy metabolism. Furthermore, biologically relevant parameters such as intracellular pH may be indirectly deduced.

### 2.3.1 Identification of Resonances

Phosphorus NMR spectra from tissues *in vivo* typically hold a limited number of resonances (Figure 2.29). The exact chemical shift position of almost all resonances is sensitive to physiological parameters like intracellular pH and ionic (e.g. magnesium) strength. By convention, the PCr resonance is used as an internal chemical shift reference and has been assigned a chemical shift of 0.00 ppm. At a pH of 7.2, with full magnesium complexation, the resonances of ATP appear at  $-7.52$  ppm ( $\alpha$ ),  $-16.26$  ppm ( $\beta$ ) and  $-2.48$  ppm ( $\gamma$ ). The resonance of inorganic phosphate appears at 5.02 ppm. Under favorable, high-sensitivity

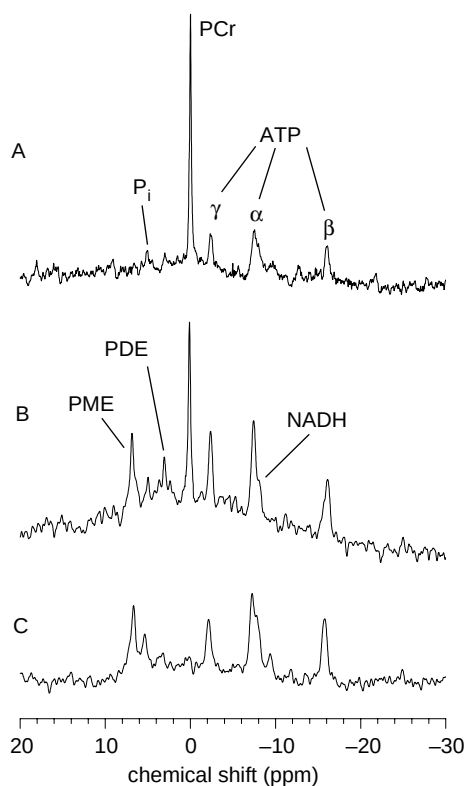


**Figure 2.29** Typical localized *in vivo*  $^{31}\text{P}$  NMR spectra from (A) rat skeletal muscle, (B) brain and (C) liver. Note the complete absence of PCr in the liver.

**Table 2.3** Chemical shifts of biologically relevant  $^{31}\text{P}$ -containing metabolites

|                                          |          |        |
|------------------------------------------|----------|--------|
| Adenosine monophosphate (AMP)            |          | 6.33   |
| Adenosine diphosphate (ADP)              | $\alpha$ | -7.05  |
|                                          | $\beta$  | -3.09  |
| Adenosine triphosphate (ATP)             | $\alpha$ | -7.52  |
|                                          | $\beta$  | -16.26 |
|                                          | $\gamma$ | -2.48  |
| Dihydroxyacetone phosphate               |          | 7.56   |
| Fructose-6-phosphate                     |          | 6.64   |
| Glucose-1-phosphate                      |          | 5.15   |
| Glucose-6-phosphate                      |          | 7.20   |
| Glycerol-1-phosphate                     |          | 7.02   |
| Glycerol-3-phosphorylcholine             |          | 2.76   |
| Glycerol-3-phosphorylethanolamine        |          | 3.20   |
| Inorganic phosphate                      |          | 5.02   |
| Phosphocreatine                          |          | 0.00   |
| Phosphoenolpyruvate                      |          | 2.06   |
| Phosphorylcholine                        |          | 5.88   |
| Phosphorylethanolamine                   |          | 6.78   |
| Nicotinamide adenine dinucleotide (NADH) |          | -8.30  |

<sup>a</sup> All chemical shifts are referenced relative to phosphocreatine at 0.00 ppm.

conditions phosphorus NMR spectra can also hold resonances from phosphomonoesters and diesters. Table 2.3 summarizes the chemical shifts of the most commonly observed  $^{31}\text{P}$ -containing metabolites. Note that PCr is completely absent in  $^{31}\text{P}$  NMR spectra from liver. The appearance of PCr in  $^{31}\text{P}$  NMR spectra from liver is a good indication of signal contamination from surrounding muscle tissue.

Besides the chemical shifts, a  $^{31}\text{P}$  NMR spectrum is further characterized by homonuclear scalar coupling for ATP and heteronuclear ( $^{31}\text{P}$ - $^1\text{H}$ ) scalar coupling for the phosphomonoesters (PME) phosphorylethanolamine and phosphorylcholine and the phosphodiester (PDE) glycerol 3-phosphorylethanolamine and glycerol 3-phosphorylcholine. The three-bond homonuclear scalar couplings for ATP have been determined as  $^3J_{\alpha\beta} = 16.3$  Hz and  $^3J_{\beta\gamma} = 16.1$  Hz in brain,  $^3J_{\alpha\beta} = 16.0$  Hz and  $^3J_{\beta\gamma} = 17.2$  Hz in skeletal muscle and  $^3J_{\alpha\beta} = 15.8$  Hz and  $^3J_{\beta\gamma} = 16.0$  Hz in myocardium [248]. Similar to most  $^{31}\text{P}$  chemical shifts, the scalar coupling constants are sensitive to the pH and the magnesium ( $\text{Mg}^{2+}$ ) concentration. The three-bond heteronuclear scalar couplings for the PME and PDE resonances are in the 6–7 Hz range and generally lead to undesirable line-broadening in that crowded spectral region. Several authors have shown that heteronuclear decoupling (see also Chapter 7) can greatly enhance the spectral resolution for the PME and PDE resonances [249, 250]. Furthermore, PME and PDE resonances can be observed with enhanced sensitivity by utilizing the heteronuclear scalar coupling in polarization transfer experiments [89–91].

### 2.3.2 Intracellular pH

The chemical shift of many phosphorus containing compounds is dependent on a number of physiological parameters, in particular intracellular pH and magnesium concentration. The cause of this phenomenon can be found in the fact that protonation (or complexation

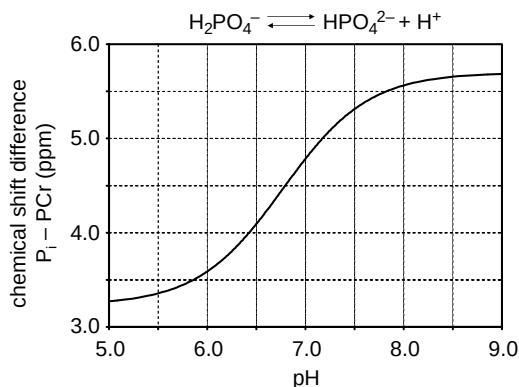


**Figure 2.30** pH calibration curve for the  $P_i$ –PCr system as used for the determination of intracellular pH with *in vivo*  $^{31}\text{P}$  MRS. The curve is described by Equation (2.2) with  $pK = 6.77$ ,  $\delta_{\text{HA}} = 3.23$  ppm and  $\delta_{\text{A}} = 5.70$  ppm.

with magnesium) of a compound changes the chemical environment of nearby nuclei and hence changes their chemical shift. When the chemical exchange between the protonated and unprotonated forms is slow, the two forms will have two separate resonance frequencies with the resonance amplitudes indicating the relative amounts. However, for most compounds observed with phosphorus NMR, the chemical exchange is fast relative to the NMR timescale and only a single, average resonance is observed. The resonance frequency is now indicative of the relative amount of the protonated and the unprotonated form, and hence the pH can be described by a modified Henderson–Hasselbach relationship according to:

$$\text{pH} = \text{pK}_{\text{A}} + \log \left( \frac{\delta - \delta_{\text{HA}}}{\delta_{\text{A}} - \delta} \right) \quad (2.2)$$

where  $\delta$  is the observed chemical shift,  $\delta_{\text{A}}$  and  $\delta_{\text{HA}}$  the chemical shifts of the unprotonated and protonated forms of compound A and  $\text{pK}_{\text{A}}$  the logarithm of the equilibrium constant for the acid–base equilibrium between HA and A.

Even though almost all resonances in  $^{31}\text{P}$  NMR spectra have pH dependence, the resonance of inorganic phosphate ( $P_i$ ) relative to PCr is most commonly used for several reasons. Its  $pK$  is in the physiological range ( $pK = 6.77$ ), it is readily observed in most tissues (with muscle being a possible exception) and its chemical shift has a large dependence on pH. The chemical shift of PCr can be assumed constant in the physiological pH range ( $pK = 4.30$ ). Figure 2.30 shows a pH curve for the  $P_i$ –PCr system with  $pK = 6.77$ ,  $\delta_{\text{HA}} = 3.23$  ppm and  $\delta_{\text{A}} = 5.70$  ppm, which represent average literature values [251–254]. It follows that the greatest sensitivity towards pH changes is achieved when  $\text{pH} \sim pK$ . While the  $P_i$ –PCr system is most widely used, it is not applicable under all conditions. For instance, PCr is not detectable in liver and kidney or under several pathological conditions like ischemia, hypoxia, anoxia and in tumors. Under normal conditions, the  $P_i$  resonance may be very low or is overlapping with other, more intense resonances. The latter can be the case for *in vivo*  $^{31}\text{P}$  NMR spectra of the heart, which are usually contaminated by 2,3-diphosphoglycerate signals from the blood. If the  $P_i$ –PCr system can be used, then the



accuracy of pH determination is typically 0.05 pH units. Following similar arguments as outlined for intracellular pH, the free magnesium concentration can be deduced from the chemical shifts of ATP [255–260].

## 2.4 Carbon-13 NMR Spectroscopy

Phosphorus MRS and proton MRS have successfully been employed in a wide range of *in vivo* NMR studies. Nevertheless, their application faces several limitations. The *in vivo*  $^{31}\text{P}$  MRS signal usually originates from a limited number of low molecular weight compounds, while  $^1\text{H}$  MRS is hindered by the small chemical shift range in which the many detectable compounds resonate.

Carbon-13 NMR can offer complementary information to that obtained with  $^1\text{H}$  and/or  $^{31}\text{P}$  MRS. Since almost all metabolically relevant compounds contain carbon,  $^{13}\text{C}$  MRS is in principle able to detect many metabolites. While carbon-13 NMR is often used in combination with  $^{13}\text{C}$ -labeled substrate infusion, as will be described in Chapter 3, natural abundance  $^{13}\text{C}$  NMR spectra also hold valuable information.

### 2.4.1 Identification of Resonances

Natural abundance  $^{13}\text{C}$  NMR spectroscopy is generally characterized by a large spectral range of  $>200$  ppm, narrow line widths and a relatively low sensitivity, due to the 1.108 % natural abundance and the low gyromagnetic ratio ( $\gamma_{^{13}\text{C}}/\gamma_{^1\text{H}} = 0.251$ ). However, when the low-sensitivity can be overcome (for example by averaging, polarization transfer, larger volumes and decoupling)  $^{13}\text{C}$  MRS allows the detection of a large number of metabolite resonances with excellent spectral resolution (Figure 2.31). Table 2.4 lists the carbon-13 chemical shifts of the most commonly encountered brain metabolites. As a rule of thumb, a  $^{13}\text{C}$  NMR spectrum can be divided into several spectral ranges that are indicative for carbon atoms in particular chemical groups. For *in vivo*  $^{13}\text{C}$  MRS chemical shifts above 150 ppm are typically indicative of carbon atoms in carbonyl groups. Carbon atoms adjacent to hydroxyl groups, like those in Glc, glycogen and mI, typically resonate in the 60–100 ppm range. Carbon atoms in CH,  $\text{CH}_2$  and  $\text{CH}_3$  groups resonate in the 45–60 ppm, 25–45 ppm and  $<25$  ppm chemical shift ranges, respectively.  $^{13}\text{C}$  NMR spectra acquired from organs other than brain are typically dominated by dominant lipid resonances in the 20–50 ppm and  $>120$  ppm ranges. Furthermore, two distinct resonances from the glycerol backbone appear at 63 and 73 ppm. The presence of inadequate spatial localization during  $^{13}\text{C}$  MRS studies of the brain is readily recognized by small, broader resonances with the most intense one typically appearing around 31 ppm. While natural abundance  $^{13}\text{C}$  NMR spectra are useful in many applications, like the identification of unknown compounds, the low sensitivity and the fact that most information can also be obtained from  $^1\text{H}$  NMR spectra, has prevented widespread application of natural abundance  $^{13}\text{C}$  MRS. However, there are two areas where  $^{13}\text{C}$  NMR is unique, namely in the detection of metabolic fluxes from  $^{13}\text{C}$ -labeled precursors (see Chapter 3) and in the detection of glycogen.

Carbohydrate reserves are mainly stored as glycogen in animals and humans. It is particularly abundant in muscle and liver, reaching concentrations up to 30–100 mmol  $\text{kg}^{-1}$  and 100–500 mmol  $\text{kg}^{-1}$ , respectively (see [114] for review). Glycogen is also present in

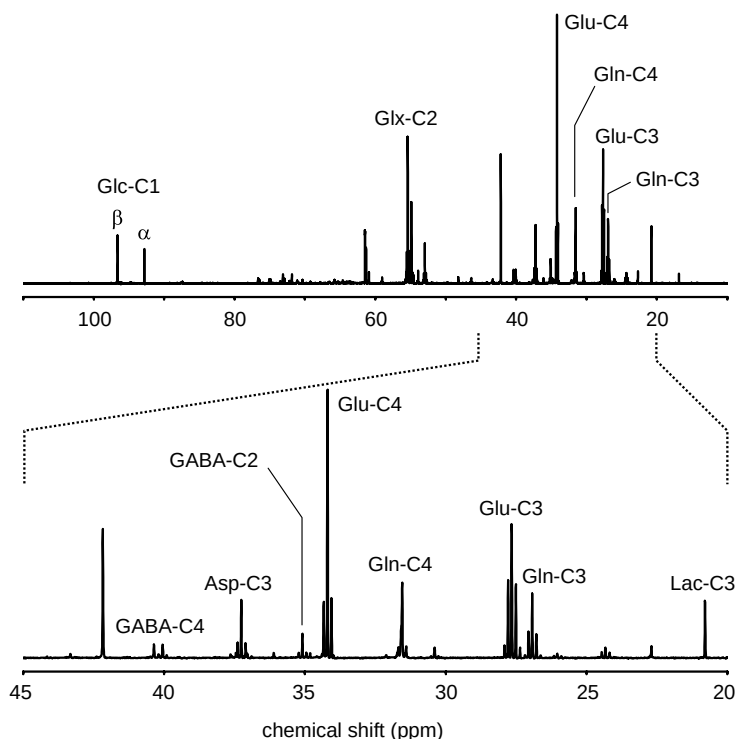


**Figure 2.31** Pulse-acquire  $^{13}\text{C}$  NMR spectrum with heteronuclear decoupling of rat brain extract. The extract was made 2 h following the intravenous infusion of  $[1-^{13}\text{C}]$ glucose and allows the detection of a wide range of  $^{13}\text{C}$ -labeled metabolites. In particular the neurotransmitters glutamate and GABA and the related compound glutamine are readily detected.

the brain, residing in astroglia at a concentration of circa  $5 \text{ mmol kg}^{-1}$  [115–117]. The regulation of glycogen synthesis and breakdown plays an important role in systemic Glc metabolism and is crucial in the understanding of diseases such as diabetes mellitus.

Despite its high molecular weight ( $10^7$ – $10^9$  Da), glycogen gives rise to relatively narrow  $^{13}\text{C}$  NMR resonances both *in vitro* and *in vivo*, indicating a high degree of internal mobility (Figure 2.32). Natural abundance  $^{13}\text{C}$  NMR detection of glycogen is typically done via the glycogen-C1 resonance at 100.5 ppm. Sillerud and Shulman [261] reported in 1983 the surprising result that the  $[1-^{13}\text{C}]$ glycogen resonance at 100.5 ppm is  $\sim 100\%$  visible, a finding which has subsequently been confirmed [122, 262–264], but also challenged [265] by others.

The appearance of  $^{13}\text{C}$  NMR spectra is, besides the wide chemical shift dispersion, dominated by heteronuclear scalar coupling since most carbon nuclei are directly bonded to one, two or three protons. As a result the acquisition of *in vivo*  $^{13}\text{C}$  NMR spectra is, with the exception of recent reports at very high magnetic fields [266], always performed in the presence of heteronuclear broadband decoupling in order to increase the sensitivity and simplify the spectral appearance. Table 2.5 summarizes the heteronuclear scalar couplings

**Table 2.4** Chemical shifts of biologically relevant  $^{13}\text{C}$ -containing metabolites

| Compound                 | Carbon atom |      |       |       |       |       |
|--------------------------|-------------|------|-------|-------|-------|-------|
|                          | C1          | C2   | C3    | C4    | C5    | C6    |
| Acetate                  | 182.6       | 24.5 |       |       |       |       |
| Alanine                  | 176.6       | 51.5 | 17.1  |       |       |       |
| Aspartate                | 175.1       | 53.2 | 37.4  | 178.4 |       |       |
| Bicarbonate              | 161.0       |      |       |       |       |       |
| Citrate                  | 179.7       | 46.8 | 76.0  | 182.3 | 46.8  | 179.7 |
| Creatine                 | 175.4       | 37.8 | 158.0 | 54.7  |       |       |
| GABA                     | 182.3       | 35.2 | 24.6  | 40.2  |       |       |
| Glycerol                 | 63.1        | 72.8 | 63.1  |       |       |       |
| $\beta$ -hydroxybutyrate | 181.2       | 47.6 | 66.8  | 22.9  |       |       |
| Glucose $\alpha$         | 92.7        | 72.1 | 73.5  | 70.4  | 72.1  | 61.4  |
| $\beta$                  | 96.6        | 79.9 | 76.5  | 70.4  | 76.5  | 61.4  |
| Glutamate                | 175.3       | 55.6 | 27.8  | 34.2  | 182.0 |       |
| Glutamine                | 174.8       | 55.1 | 27.1  | 31.7  | 178.5 |       |
| Glycine                  | 173.3       | 42.5 |       |       |       |       |
| Glycogen                 | 100.5       | –    | 74.0  | 78.1  | 72.1  | 61.4  |
| Myo-inositol             | 73.3        | 73.1 | 73.3  | 71.9  | 75.1  | 71.9  |
| Lactate                  | 183.3       | 69.3 | 21.0  |       |       |       |
| Malate                   | 182.1       | 71.7 | 43.9  | 180.9 |       |       |
| NAA                      | 179.7       | 54.0 | 40.3  | 179.7 | 174.3 | 22.8  |
| Succinate                | 183.4       | 35.3 | 35.3  | 183.4 |       |       |
| Taurine                  | 48.4        | 36.2 |       |       |       |       |

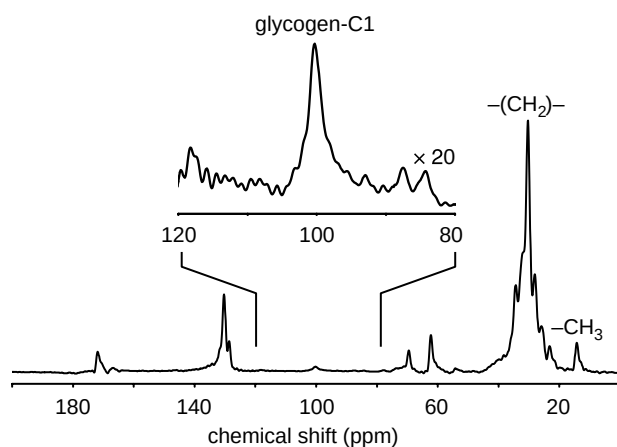


**Figure 2.32** Glycogen detection in human skeletal muscle at 4 T with direct, pulse-acquire  $^{13}\text{C}$  NMR spectroscopy ( $TR = 1000$  ms,  $NA = 3000$ ) in the presence of heteronuclear decoupling. Besides the singlet resonance of glycogen-C1 at 100.5 ppm, the  $^{13}\text{C}$  NMR spectrum is dominated by resonances from lipids. (Courtesy of G. I. Shulman.)

**Table 2.5** Homonuclear  $^{13}\text{C}$ - $^{13}\text{C}$  and heteronuclear  $^1\text{H}$ - $^{13}\text{C}$  scalar coupling constants over one ( $^1J_{\text{CC}}$ ,  $^1J_{\text{HC}}$ ), two ( $^2J_{\text{HC}}$ ) and three ( $^3J_{\text{HC}}$ ) chemical bonds for biologically relevant  $^{13}\text{C}$ -containing metabolites

| Compound         | Interaction  | Scalar coupling constant (Hz) |                   |                   |                   |
|------------------|--------------|-------------------------------|-------------------|-------------------|-------------------|
|                  |              | $^1J_{\text{CC}}$             | $^1J_{\text{HC}}$ | $^2J_{\text{HC}}$ | $^3J_{\text{HC}}$ |
| Acetate          | C2-H         | —                             | 127.1             | —                 | —                 |
| Alanine          | C3-H         | —                             | 129.7             | 4.5               | —                 |
| Aspartate        | C2-H         | —                             | 145.1             | 4.3               | —                 |
|                  | C1-C2        | 53.8                          | —                 | —                 | —                 |
|                  | C2-C3        | 36.4                          | —                 | —                 | —                 |
|                  | C3-C4        | 50.8                          | —                 | —                 | —                 |
|                  | C2-H         | —                             | 144.3             | 3.4               | —                 |
| GABA             | C3-H         | —                             | 129.3             | 4.4               | —                 |
|                  | C2-C3, C3-C4 | 35.1                          | —                 | —                 | —                 |
|                  | C2-H         | —                             | 127.2             | 4.0               | 4.0               |
|                  | C3-H         | —                             | 129.4             | 3.9               | —                 |
|                  | C4-H         | —                             | 143.1             | 5.2               | 5.2               |
| Glucose $\alpha$ | C1-H         | —                             | 169.8             | —                 | —                 |
| Glucose $\beta$  | C1-H         | —                             | 161.3             | —                 | —                 |
| Glutamate        | C1-C2        | 53.4                          | —                 | —                 | —                 |
|                  | C2-C3, C3-C4 | 34.6                          | —                 | —                 | —                 |
|                  | C4-C5        | 51.3                          | —                 | —                 | —                 |
|                  | C2-H         | —                             | 145.2             | 4.2               | 4.1               |
|                  | C3-H         | —                             | 131.4             | 4.3               | —                 |
| Glutamine        | C4-H         | —                             | 126.7             | 4.5               | 4.3               |
|                  | C1-C2        | 53.4                          | —                 | —                 | —                 |
|                  | C2-C3, C3-C4 | 34.9                          | —                 | —                 | —                 |
|                  | C4-C5        | 48.4                          | —                 | —                 | —                 |
|                  | C2-H         | —                             | 145.4             | 4.2               | 3.8               |
| Lactate          | C3-H         | —                             | 131.4             | 4.7               | —                 |
|                  | C4-H         | —                             | 128.3             | 4.5               | 4.5               |
|                  | C2-H         | —                             | 145.6             | 4.4               | —                 |
|                  | C3-H         | —                             | 127.8             | 3.5               | —                 |

for the most commonly observed metabolites, including the neurotransmitters Glu and GABA. Homonuclear  $^{13}\text{C}$ - $^{13}\text{C}$  scalar couplings do not have to be considered in natural abundance  $^{13}\text{C}$  NMR as the probability that two adjacent carbon nuclei are both of the  $^{13}\text{C}$  isotope is less than 0.015 %. However, during the infusion of 99 %  $^{13}\text{C}$ -enriched substrate, the probability of  $^{13}\text{C}$  isotopes in adjacent positions can increase to >20 %. The splitting of resonances due to homonuclear  $^{13}\text{C}$ - $^{13}\text{C}$  scalar coupling gives rise to so-called isotopomer resonances and isotopomer analysis can provide additional information on the underlying metabolic pathways (see also Chapter 3). Table 2.5 summarizes the homonuclear scalar coupling constants for the most commonly observed metabolites.

## 2.5 Sodium-23 and Potassium-39 NMR Spectroscopy

Sodium ( $^{23}\text{Na}$ ) and potassium ( $^{39}\text{K}$ ) are inhomogeneously distributed in cerebral tissues. Normal cells maintain a concentration gradient across the cell membrane of intracellular

The NMR detection of intracellular sodium is complicated by the small chemical shift dispersion of sodium-23 and the large overlapping signal from extracellular sodium. *In vivo*  $^{23}\text{Na}$  and  $^{39}\text{K}$  NMR spectra are further characterized by a partial NMR visibility of the resonances observed [258, 267–271]. As discussed in Chapter 1, a nucleus with  $I \geq 1$  has more than one allowed single quantum NMR transition. Consider the energy level (or Zeeman) diagram of spin  $I = 3/2$  shown in Figure 2.33. It can be seen that three single quantum transitions, i.e.  $\Delta m = \pm 1$ , are possible (i.e.  $3/2 \rightarrow 1/2$ ,  $1/2 \rightarrow -1/2$ , and  $-1/2 \rightarrow -3/2$ ). Time-dependent quantum mechanical perturbation theory shows that the three transitions

have relative intensities of 3:4:3. When the electric field (arising from the quadrupole moment) sensed by the nucleus is spatially homogeneous, the three transitions have the same resonance frequency and the compound would be 100 % NMR visible. However, static and/or dynamic quadrupolar effects can make the two outer transitions ( $3/2 \rightarrow 1/2$  and  $-1/2 \rightarrow -3/2$ ) disappear from the spectrum, resulting in a NMR visibility of only 40 %.

Static quadrupolar effects cause a static electrical field gradient at the nucleus, shifting the resonance frequencies of the two outer transitions away from that of the inner transition. The frequency of the inner transition is, to first order, insensitive to a static field gradient. The extent of splitting depends on the strength of the field gradient and its orientation with respect to the main magnetic field. Depending on the properties of the system (i.e. crystalline, liquid crystalline, isotropic solution) the splitting can range between kHz and MHz. In biological tissues (which can usually be regarded as macroscopically unoriented), the frequencies of the outer transitions from different nuclei will be dispersed over the entire spectral range, making them in practice NMR invisible. This is referred to as heterogeneous broadening.

Dynamic quadrupolar effects arise when time-dependent fluctuations of electrical fields are present at the nucleus. Even if the quadrupolar splitting averages to zero over the fluctuations, the frequencies of the fluctuations can be in the correct range to induce relaxation of the resonances. When the transverse relaxation rate of the outer transitions is faster than that of the inner transition, the resonances arising from the outer transitions will be broader. This is referred to as homogeneous broadening. In biological samples, static and dynamic quadrupole effects are likely to occur simultaneously. Therefore, the line width for resonances arising from the outer transitions are too broad to be detectable, leading to a 40 % NMR visibility as was also the case for static quadrupolar effects.

There are essentially two methods by which the intra- and extracellular contributions to the single  $^{23}\text{Na}$  (and  $^{39}\text{K}$ ) resonance can be separated: (1) the use of so-called shift reagents [272–277]; and (2) the utilization of relaxation-allowed multiple quantum coherence filtering [278–283]. With the first method, a shift reagent is added to the perfusion medium in the case of perfused heart studies. Shift reagents are in general anionic chelate complexes of paramagnetic lanthanides ( $\text{Dy}^{3+}$ ,  $\text{Tm}^{3+}$  and  $\text{Tb}^{3+}$ ). Popular shift reagents are dysprosium bis(tripolyphosphate),  $\text{Dy}(\text{PPP})_2^{7-}$ , which induces very large shifts but can be toxic after decomposition, and dysprosium triethylene tetraamine hexaacetate,  $\text{DyTTTHA}^{3-}$ , which induces smaller shifts but is much less poisonous. Binding of  $\text{Na}^+$  to the shift reagent causes a large shift in resonance frequency. Since the exchange between the free and complexed form of the cation is very fast on the NMR timescale, one average chemical shift will be observed. Being a chemical equilibrium, the chemical shift can be affected by the absolute and relative concentrations of the shift reagent and cation, the temperature, pH, the presence of other cations and ionic strength. Because all shift reagents are of an anionic character they can not cross the cellular membrane. Therefore the chemical shift of the intracellular cations (e.g. sodium) is unaffected. When present in sufficient concentration, a shift reagent can completely separate the intra- and extracellular sodium resonances (Figure 2.34). Shift reagents are mainly used on cell systems and perfused organs, partly due to their toxicity at the required concentrations.

A noninvasive alternative for shift reagents is to utilize differences in rotational motion of intra- and extracellular sodium for multiple quantum coherence filtering. The

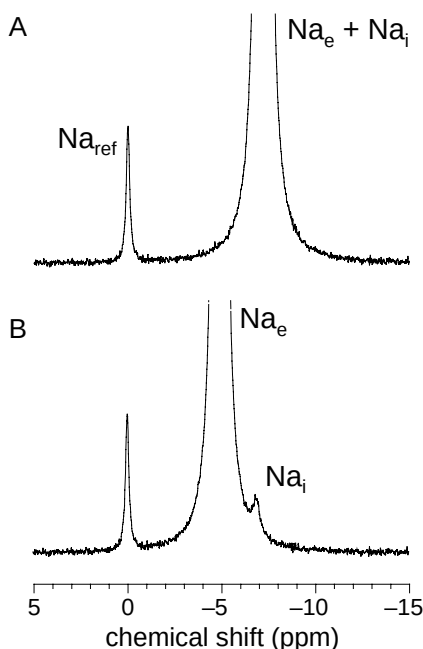


**Figure 2.34** Effect of the addition of a shift reagent on the chemical shift position of sodium resonances in a Langendorff perfused heart. (A) Without shift reagent the intra- and extracellular sodium resonances,  $\text{Na}_i$  and  $\text{Na}_e$ , respectively, completely overlap. (B) In the presence of 3.5 mM  $\text{TmDOTP}^{5-}$  shift reagent, the  $\text{Na}_e$  resonance shifts to a higher frequency such that the  $\text{Na}_i$  becomes visible.  $\text{Na}_{\text{ref}}$  is a reference compound (250 mM  $\text{Na}^+$  in solution with 5 mM  $\text{TmDOTP}^{5-}$  shift reagent) used for signal quantification. (Courtesy of J. van Emous and C. J. A. van Echteld.)

principles underlying this discrimination rely on the fact that bi-exponential relaxation of spins with  $I \geq 1$  induces violations of the coherence transfer selection rules (e.g.  $\Delta m = \pm 1$ ). This has been described and analyzed by representing the evolution of coherence in terms of spherical tensor operators. In cases when the extreme narrowing condition is fulfilled (see Chapter 3, i.e.  $\omega_0 \tau_c \ll 1$ , where  $\omega_0$  is the Larmor frequency and  $\tau_c$  is the rotation correlation time), the outer two transitions relax in an identical manner to the inner transition, leading to a single-exponential decay. When the extreme narrowing condition is not fulfilled (e.g. for motionally restricted nuclei), bi-exponential relaxation will be observed as described for dynamic quadrupole effects. Therefore, by utilizing a multiple quantum filter (i.e. a pulse sequence which preserves coherence of a particular order and eliminates all other coherence orders by phase cycling and/or magnetic field gradients, see Chapter 8 for more details), it is possible to discriminate sodium on the basis of its correlation time (and related bi-exponential relaxation). Under the assumption that extracellular sodium (free and mobile) satisfies the extreme narrowing condition and intracellular sodium (being partly bound) does not, a double quantum filter should be able to separate intra- and extracellular sodium. The method of relaxation-allowed multiple

quantum filtering is completely noninvasive and therefore very suitable for repeated *in vivo* measurements. The main problem of the technique is that if the extracellular sodium does not completely satisfy the extreme narrowing condition (e.g. when it interacts with binding sites on the cell surface), the intracellular sodium resonance is rapidly overwhelmed by the extracellular sodium [283]. This can be alleviated to a large extent by selectively quenching the extracellular double-quantum sodium with a relaxation agent (e.g. a Gd-chelate) at a low, nonperturbing and nontoxic concentration [281, 282]. Since the reagent is exclusively localized in the extracellular space, the intracellular sodium remains unaffected. At present the use of relaxation agents has not found widespread applications in *in vivo* cation NMR.

## 2.6 Fluorine-19 NMR Spectroscopy

Proton, phosphorus-31 and to a lesser degree carbon-13 nuclei are most frequently used for *in vivo* NMR spectroscopy. This is not surprising, considering the wide range of metabolites that can be measured with these nuclei. One of the most sensitive nuclei, fluorine-19, has received much less attention. Fluorine-19 (spin  $I = 1/2$ ) is 100 % naturally abundant and has a NMR sensitivity of 83 % relative to protons. The chemical shift range is more than four times that of phosphorus, while line widths are comparable. Since there are no endogenous fluorine-19 containing compounds in biological tissues,  $^{19}\text{F}$  MRS is not hampered by interference from background signals (such as water in *in vivo*  $^1\text{H}$  MRS).

$^{19}\text{F}$  MRS can be used *in vivo* to monitor the uptake and metabolism of drugs [284–289], to determine cerebral blood flow [290–293], to study the metabolism of anaesthetics [294–298], and with the aid of specific probe molecules, to measure a variety of parameters such as pH, oxygen levels and metal ion concentration [299–305]. In the next few paragraphs, a short description of the potential of  $^{19}\text{F}$  MRS will be given. More detailed reviews on applications of *in vivo*  $^{19}\text{F}$  MRS can be found in the literature [306–308].

### 2.6.1 Identification of Resonances

Fluorine-19 has a very large chemical shift range, stretching out over 300 ppm for organic compounds. The chemical shifts of fluorine-containing compounds relevant for *in vivo* NMR spectroscopy are listed in Table 2.6. Trifluoroacetate is used as a chemical shift reference ( $\delta = 0.00$ ), but  $^{19}\text{F}$  NMR does not have a single, generally accepted reference compound. For *in vivo* applications, the reference is inevitably an external reference compound (since no endogenous fluorine compounds exist) with the associated problems of magnetic susceptibility differences between sample and reference. This can lead to errors of several ppm, making *in vivo* assignments of resonances difficult. Although the total chemical shift range spans more than 300 ppm, spectral overlap is a significant problem as many relevant compounds have almost identical chemical environments and hence almost the same chemical shift. Furthermore, many fluorinated compounds also contain protons which lead to heteronuclear scalar couplings and a further complication of  $^{19}\text{F}$  NMR spectra. It has been shown that the *in vivo* spectral resolution can be greatly improved by proton-decoupling [309].



**Table 2.6** Chemical shifts of biologically relevant  $^{19}\text{F}$ -containing metabolites

| Compound                                        | Chemical shift (ppm) |
|-------------------------------------------------|----------------------|
| Halothane                                       | −2.2 to +1.7         |
| Enflurane $\text{CF}_2$                         | −11.2 to −9.4        |
| CHClF                                           | −81.1 to −80.2       |
| Isoflurane $\text{CF}_3$                        | −3.4                 |
| $\text{CHF}_2$                                  | −9.7                 |
| 5-Deoxy-5-fluorouridine                         | −90.4 to −88.4       |
| 5-Fluorouracil                                  | −93.9                |
| 5-Fluorouridine                                 | −90.0                |
| Fluorocytidine                                  | −90.0                |
| 5-Fluorocytosine                                | −92.0                |
| 2-Fluoro-2-deoxyglucose $\alpha$                | −123.8               |
| $\beta$                                         | −123.7               |
| 2-Fluoro-2-deoxyglucose-6-phosphate $\alpha$    | −123.9               |
| $\beta$                                         | −123.8               |
| 2-Fluoro-2-deoxy-6-phosphogluconate             | −120.6               |
| 2-Fluoro-2-deoxy-D-SORBITAL                     | −125.0               |
| 5-Fluoro-5-deoxy-L-SORBOSE $\alpha$             | −123.4               |
| 5-Fluoro-5-deoxy-L-SORBOSE-1-PHOSPHATE $\alpha$ | −122.9               |
| 2-Fluoro-2-deoxy-L-GLYCERALDEHYDE               | −127.5               |
| 2-Fluoro-2-deoxyglycerol                        | −128.9               |
| Fluorodeoxymannose $\alpha$                     | −129.1               |
| $\beta$                                         | −147.5               |
| 3-Fluoro-2-deoxyglucose $\alpha$                | −124.5               |
| $\beta$                                         | −119.5               |
| 3-Fluoro-3-deoxy-D-SORBITAL                     | −137.7               |
| 3-Fluoro-3-deoxy-D-FRUCTOSE                     | −133.1               |
| Fluoro- $\beta$ -alanine                        | −113.0               |
| Fluorocitrate                                   | −116.0               |
| Fluorouridopropionic acid                       | −111.0               |
| Fluoroacetate                                   | −142.0               |

<sup>a</sup> All chemical shifts are referenced relative to trifluoroacetate at 0.00 ppm.

## 2.6.2 Fluorinated Drugs, Anaesthetics, and Fluorodeoxyglucose Metabolism

Nucleoside analogs are effective chemotherapeutic agents in tumor treatment, in particular the fluoropyrimidine anticancer agents, 5-fluorouracil (5-FU) and 5'-deoxy-5-fluorouridine (5'dFUrd). Both drugs (where 5'dFUrd is first converted to 5-FU) are metabolized in tumors to the active compound, fluorodeoxyuridine monophosphate (FdUMP) which inhibits DNA replication. A second cytostatic effect may be caused by the extensive incorporation of 5-FU into RNA, thereby altering the structure and function of some cellular RNAs. The relative importance of these effects to the anticancer properties of fluoropyrimidines is not known.  $^{19}\text{F}$  NMR is uniquely suited to monitor the metabolism of fluoropyrimidines *in vivo* [284–287]. The detection of their activation products may be useful for the design of drugs while the *in vivo* estimation of drug concentrations may be used to achieve optimal activation or decrease unwanted side-effects.

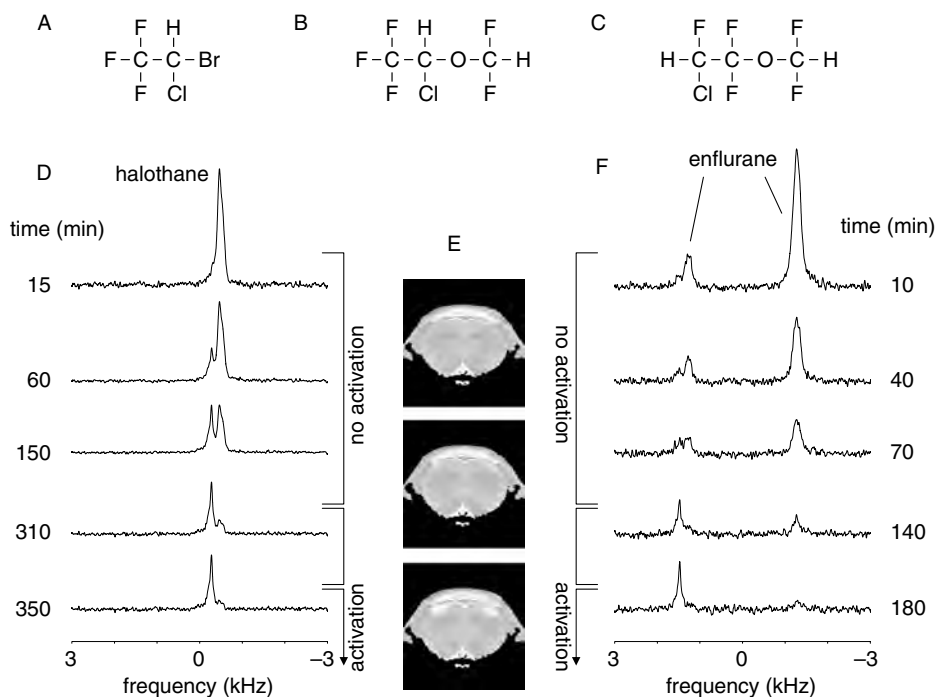


**Figure 2.35** Chemical structures of commonly used fluorinated anaesthetics, namely (A) halothane, (B) isoflurane and (C) enflurane. (D) Halothane and (F) enflurane elimination from rat brain following the administration of 1 % of the corresponding anaesthetic for 2 h. Note that both anaesthetics break down over time, presumably into trifluoroacetate and difluoromethoxy-2-difluoroacetic acid for halothane and enflurane, respectively. Further note that the washout of enflurane is considerably faster than for halothane. (E) BOLD fMRI activation maps (yellow) overlaying an anatomical MR image through the somatosensory cortex during double forepaw stimulation. The appearance of BOLD activation is correlated with the washout of the fluorinated anaesthetics. (See color plate 1).

Several inhalation anaesthetics which are commonly used in experimental and clinical settings are fluorinated and could, in principle, be monitored by  $^{19}\text{F}$  MRS [294–298, 310, 311]. These include halothane, isoflurane and enflurane of which the chemical structures are shown in Figure 2.35A–C. As an example, Figure 2.35D and F shows the clearance of halothane and enflurane from rat brain as measured by unlocalized  $^{19}\text{F}$  NMR spectroscopy (the inhalation anaesthetics are replaced by intravenous infusion of  $\alpha$ -chloralose at time  $t = 0$ ). The washout of halothane ( $t_{1/2} = 123$  min) is considerably slower than for enflurane ( $t_{1/2} = 42$  min), whereby both anaesthetics show significant metabolism. The metabolites for halothane and enflurane include trifluoroacetate and 2,2-difluoromethoxy-2-difluoroacetic acid/fluoride ions, respectively and can be present for several days.

Many functional MRI studies on animals start with a preparation period under halothane or enflurane anaesthesia during which surgery can be performed. However, leading back to the 2-deoxyglucose autoradiography experiments of Ueki *et al.* [312], it was shown

that halothane did not preserve the neuronal-hemodynamic coupling leading to a very small functional response during somatosensory stimulation.  $\alpha$ -Chloralose on the other hand showed robust and large functional responses. Because of this result [312], which was confirmed by others [313], the standard protocol for fMRI experiments on animals involves a discontinuation of the gaseous anaesthesia following the preparation period and a switch to  $\alpha$ -chloralose infusion. However, while only sporadically or anecdotally reported (e.g. [314]), it is well known that strong and robust fMRI activation is not obtained until several hours following the switch to  $\alpha$ -chloralose. Figure 2.35E shows BOLD fMRI images of double forepaw stimulation in relation to the time of halothane or enflurane discontinuation. No significant BOLD signal is obtained during the first 4 and 1.5 h following the discontinuation of halothane and enflurane, respectively. The BOLD fMRI signal becomes robust and reliable after circa 6 and 2.5 h for halothane and enflurane, respectively. These time points correspond roughly with the complete washout of both gaseous anaesthetics from the brain. While the results in Figure 2.35 are not conclusive and require further investigations, they do show the utility of  $^{19}\text{F}$  NMR spectroscopy to study the dynamics and metabolism of fluorinated anaesthetics *in vivo*.

In order to assess brain metabolic activity, positron emission tomography (PET) is often used to detect the uptake of radioactive  $^{18}\text{F}$ -labeled 2-fluoro-2-deoxy-glucose (FDG) [315]. In the brain FDG is phosphorylated by the enzyme hexokinase to 2-fluoro-2-deoxy-glucose-6-phosphate (FDG-6P) in an analogous manner to the metabolism of glucose. Unlike glucose-6-phosphate, FDG-6P is assumed to be metabolically inert. While PET has excellent sensitivity and good spatial resolution when compared with MRS, it is not able to distinguish metabolites from the original compound since the technique relies on the nonspecific detection of radioactivity. Several groups have used the  $^{19}\text{F}$  analog of FDG to study brain metabolism with  $^{19}\text{F}$  NMR which, through the different chemical shifts of the metabolites, does yield specific information [316–320]. *In vivo*  $^{19}\text{F}$  MRS studies have revealed that FDG-6P is not completely metabolically stable, but is partly converted to several other metabolites including 2-fluoro-2-deoxyglycerol and 2-fluoro-2-deoxy-D-sorbitol via the aldose reductase sorbitol pathway.

### 2.6.3 Fluorinated Probes

There are a number of fluorinated compounds ('probes') whose chemical shift is sensitive to physiological variables such as pH, oxygen tension and the free concentration of intracellular metal ions. Some probes can be made to target specific locations, for instance to discriminate between normal and tumor tissue.

Just as with  $^{31}\text{P}$  MRS, an adequate pH indicator must have an ionizable group with a pK value close to the pH to be measured and the ionizable group should be in the vicinity of the  $^{19}\text{F}$  nucleus, such that the chemical shift will change upon protonation/deprotonation. Furthermore, the compound should be of very low toxicity, so that sufficient quantities can be loaded into the cell for sufficient signal-to-noise. This is usually achieved by esterification of the compound to increase its membrane solubility whereby it will spontaneously cross cell membranes. Once inside the cell, esterases hydrolyze the ester bond trapping the membrane-impermeable pH indicator in the intracellular compartment.

Several compounds have specifically been designed for use in  $^{19}\text{F}$  MRS. Judged on their pK and sensitivity, which is expressed as the change in chemical shift following a pH change of one pH unit away from the pK, F-anil and Fquene [301, 304] give the most

accurate pH values. Although at this time only used on perfused organs and cell systems, fluorinated and nontoxic pH indicators may be of great value *in vivo*, especially when other NMR methods are not an option.

A major advance in the measurement of intracellular metal ion concentration was made by the synthesis of  $^{19}\text{F}$ -labeled probes for intracellular calcium ( $\text{Ca}^{2+}$ ) concentration by Smith *et al.* [299]. The synthesized probes are fluorinated analogs of BAPTA [1,2-bis-(*o*-aminophenoxy)ethane-*N, N, N', N'*-tetraacetic acid], in which the chemical shift of the fluorine nuclei is sensitive to the binding of cations. The exchange of cations from certain fluorinated BAPTA analogs is slow relative to the Larmor frequency, resulting in the presence of two  $^{19}\text{F}$  resonances, one corresponding to the cation-complexed chelator and the other to the uncomplexed chelator. The determination of intracellular calcium concentration is obtained from the relative peak areas and a predetermined titration curve. The presence of other metal ions such as zinc, magnesium and iron potentially interferes with an accurate determination of the calcium concentration. However, because the different metal-chelate complexes have different chemical shifts, they can be identified in the NMR spectrum and be accounted for in the calculations.

As with fluorinated pH probes, fluorinated BAPTA is introduced as a membrane-permeable ester. Free, membrane-impermeable, fluorinated BAPTA is formed by the hydrolysis of the ester bond. Fluorinated BAPTA has been used to measure calcium levels in perfused hearts [321, 322] and brain slices [303, 323]. The main problem with this type of intracellular calcium probes is that they have to chelate a significant portion of the free calcium ion pool. This challenges the physiological condition of the organ under investigation.

The fluorinated probes are not limited to the detection of calcium. Specific probes have been developed for the determination of sodium [302] and magnesium [305] concentrations. Furthermore, some fluorinated probes are sensitive to oxygen [300, 324]. Especially, the spin-lattice  $T_1$  relaxation time of perfluorohydrocarbons is dependent on the binding of oxygen, which allows the estimation of oxygen levels. Until this point, the fluorinated probes have only been used for  $^{19}\text{F}$  MRS studies on animals or perfused organs. These experiments have demonstrated the potential of  $^{19}\text{F}$  MRS to measure important parameters *in vivo* and it is to be expected that  $^{19}\text{F}$  MRS will find increased applications in both experimental and clinical studies.

## 2.7 Exercises

- 2.1** In a proton spectrum acquired from rat brain at 7.05 T, the water resonance appears on-resonance while the NAA methyl resonance appears –801 Hz off-resonance. On a phantom the relation between the temperature  $T$  (in Kelvin) and the chemical shift difference (in ppm) between water and NAA,  $\delta_{\text{water-NAA}}$ , was established as:

$$T = -95.24\delta_{\text{water-NAA}} + 564.15$$

- A** Calculate the brain temperature (in Kelvin) using the phantom calibration data.  
**B** Following a period of ischemia a proton spectrum is acquired in which the water appears +11 Hz off-resonance. The NAA methyl resonance now appears at –796 Hz off-resonance.  
 Calculate the brain temperature (in Kelvin).

- 2.2 The proton NMR spectrum from rat brain shows an unknown resonance at 3.40 ppm. Name at least five methods by which a full or partial assignment can be made.
- 2.3 Choline, phosphorylcholine and glycerophosphoryl choline all predominantly resonate at 3.22 ppm. Name at least two methods by which the three compounds can be detected separately.
- 2.4 The chemical shift difference between  $P_i$  and PCr equals 4.62 ppm. Calculate the intracellular pH with the parameters used in Figure 2.30.
- 2.5 **A** Using the chemical shift and scalar coupling information provided in Tables 2.4 and 2.5 and the structure shown in Figure 2.7, sketch the high-field ( $B_0 = 11.75$  T) pulse-acquire  $^{13}\text{C}$  NMR spectrum of a mixture of 0.4 mM  $[2-^{13}\text{C}]$ aspartate, 0.6 mM  $[3-^{13}\text{C}]$ aspartate and 0.6 mM  $[2,3-^{13}\text{C}_2]$ aspartate in the presence of heteronuclear broadband decoupling during acquisition. Assume equal  $T_1$  and  $T_2$  relaxation parameters for all resonances and ignore the contributions of natural abundance  $^{13}\text{C}$  NMR resonances. Further assume weak scalar-coupling for all resonances and ignore heteronuclear scalar coupling over more than 1 chemical bond.  
**B** Sketch the  $^{13}\text{C}$  NMR spectrum in the *absence* of heteronuclear decoupling.  
**C** Sketch the  $^1\text{H}$  NMR spectra in the presence and absence of heteronuclear decoupling. Assume weak scalar coupling for all resonances and ignore heteronuclear scalar coupling over more than one chemical bond.
- 2.6 The macromolecular resonances at 9.4 T are characterized by  $T_1$  relaxation time constants in the range of 300–500 ms. Selective observation of macromolecular resonances can be achieved with a double inversion recovery method ( $90^\circ\text{--TR--}180^\circ\text{--TI1--}180^\circ\text{--TI2--observe}$ ) with  $\text{TR} = 3250$  ms,  $\text{TI1} = 2100$  ms and  $\text{TI2} = 630$  ms. Calculate the signal recovery for metabolites with  $T_1 = 1250$ , 1500 and 1750 ms and for macromolecules with  $T_1 = 300$ , 400 and 500 ms. Comment on the results regarding macromolecule detection.
- 2.7 **A** In a proton spectrum from human brain the tCho/tCr ratio, as determined by numerical integration, is 2.0 when measured at  $\text{TE} = 10$  ms, while it is 1.5 when measured at  $\text{TE} = 75$  ms. Given that the  $T_2$  relaxation time for tCho and tCr are 200 and 100 ms, respectively, give a possible explanation for the observed ratios.  
**B** Discuss the required steps to obtain identical tCho/tCr ratios at all echo times.
- 2.8 Draw the theoretical, high-resolution, pulse-acquire  $^{19}\text{F}$  NMR spectra of halothane, isoflurane and enflurane obtained with and without proton decoupling. Assume weak coupling for all interactions,  $^2J_{\text{HF}} = 40$  Hz,  $^3J_{\text{HF}} = 8$  Hz,  $^3J_{\text{FF}} = 25$  Hz and magnetically equivalent nuclei within methyl and methylene groups. Only consider protons and fluorine atoms.
- 2.9 Sketch all nine stereoisomers of inositol and indicate the magnetically equivalent nuclei.
- 2.10 **A** A given glycogen pool is characterized by a  $T_2$  relaxation time constant of 10 ms. When the residual magnetic field inhomogeneity is 5 Hz and the observed glycogen peak can be described by a pure Lorentzian lineshape, calculate the integration boundaries for which the resulting integral represents 90 and 95 % of the glycogen resonance. Comment on the result regarding glycogen quantification.  
**B** Calculate the integral as a percentage of the maximum integral when the integration boundaries are limited to  $\pm 2\text{FWHM}$ .
- 2.11 Name at least five factors that can affect the chemical shift of a metabolite.

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# 3

## *In Vivo* NMR Spectroscopy – Dynamic Aspects

### 3.1 Introduction

The chemical specificity of NMR spectroscopy, observed through the chemical shift and scalar coupling patterns, allows the detection of more than 15 metabolites *in vivo* and more than 50 chemical compounds in bodily fluids *in vitro* [1–3]. The detection and quantification of a wide range of metabolites has led to the characterization of disease progression, allows the study of intervention by medication or surgery and allows identification or categorization of diseases by observing specific metabolic markers. For example, using pattern recognition algorithms on  $^1\text{H}$  NMR spectra, a wide range of tumor types can be reliably identified [4]. In epilepsy research, *N*-acetyl aspartate has been identified as an important marker for the detection of the foci of epileptic seizures [5], while the cerebral levels of GABA and homocarnosine have shown to be correlated with the frequency of epileptic seizures [6].

However, despite its great importance, detection of static metabolite concentrations alone provides only a partial description of metabolism. *In vivo* metabolism is largely characterized by dynamic processes, like enzyme-catalyzed chemical exchange, transfer of chemical groups through entire metabolic pathways and, specific for NMR, relaxation processes. This chapter is dedicated to the description of dynamic processes *in vivo* that can be measured by NMR spectroscopy. The dynamics of  $T_1$  and  $T_2$  relaxation are discussed in Section 3.2. Using appropriate experimental techniques, NMR can be sensitized to a wide variety of dynamic processes, of which chemical exchange and diffusion are discussed in Sections 3.3 and 3.4, respectively. When combined with the infusion of exogenous compounds, NMR allows the detection of metabolic fluxes noninvasively *in vivo*, and will be the subject of Section 3.5. A promising new field of NMR focuses on the use of hyperpolarized compounds of which the principles are discussed in Section 3.6.

## 3.2 Relaxation

### 3.2.1 General Principles of Dipolar Relaxation

So far relaxation has been described as the process by which the spins return to the Boltzmann equilibrium state following a perturbation. The restoration of the longitudinal equilibrium magnetization is characterized by the longitudinal or spin-lattice relaxation time constant  $T_1$ , while the disappearance of transverse magnetization is described by the transverse or spin-spin relaxation time constant  $T_2$ . In this section the nature of the processes responsible for relaxation will be considered.

It was shown that the relative populations of the nuclear spin states can be altered by application of an oscillating RF field applied in the transverse plane. A more general formulation is that any fluctuating magnetic field oscillating near the Larmor frequency will induce transitions between the spin states. Fluctuating magnetic fields originating from the environment (i.e. lattice) will exchange energy with the spins until thermal equilibrium has been established, the process called relaxation. For spins in solution, the surroundings indeed provide randomly fluctuating magnetic fields. Particularly, dipole–dipole interactions between spins as shown in Figure 3.1 are the main source of fluctuating magnetic fields. The magnetic moment of one spin affects the local field at another spin position

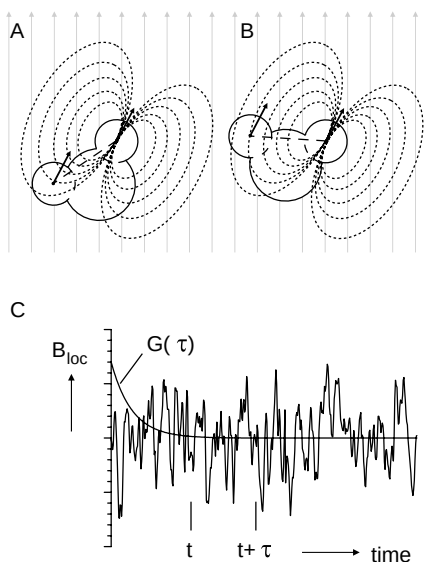


**Figure 3.1** Dipole–dipole interactions for water. (A) The magnetic moment of one proton in an external magnetic field  $B_0$  (parallel gray lines) perturbs the magnetic field at the other proton within the same water molecule. (B) As the water rotates and translates randomly, the direction and magnitude of the dipole–dipole interaction changes, giving rise to the time-varying magnetic fields required for relaxation. (C) An example of the amplitude of the time-varying magnetic field at a particular nucleus. The correlation function  $G(\tau)$  is a measure of the correlation of the magnetic field at two time points. When  $\tau$  is large, no correlation exists and  $G(\tau)$  equals zero.

in a random manner (both in amplitude and orientation), due to Brownian motion and molecular tumbling. When these fluctuating fields exhibit frequency components close to the Larmor frequency they will induce relaxation. Figure 3.1C shows a typical (random) distribution of the local field at a nucleus as a function of time. To describe relaxation, the frequency components in the local field as shown in Figure 3.1C need to be known. If the behavior of the fluctuating magnetic field can be described in an analytical form, the frequency components can easily be extracted by Fourier analysis. For this purpose the correlation function  $G(\tau)$  is introduced which is defined as:

$$G(\tau) = \overline{B_{\text{loc}}(t)B_{\text{loc}}(t + \tau)} \quad (3.1)$$

where the bar indicates that the ‘ensemble average’ (i.e. an average over all spins in a macroscopic part of the sample) needs to be taken. The correlation function  $G(\tau)$ , which is independent of  $t$ , is a measure for the correlation between the local magnetic fields as time  $t$  progresses. For a short delay  $\tau$ , the orientation and amplitude of the local magnetic field will not have changed much and the ensemble average would be large (i.e. high correlation). After longer  $\tau$  delays the local magnetic field is drastically altered due to Brownian motion and molecular tumbling, leading to a low correlation. Clearly, the correlation function  $G(\tau)$  is a decaying function and is usually taken as a decaying exponential, i.e.:

$$G(\tau) = \overline{B_{\text{loc}}^2} e^{-|\tau|/\tau_c} \quad (3.2)$$

where  $\tau_c$  is the (rotation) correlation time. For random molecular tumbling,  $\tau_c$  roughly corresponds to the average time for a molecule to rotate over 1 rad. Note that for mobile spins in solution  $\tau_c$  is short ( $10^{-12}$ – $10^{-10}$  s), while for immobile spins (in solids)  $\tau_c$  is longer ( $10^{-8}$ – $10^{-6}$  s).

Now the frequency components in the analytical time-domain correlation function can be analyzed by Fourier analysis according to:

$$J(\omega) = \int_{-\infty}^{+\infty} G(\tau) e^{-i\omega\tau} d\tau \quad (3.3)$$

Substitution of Equation (3.2) for  $G(\tau)$ , followed by integration gives a Lorentzian form for the spectral density function  $J(\omega)$ :

$$J(\omega) = 2\overline{B_{\text{loc}}^2} \frac{\tau_c}{1 + \omega^2\tau_c^2} \quad (3.4)$$

Spectral density functions are fundamental to the theoretical description of relaxation [7]. They allow the motional characteristics (described by  $\tau_c$ ) to be expressed in terms of the power at frequency  $\omega$ . The factor 2 in Equation (3.4) arises from the fact that a FID can only exist for times greater than zero, whereas the correlation function extends symmetrically on either side of  $\tau = 0$ . Figure 3.2 shows the spectral density function for three cases, i.e. very fast rotational motion,  $\omega^2\tau_c^2 \ll 1$ , intermediate rotational motion,  $\omega^2\tau_c^2 \approx 1$  and slow rotational motion,  $\omega^2\tau_c^2 \gg 1$ . As expected, slow rotational motions emphasize the low frequency components, while fast rotational motion also has significant power at high frequencies.

The remaining point in understanding (dipole–dipole) relaxation is to establish relations between the relaxation time constants  $T_1$  and  $T_2$  and the frequency components given by



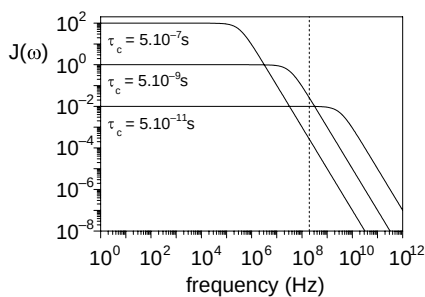


**Figure 3.2** Spectral density profiles as a function of the frequency for three correlation times  $\tau_c$ , i.e.  $5 \times 10^{-7}$ ,  $5 \times 10^{-9}$  and  $5 \times 10^{-11}$  s, corresponding to slow (macromolecular), intermediate and fast (free water) motion. The double-exponential axes disguise the Lorentzian shape of the spectral density functions. Note that the total area under all three curves is identical.

the spectral density function. For this purpose assume two spins I and S which are not scalar coupled, but do have a dipole–dipole coupling, such that dipole–dipole relaxation (which is normally the dominant relaxation pathway for spin 1/2 nuclei in solution) is feasible. The energy level diagram for these two spins is shown in Figure 3.3, together with the transition probabilities  $W_I$ ,  $W_S$ ,  $W_0$  and  $W_2$  for a spin to switch energy levels. For instance  $W_2$  gives the probability that both spins are involved in the transition  $\alpha \rightarrow \beta$  (or reverse), which gives rise to longitudinal relaxation. At some point in time, the energy levels are occupied by a certain number of spins, given by  $N_{\alpha\alpha}$ ,  $N_{\alpha\beta}$ ,  $N_{\beta\alpha}$ , and  $N_{\beta\beta}$ . Relaxation processes can be described by the rate at which the occupancy of the energy levels changes with time [8], i.e.:

$$\frac{dN_{\alpha\alpha}}{dt} = -(W_I + W_S + W_2)N_{\alpha\alpha} + W_I N_{\beta\alpha} + W_S N_{\alpha\beta} + W_2 N_{\beta\beta} \quad (3.5a)$$

$$\frac{dN_{\alpha\beta}}{dt} = -(W_0 + W_I + W_S)N_{\alpha\beta} + W_0 N_{\beta\alpha} + W_I N_{\beta\beta} + W_S N_{\alpha\alpha} \quad (3.5b)$$

$$\frac{dN_{\beta\alpha}}{dt} = -(W_0 + W_I + W_S)N_{\beta\alpha} + W_0 N_{\alpha\beta} + W_I N_{\alpha\alpha} + W_S N_{\beta\beta} \quad (3.5c)$$

$$\frac{dN_{\beta\beta}}{dt} = -(W_I + W_S + W_2)N_{\beta\beta} + W_I N_{\alpha\beta} + W_S N_{\beta\alpha} + W_2 N_{\alpha\alpha} \quad (3.5d)$$

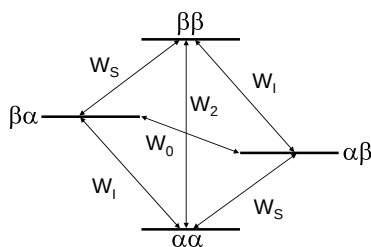


**Figure 3.3** Energy level diagram for a dipolar coupled two spin system.  $W_I$  and  $W_S$  represent the transition probabilities that spin I or S changes energy level, respectively.  $W_0$  and  $W_2$  correspond to the probability of a zero or double quantum transition.

The longitudinal magnetization for each spin is proportional to the difference in population across the relevant transitions and is given by:

$$I_z \propto (N_{\beta\beta} - N_{\alpha\beta}) + (N_{\beta\alpha} - N_{\alpha\alpha}) \quad (3.6)$$

$$S_z \propto (N_{\beta\beta} - N_{\beta\alpha}) + (N_{\alpha\beta} - N_{\alpha\alpha}) \quad (3.7)$$

Combining Equation (3.5a–d) and Equations (3.6) and (3.7) will give the time dependency of the longitudinal magnetization:

$$\frac{dI_z}{dt} = -(W_0 + 2W_I + W_2)(I_z - I_0) - (W_2 - W_0)(S_z - S_0) \quad (3.8)$$

$$\frac{dS_z}{dt} = -(W_0 + 2W_S + W_2)(S_z - S_0) - (W_2 - W_0)(I_z - I_0) \quad (3.9)$$

where  $I_0$  and  $S_0$  are the Boltzmann equilibrium magnetization for spins I and S, respectively. In essence these equations are the Bloch equations in analogy with Equation (1.38). However, besides the term  $(W_0 + 2W_{I,S} + W_2)$  for direct relaxation, these equations also include a term  $(W_2 - W_0)$  representing the IS interaction (i.e. the effect of spin I on spin S and vice versa) for cross relaxation. Therefore, in general the recovery of longitudinal magnetization is a linear combination of two exponentials (direct and cross relaxation), rather than a simple single exponential. However, when  $\omega_I = \omega_S$  (for instance the two equivalent protons in water) the observed magnetization is given by  $I_z + S_z$  and the differential equation for the recovery of longitudinal magnetization can be simplified to:

$$\frac{d(I_z + S_z)}{dt} = -2(W_I + W_2)[(I_z + S_z) - (I_0 + S_0)] \quad (3.10)$$

Comparing Equation (3.10) with the classical Bloch equation [Equation (1.38)] gives the relation between the longitudinal relaxation rate constant  $(1/T_1)$  and the transition probabilities  $(W_I$  and  $W_2)$  according to:

$$\frac{1}{T_1} = 2W_I + 2W_2 \quad (3.11)$$

It is important to note that Equation (3.11) only holds for identical spins. Only in that case can the recovery of longitudinal magnetization be described by a single exponential function.

To proceed it is necessary to derive explicit expressions for the transition probabilities  $W_0$ ,  $W_I$ ,  $W_S$  and  $W_2$ . This can be achieved with quantum mechanics by evaluating Equation (3.5a–d) using the relevant eigenstates, energies and dipolar Hamiltonian operators [8]. These derivations are beyond the scope of the book, but when the result of these calculations is substituted in Equation (3.11) an analytical expression for dipolar longitudinal relaxation is obtained, given by:

$$\frac{1}{T_1} = \frac{3}{10} \frac{\gamma^4 h^2}{4\pi^2 r^6} \left( \frac{\tau_c}{1 + \omega^2 \tau_c^2} + \frac{4\tau_c}{1 + 4\omega^2 \tau_c^2} \right) \quad (3.12)$$

where  $r$  is inter-nuclear distance over which the dipolar interactions take place.

Equation (3.12) gives the relation between the relaxation rate constant ( $1/T_1$ ) and the spectral density functions, since:

$$\frac{1}{T_1} = \frac{3}{10} \frac{\gamma^4 \hbar^2}{4\pi^2 r^6} (J(\omega) + 4J(2\omega)) \quad (3.13)$$

$T_1$  relaxation is therefore affected by local magnetic fields oscillating at the Larmor frequency and twice the Larmor frequency.

Similarly, the expression for the transverse or spin-spin relaxation rate constant is given by:

$$\frac{1}{T_2} = \frac{3}{20} \frac{\gamma^4 \hbar^2}{4\pi^2 r^6} \left( 3\tau_c + \frac{5\tau_c}{1 + \omega^2 \tau_c^2} + \frac{2\tau_c}{1 + 4\omega^2 \tau_c^2} \right) \quad (3.14)$$

Equation (3.14) is only valid for identical spins for which the transverse relaxation is mono-exponential. The  $T_2$  relaxation rate constant exhibits several components of the Lorentzian spectral density function, i.e.:

$$\frac{1}{T_2} = \frac{3}{20} \frac{\gamma^4 \hbar^2}{4\pi^2 r^6} (3J(0) + 5J(\omega) + 2J(2\omega)) \quad (3.15)$$

Besides the frequency components contributing to  $T_1$  relaxation [i.e.  $J(\omega)$  and  $J(2\omega)$ ],  $T_2$  relaxation is also affected by low frequency components. The implications of Equations (3.13) and (3.15) is that in the presence of slow molecular motion (e.g. water bound to macromolecular structures)  $T_2$  becomes very small, since the low frequencies dominate. The  $T_1$  relaxation constant is not affected by low frequency fluctuations.

With Equations (3.13) and (3.15) the dependence of  $T_1$  and  $T_2$  on molecular motion, i.e. the rotation correlation time  $\tau_c$  and field strength  $B_0$  ( $= \omega_0/\gamma$ ) can be calculated. Figure 3.4 shows the  $\tau_c$  dependence of  $T_1$  and  $T_2$ . Note that in the ‘extreme narrowing’ situation

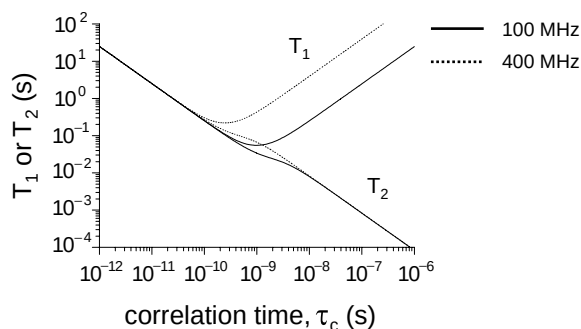


**Figure 3.4** Dependence of the longitudinal and transverse relaxation times  $T_1$  and  $T_2$  as a function of the correlation time  $\tau_c$  for two external magnetic field strengths corresponding to Larmor frequencies of 100 and 400 MHz for protons. The root-mean-square random fluctuating field  $B_{loc}$  was adjusted to obtain known  $T_1$  and  $T_2$  values in the extreme narrowing limit.

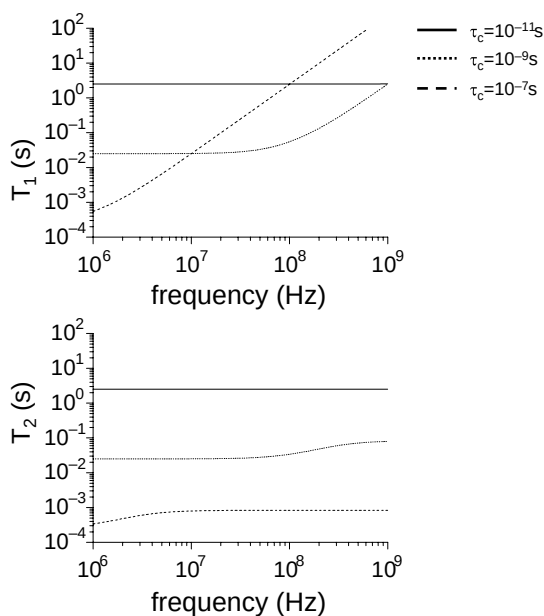


**Figure 3.5** Magnetic field dependence of  $T_1$  and  $T_2$  relaxation times for three rotation correlation times  $\tau_c$ . For intermediate and slow rotational processes, the  $T_1$  relaxation time shows a significant increase with increasing magnetic field strength. The transverse relaxation time is virtually independent of the external magnetic field strength. Note that these curves are only valid for pure dipole–dipole interactions.

(i.e. very fast rotational motion, such that  $\omega^2\tau_c^2 \ll 1$ ),  $T_1 = T_2$ , since:

$$\frac{1}{T_1} = \frac{1}{T_2} = \frac{3}{2} \frac{\gamma^4 \hbar^2}{4\pi^2 r^6} \tau_c \quad (3.16)$$

Therefore in cerebrospinal fluid (CSF) where the majority of the water molecules are highly mobile, the  $T_2$  relaxation time is very long and approaches the  $T_1$  relaxation time. In the case of slow molecular motion (i.e.  $\tau_c > 10^{-9}$  s),  $T_2$  becomes very short, leading to broad unobservable resonances. Nevertheless, such immobile protons can occasionally be studied using magnetization transfer techniques (see Section 3.3.6). Figure 3.5 shows the field dependence of  $T_1$  and  $T_2$  for various rotation correlation times. Especially  $T_1$  relaxation shows a strong dependence on the magnetic field in the regime of intermediate ( $\tau_c \sim 10^{-9}$  s) and slow ( $\tau_c \sim 10^{-7}$  s) rotation correlation times. This dependency should always be taken into account when comparing NMR experiments at different magnetic field strengths.  $T_2$  relaxation shows virtually no dependence on the applied field strength.

Besides the dipole–dipole interactions between two identical nuclei, the required fluctuating dipolar fields can also arise from unpaired electrons, if present. Since the electron magnetic moment is  $>650$  times that of a proton, such relaxation can be extremely effective (i.e. resulting in very short  $T_1$  and  $T_2$  relaxation times), provided that the distance between electron and nucleus is small enough. Because of the high efficiency of

paramagnetic centers, only trace amounts are necessary for a significant increase in relaxation rates. Endogenous paramagnetic compounds are molecular oxygen and deoxy-hemoglobin. Exogenous paramagnetic compounds, as used for  $T_2^*$  image contrast, are often chelates of lanthanides (e.g. gadolinium-DTPA). Water is especially sensitive to paramagnetic centers, since it can come in close proximity to the unpaired electron.

### 3.2.2 Nuclear Overhauser Effect

A phenomenon closely related to magnetic dipolar interactions is the nuclear Overhauser effect (nOe) [9,10]. Originally, the nOe was defined as a change in the integrated NMR absorption intensity of a nuclear spin when the NMR absorption of another spin is saturated. Since the nOe arises from dipolar relaxation, the formalism of dipole–dipole relaxation [Equations (3.5)–(3.9)] can be used for a quantitative description. Consider again the energy level diagram for a dipolar coupled IS spin system (Figure 3.3). The time dependence of the longitudinal magnetization is given by Equations (3.8) and (3.9). This pair of coupled differential equations indicates that in general the IS spin system will not relax mono-exponentially after a perturbation. [Note that Equation (3.10) was derived for identical spins]. Suppose that spin S is completely saturated, so that the energy levels involved are equally populated (i.e.  $N_{\alpha\alpha} = N_{\alpha\beta}$  and  $N_{\beta\alpha} = N_{\beta\beta}$  making  $S_z = 0$ ) [see Equation (3.7)]. When the IS spin system (while being saturated) reaches a steady-state situation, i.e.  $dI_z/dt = 0$ , Equation (3.8) reduces to:

$$0 = -(W_0 + 2W_1 + W_2)(I_z - I_0) + (W_2 - W_0)S_0 \quad (3.17)$$

or

$$\frac{I_z}{I_0} = 1 + \frac{S_0}{I_0} \left( \frac{W_2 - W_0}{W_0 + 2W_1 + W_2} \right) \quad (3.18)$$

Equation (3.18) can be rewritten to a more practical form which is known as the nOe:

$$\text{nOe} = 1 + \frac{\gamma_S}{\gamma_I} \left( \frac{W_2 - W_0}{W_0 + 2W_1 + W_2} \right) \quad (3.19)$$

In the extreme narrowing limit ( $\omega^2\tau_c^2 \ll 1$ ), Equation (3.19) reduces to:

$$\text{nOe} = 1 + \frac{\gamma_S}{2\gamma_I} = 1 + \eta \quad (3.20)$$

where  $\eta$  is sometimes called the nuclear Overhauser enhancement. Equation (3.20) gives the maximum observable nOe. For heteronuclear double-resonance experiments, involving low-sensitivity nuclei like  $^{13}\text{C}$ - $\{^1\text{H}\}$  and  $^{31}\text{P}$ - $\{^1\text{H}\}$ , the nOe generates a useful enhancement in signal intensity of the low-sensitivity nucleus upon saturation of the protons. The maximal nOe for  $^{13}\text{C}$ - $\{^1\text{H}\}$  (i.e. the nOe on  $^{13}\text{C}$  is generated by dipolar interactions with  $^1\text{H}$ ) and  $^{31}\text{P}$ - $\{^1\text{H}\}$  are 2.988 and 2.235, respectively. If dipolar relaxation accounts only for a part of all relaxation mechanisms, the value of the nuclear Overhauser enhancement  $\eta$  is proportionally reduced. With *in vivo* applications, typical nOe values are 1.3–2.9 and 1.4–1.8 for  $^{13}\text{C}$ - $\{^1\text{H}\}$  [11–14] and  $^{31}\text{P}$ - $\{^1\text{H}\}$  [14–17], respectively. Figure 3.6 shows a typical *in vivo*  $^{31}\text{P}$  NMR example of nuclear Overhauser enhancement in human calf muscle.

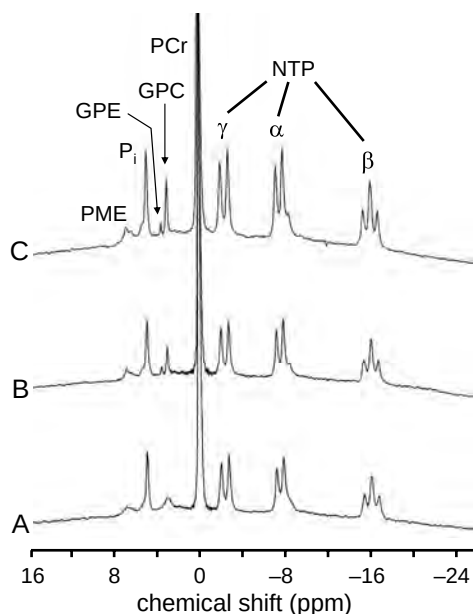


**Figure 3.6** (A) Nonlocalized  $^{31}\text{P}$  NMR spectrum from human calf muscle ( $TR = 20\text{ s}$ , 32 averages, 1.5 T). (B)  $^{31}\text{P}$  NMR spectrum obtained with proton decoupling and (C) proton decoupling and full  $n\text{Oe}$  generation. Note that decoupling increases the spectral resolution, while  $n\text{Oe}$  generation increases the signal intensity. [Reproduced with permission from T. R. Brown et al. *Magn. Reson. Med.* **33**, 417–421 (1995), Copyright John Wiley & Sons, Inc].

### 3.2.3 Alternative Relaxation Mechanisms

The relaxation theory outlined above is generally referred to as the Bloembergen–Purcell–Pound (BPP) theory [7]. After its publication in 1948 the theory was an immediate success, since it could accurately describe dipole–dipole relaxation in liquids such as water and glycerol, as well as in solids. However, apart from dipole–dipole interactions a variety of other relaxation mechanisms may be operative. In principle, any interaction which causes fluctuating magnetic fields can induce relaxation. The interactions which can generate the appropriate conditions for relaxation are:

1. magnetic dipole–dipole interactions;
2. electric quadrupole interactions;
3. chemical shift anisotropy;
4. spin rotation interactions;
5. scalar coupling interactions;

A nucleus with spin  $I > 1/2$  possesses an electric quadrupole moment since the charge distribution is no longer spherical (as is the case for spin  $I = 1/2$ ). The electric quadrupole moment interacts with local electric field gradients. Fluctuations in the strength of this interaction, as caused by molecular motion (tumbling), will induce relaxation. In the

extreme narrowing limit, the relaxation rates solely caused by quadrupole interactions are given by:

$$\frac{1}{T_1} = \frac{1}{T_2} = \frac{3}{10} \left( \frac{2I + 3}{I^2(2I - 1)} \right) \left( 1 + \frac{\eta^2}{3} \right) \left( \frac{\pi e^2 Q q}{h} \right)^2 \tau_c \quad (3.21)$$

where  $\eta$  is an asymmetry parameter, which measures the deviation of the electronic environment from spherical symmetry.  $(\pi e^2 Q q / h)$  is the quadrupole coupling constant, which depends on the nuclear quadrupole moment  $Q$  and the electric field gradient  $q$ . As described for  $^{23}\text{Na}$  and  $^{39}\text{K}$  in Section 2.5, most quadrupolar nuclei relax predominantly according to Equation (3.21), making the relaxation time constant  $T_1$  in the order of milliseconds. The quadrupolar interaction does not contribute to relaxation when the quadrupole coupling constant is zero due to molecular symmetry.

In Chapter 1 the chemical shift was presented as a single number, proportional to the effective magnetic field at the nucleus, which included the effects of the external magnetic field, as well as electronic shielding of the nucleus. However, because the chemical shift of a nucleus depends upon the orientation of the molecule relative to the main magnetic field direction, a proper representation of the chemical shift (or shielding constant) is a  $3 \times 3$  chemical shift tensor (see Appendix A1). Rapid molecular motions in the liquid state result in an averaging of all possible orientations and chemical shifts, leading to in an average chemical shift represented by the trace of the tensor. Even though the observed chemical shift may not be affected by chemical shift anisotropy (as is the case in the extreme narrowing limit), the nucleus will nevertheless experience fluctuations in the local magnetic field as the molecule tumbles. These fluctuations provide yet another mechanism for relaxation. The relaxation rate due to chemical shift anisotropy is dependent upon the Larmor frequency  $\omega$ , the molecular correlation time  $\tau_c$  and the main magnetic field strength  $B_0$  and in the extreme narrowing limit is given by:

$$\frac{1}{T_1} = \frac{2}{15} \gamma^2 B_0^2 (\sigma_{\parallel} - \sigma_{\perp})^2 \tau_c \quad (3.22)$$

$$\frac{1}{T_2} = \frac{7}{45} \gamma^2 B_0^2 (\sigma_{\parallel} - \sigma_{\perp})^2 \tau_c \quad (3.23)$$

where  $\sigma_{\parallel}$  and  $\sigma_{\perp}$  refer to shielding parallel and perpendicular to the symmetry axis, when axial symmetry is assumed. In contrast to the situation under exclusive dipole–dipole relaxation,  $T_1$  does not equal  $T_2$  under extreme narrowing conditions when relaxation via chemical shift anisotropy is involved. Note that the relaxation rates through chemical shift anisotropy are proportional to the square of the main magnetic field. It can therefore be expected that this mechanism becomes increasingly important as the magnetic field strength  $B_0$  increases. Unlike dipole–dipole relaxation, the relaxation rates under exclusive chemical shift anisotropy will increase with increasing  $B_0$ , such that the exact  $B_0$  dependence of  $T_1$  and  $T_2$  relaxation will strongly depend on the relative contributions of the different mechanisms.

For  $^{31}\text{P}$  MRS, dipolar relaxation and chemical shift anisotropy are the two major, competing relaxation mechanisms [18, 19]. Experiments on model solutions indicate that chemical shift anisotropy is the dominant mechanism for relaxation of  $^{31}\text{P}$  nuclei in ATP at high magnetic fields, whereas dipolar interactions dominate the  $T_1$  relaxation rate at lower fields and

for the monophosphate groups in phosphocreatine and inorganic phosphate. An advantage of the reduced  $T_1$  relaxation times for  $^{31}\text{P}$  NMR at higher magnetic fields is that shorter repetition times can be employed, such that S/N per unit of time can be improved. However, chemical shift anisotropy also reduces the  $T_2$  relaxation time, thereby significantly compromising the enhancement in spectral resolution that may be expected upon increasing the magnetic field strength. If chemical shift anisotropy is a dominant mechanism, the line widths increase linearly with  $B_0^2$ , whereas the frequency range only increases according to  $B_0$ . It follows that the best spectral resolution is not necessarily obtained at the highest magnetic field.

Reports on the measurement of  $^{31}\text{P}$  longitudinal and transverse relaxation times *in vivo* [17,20–34] only qualitatively support the notion that  $^{31}\text{P}$   $T_1$  relaxation reduces at higher magnetic fields. This can be largely attributed to the large variation between reports. A coherent trend that can be extracted is that the transverse relaxation rate of ATP is significantly higher than that of the other phosphorus containing metabolites. It seems unlikely that this large difference is solely based on dipole–dipole interactions and chemical shift anisotropy. It has been suggested that the short transverse relaxation time of ATP is partly due to exchange between free and bound states of ATP. Interactions with enzymes like creatine kinase and ATPases, for example, which effectively act as solid matrices may result in long  $T_1$  and short  $T_2$  relaxation times. Furthermore, the strong dipole–dipole interaction between ATP and the unpaired electron of a complexed paramagnetic ion (like  $\text{Mn}^{2+}$ ) could also play a role.

Note that some of the reported  $T_2$  values of ATP in the literature [27, 28] represent an underestimation of the true  $T_2$  relaxation time due to interference of homonuclear scalar coupling. Especially in the early stages of *in vivo*  $^{31}\text{P}$  MRS the homonuclear scalar coupling was ignored, leading to a sinusoidally modulated  $T_2$  relaxation curve. The introduction of selective refocusing pulses and/or homonuclear decoupling [29–31,35] refocuses homonuclear scalar coupling evolution, thereby allowing the accurate determination of  $T_2$ .

Spin rotation relaxation arises from magnetic fields at the nucleus generated by coherent rotational motion of the entire molecule, which can couple with the nuclear spin. Interruption of this coupling (e.g. by collisions) provides a relaxation mechanism. This effect is most significant for small, symmetric molecules with short correlation times or for similar parts of molecules, like methyl groups. This mechanism is of little importance for most molecules observed with *in vivo* NMR.

As described in Section 1.10, apart from direct, through-space dipolar interaction two nuclear spins I and S can also experience indirect coupling through the electrons in the chemical bond. This is referred to as scalar coupling, the strength of which is independent of the orientation of the molecules within the applied field. A scalar interaction, which involves a magnetic field produced by spin S acting (indirectly) on spin I (and vice versa) can lead to relaxation of spin I, if a time dependence in the scalar coupling occurs. This can happen if the scalar coupling constant becomes time dependent due to exchange processes or if the energy level populations of spin S become time dependent as a result of relaxation of spin S. These two possible causes for scalar relaxation are known as scalar relaxation of the first and second kind, respectively. Scalar relaxation is most commonly observed when S is a quadrupole nucleus with short relaxation times. Most often scalar relaxation only has a pronounced effect on  $T_2$  relaxation, leading for instance to line broadening in the  $^1\text{H}$  spectrum of nuclei coupled to  $^{14}\text{N}$ .



### 3.2.4 In Vivo Relaxation

From the preceding discussions it is clear that relaxation in general is a complex process involving several competing mechanisms. Relaxation in biological tissues is complicated even further due to compartmentalization. In contrast to metabolites, the relaxation characteristics of water have been extensively studied and it has been established that dipole–dipole interactions give rise to the dominant relaxation pathway. However, applying the BPP theory as outlined earlier to water in biological tissues gives incorrect results. This is mainly because BPP theory assumes an isotropic molecular motion, while in tissues the exchange of molecules between different molecular environments plays an important role. The relaxation of water in tissues can largely be explained by hydration-induced changes in rotational motion of the water in the vicinity of macromolecular surfaces [36–39]. Figure 3.7 shows a schematic drawing of water in the vicinity of a protein. Besides the ‘bulk’ fraction of the water which can freely rotate (and hence has a short  $\tau_c \sim 10^{-10}$  s), there is also a fraction of the water which forms a hydration layer surrounding the protein. Normally this hydration layer has a thickness of several water molecules. The molecules in a hydration layer are more structured as a result of hydrogen or ionic bonds to hydrophilic or ionic sites on the protein. The binding reduces the rotational mobility of the water in the hydration layer, resulting in longer correlation times  $\tau_c$  ( $\tau_c$  can be as long as  $10^{-6}$ – $10^{-5}$  s for ionic bonds). Although Figure 3.7 shows a hydration layer surrounding a protein, these layers can form along any hydrophilic surface, including membranes and DNA. When a fast exchange between the bulk water and the rotationally restricted water is assumed, the

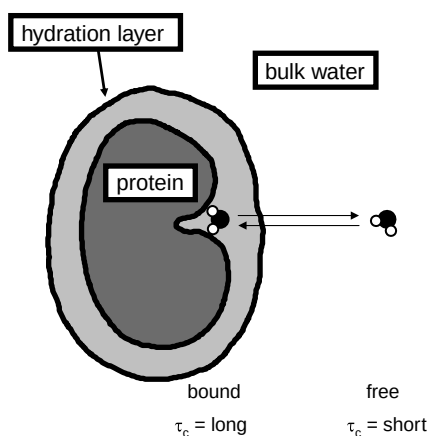


**Figure 3.7** Two-compartment model for water in biological systems. Most of the water possesses bulk properties in that it is rotationally mobile (short rotation correlation time  $\tau_c$ ). A part of the water forms a hydration layer (of  $\sim 3$  water molecules) around proteins or other hydrophilic structures. This structured water is characterized by restricted mobility (long rotation correlation time  $\tau_c$ ) and consequently a significantly reduced  $T_2$  relaxation time. The structured and bulk water pools are in fast exchange such that the relaxation characteristics of the immobile pools can significantly shorten the observed relaxation times. The two-compartment model can easily be extended to more compartments, for instance by the discrimination between dipolar and ionic bound water.

**Table 3.1**  $T_1$  and  $T_2$  relaxation time constant ranges for human tissues at different magnetic field strengths

| Tissue type         | Magnetic field strength (T) |            |            |            |            |            |
|---------------------|-----------------------------|------------|------------|------------|------------|------------|
|                     | 1.5                         |            | 3.0        |            | 4.0        |            |
|                     | $T_1$ (ms)                  | $T_2$ (ms) | $T_1$ (ms) | $T_2$ (ms) | $T_1$ (ms) | $T_2$ (ms) |
| Brain, gray matter  | 900–1100                    | 90–110     | 1100–1500  | 70–90      | 1400–1700  | 50–70      |
| Brain, white matter | 800–900                     | 70–90      | 950–1150   | 50–70      | 1050–1200  | 40–60      |
| CSF                 | >2000                       | >500       | >2000      | >500       | >3000      | >500       |
| Muscle              | 900–1100                    | 30–50      | 1200–1450  | 30–50      | 1200–1450  | 25–40      |
| Liver               | 500–600                     | 40–50      | 750–850    | 40–50      | 800–900    | 30–50      |

relaxation rate can simply be calculated as the sum of the two fractions:

$$\frac{1}{T_{1,\text{observed}}} = \frac{f_{\text{free}}}{T_{1,\text{free}}} + \frac{f_{\text{restricted}}}{T_{1,\text{restricted}}} \quad (3.24)$$

where  $f_{\text{free}} / T_{1,\text{free}}$  and  $f_{\text{restricted}} / T_{1,\text{restricted}}$  are the fractions and the  $T_1$  relaxation times of the free and restricted water, respectively, which can be directly calculated from BPP theory. A similar expression can be derived for the transverse relaxation rate. This two-compartment model can be further refined to discriminate between ionic bound water (which behaves as if it were a solid) and hydrogen bound water (which behaves as a viscous liquid with  $\tau_c \sim 10^{-9}$  s). Using this two-compartment, fast exchange model or other more extensive multi-compartment models, a semi-quantitative description of water relaxation in biological tissues has been established. Table 3.1 gives average values of  $T_1$  and  $T_2$  relaxation times for water in different human tissues at several magnetic field strengths as reported in literature [36,39–49]. Despite the large inter-study variation, a steady increase in longitudinal relaxation time  $T_1$  with increasing magnetic field strength can be deduced, indicating that dipole–dipole interactions are a dominant relaxation mechanism. On average the spin-spin relaxation is relatively constant with a tendency towards a decrease with increasing magnetic fields. In order to minimize intra-field variations and establish the magnetic field dependence of water  $T_1$  and  $T_2$  relaxation times, Table 3.2 and Figure 3.8A and B summarize the results of a single study at three different magnetic fields, 4.0, 9.4 and 11.7 T [50]. It follows that for all rat brain structures, the  $T_1$  relaxation increases and the  $T_2$  relaxation decreases with increasing magnetic field strength.

Reports on the relaxation times of cerebral metabolites in human [51–61] and animal [50,62–65] brain are more sparse, which, in combination with the greatly decreased sensitivity of metabolite detection, leads to even larger inter-study variations. As a result, Table 3.3 and Figure 3.8C and D summarize the results of a single study performed at multiple fields [50]. To obtain values at intermediate fields, the experimentally measured  $T_1$  and  $T_2$  relaxation times were modeled to the functions  $A[B_0]^B$  and  $C\exp(-B_0/D)$ , respectively, where  $A$ – $B$  were determined by least-squares curve fitting. The  $T_1$  function was also used by Bottomley *et al.* [36] for water and can adequately fit  $T_1$  relaxation data over the range 0–500 MHz. It follows that the relaxation pattern of metabolites tracks that

**Table 3.2** Water  $T_1$  and  $T_2$  relaxation time constants (in ms) for rat brain<sup>a</sup> at 4.0, 9.4 and 11.7 T

| Structure    | cc            | cx            | ob            | hc            | cw            | cg            | st            | th            | mb            |
|--------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| 4.0 T $T_1$  | <b>1096.8</b> | <b>1285.8</b> | <b>1640.6</b> | <b>1334.1</b> | <b>1046.9</b> | <b>1352.6</b> | <b>1288.2</b> | <b>1169.4</b> | <b>1064.4</b> |
| SD           | 49.3          | 77.0          | 20.8          | 97.4          | 53.3          | 82.6          | 87.3          | 69.5          | 17.3          |
| 9.4 T $T_1$  | <b>1752.1</b> | <b>1948.4</b> | <b>2129.1</b> | <b>2059.7</b> | <b>1660.3</b> | <b>2097.2</b> | <b>1927.0</b> | <b>1793.1</b> | <b>1786.5</b> |
| SD           | 52.1          | 51.9          | 63.7          | 66.1          | 79.3          | 68.2          | 54.7          | 64.3          | 81.9          |
| 11.7 T $T_1$ | <b>1861.3</b> | <b>2073.4</b> | <b>2304.3</b> | <b>2222.8</b> | <b>1745.1</b> | <b>2109.4</b> | <b>2046.5</b> | <b>1903.2</b> | <b>1893.2</b> |
| SD           | 73.5          | 100.7         | 63.4          | 63.2          | 36.0          | 95.0          | 55.3          | 61.2          | 44.3          |
| 4.0 T $T_2$  | <b>57.9</b>   | <b>65.2</b>   | <b>80.2</b>   | <b>72.0</b>   | <b>58.8</b>   | <b>65.3</b>   | <b>69.7</b>   | <b>61.7</b>   | <b>60.6</b>   |
| SD           | 1.6           | 2.4           | 2.0           | 1.3           | 1.5           | 2.0           | 2.0           | 2.1           | 1.9           |
| 9.4 T $T_2$  | <b>35.8</b>   | <b>42.1</b>   | <b>48.1</b>   | <b>45.4</b>   | <b>37.2</b>   | <b>41.7</b>   | <b>43.5</b>   | <b>40.6</b>   | <b>40.3</b>   |
| SD           | 1.2           | 1.2           | 1.9           | 1.8           | 2.0           | 1.6           | 2.4           | 1.2           | 1.4           |
| 11.7 T $T_2$ | <b>30.7</b>   | <b>36.2</b>   | <b>38.9</b>   | <b>38.9</b>   | <b>27.1</b>   | <b>37.3</b>   | <b>36.4</b>   | <b>33.8</b>   | <b>33.8</b>   |
| SD           | 1.0           | 1.0           | 1.1           | 1.3           | 1.3           | 2.3           | 2.2           | 1.6           | 1.9           |

<sup>a</sup> cc, corpus callosum; cx, cerebral cortex; ob, olfactory bulb; hc, hippocampus; cw, cerebellar white matter; cg, cerebellar gray matter; st, striatum; th, thalamus; mb, mid-brain.

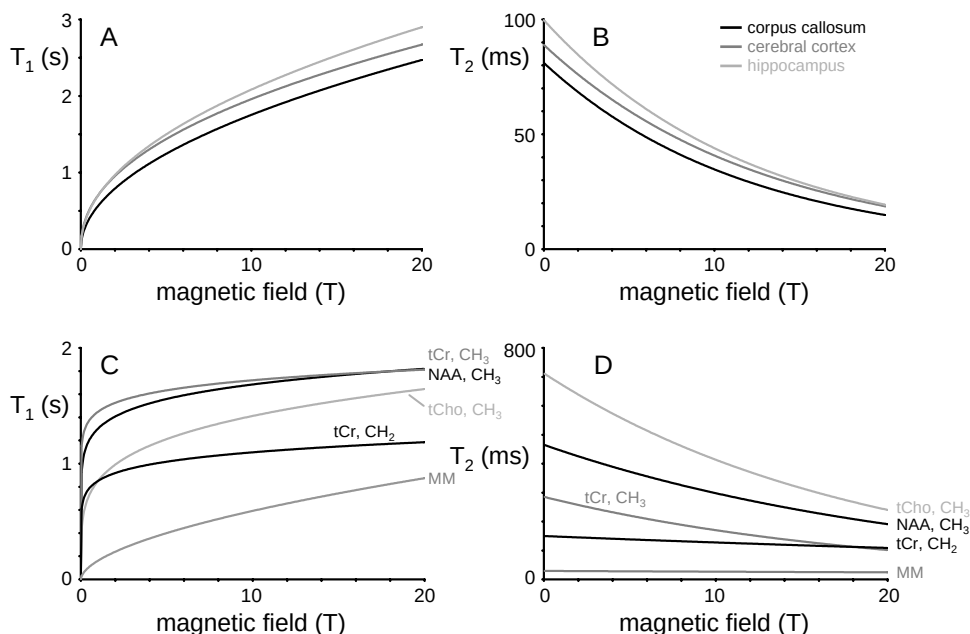


**Figure 3.8** Magnetic field dependence of the longitudinal relaxation time constant  $T_1$  (A, C) and transverse relaxation time constant  $T_2$  (B, D) for water (A, B) and metabolites/macromolecules (C, D). The curves are extrapolated from measured values at 4.0, 9.4 and 11.7 T. See text for more details.

of water, with increasing  $T_1$  and decreasing  $T_2$  relaxation times at higher magnetic fields. For macromolecular resonances the  $T_1$  relaxation rapidly increases with magnetic field strength, whereas the  $T_2$  relaxation is virtually field independent.

The sharp decrease of water  $T_2$  relaxation times with increasing magnetic field strength is in apparent contradiction with BPP dipolar relaxation theory, which predicts field-independent  $T_2$  relaxation for a wide range of rotation correlation times (see Figure 3.5.) Michaeli *et al.* [66] explained the field-dependent  $T_2$ s as the result of increased dynamic dephasing due to increased local (microscopic) susceptibility gradients. It is well-known that macroscopic, as well as microscopic magnetic field inhomogeneity due to susceptibility differences between tissues (and air) increases linearly with magnetic field strength (see Chapter 10). As molecules diffuse through these microscopic field gradients they lose phase coherence, resulting in a shorter apparent  $T_2$  relaxation time constant,  $T_2^+$ . Since the dynamic dephasing process is dependent on diffusion, the decrease in observed  $T_2$  relaxation times is expected to be greatest for water (diffusion coefficient  $D \sim 0.7 \mu\text{m}^2 \text{ms}^{-1}$ ) and smallest for large macromolecules ( $D < 0.02 \mu\text{m}^2 \text{ms}^{-1}$ ). Metabolites, with intermediate diffusion coefficients ( $D \sim 0.2 \mu\text{m}^2 \text{ms}^{-1}$ ), are expected to display a moderate decrease in  $T_2$  with increasing magnetic field strength.

From the presented data, predictions can be made about the magnetic field dependence of the signal-to-noise ratio (SNR) and the spectral resolution. Ignoring relaxation parameters the SNR is expected to increase linearly with magnetic field strength  $B_0$  [67]. The NMR

**Table 3.3** Metabolite and macromolecule  $T_1$  and  $T_2$  relaxation time constants (in ms) for rat brain at 4.0, 9.4 and 11.7 T

| Metabolite   | tCr           | ml            | Tau           | tCh           | tCr           | Glx           | NAA           | MM4          | MM2,3        | MM1          |
|--------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|--------------|--------------|--------------|
| 4.0 T $T_1$  | <b>1003.4</b> |               |               | <b>1451.4</b> | <b>1614.0</b> | <b>1262.6</b> | <b>1520.6</b> |              | <b>360.9</b> | <b>353.5</b> |
| SD           | 81.2          |               |               | 69.1          | 35.2          | 28.8          | 51.3          |              | 38.1         | 38.9         |
| 9.4 T $T_1$  | <b>1039.7</b> | <b>1370.1</b> | <b>2328.9</b> | <b>1348.3</b> | <b>1679.2</b> | <b>1497.5</b> | <b>1674.0</b> | <b>530.6</b> | <b>580.9</b> | <b>632.6</b> |
| SD           | 73.5          | 120.4         | 358.6         | 20.6          | 67.7          | 106.5         | 31.1          | 42.4         | 22.1         | 30.3         |
| 11.7 T $T_1$ | <b>1156.4</b> | <b>1638.1</b> | <b>2447.5</b> | <b>1629.6</b> | <b>1767.3</b> | <b>1450.5</b> | <b>1713.5</b> | <b>627.1</b> | <b>634.1</b> | <b>762.2</b> |
| SD           | 84.5          | 170.5         | 167.8         | 108.7         | 80.2          | 146.8         | 44.1          | 42.9         | 25.1         | 30.4         |
| 4.0 T $T_2$  | <b>140.7</b>  |               |               | <b>569.8</b>  | <b>232.9</b>  |               | <b>391.9</b>  |              | <b>31.7</b>  | <b>23.4</b>  |
| SD           | 11.5          |               |               | 28.8          | 5.9           |               | 15.1          |              | 2.5          | 1.1          |
| 9.4 T $T_2$  | <b>127.8</b>  |               |               | <b>441.3</b>  | <b>171.1</b>  |               | <b>294.3</b>  | <b>22.7</b>  | <b>33.5</b>  | <b>27.4</b>  |
| SD           | 5.7           |               |               | 36.2          | 3.9           |               | 5.1           | 2.5          | 1.9          | 2.3          |
| 11.7 T $T_2$ | <b>124.8</b>  |               |               | <b>365.7</b>  | <b>159.1</b>  |               | <b>285.2</b>  | <b>19.9</b>  | <b>31.4</b>  | <b>25.3</b>  |
| SD           | 7.3           |               |               | 73.1          | 6.6           |               | 26.5          | 2.3          | 2.1          | 1.9          |

signal induced in a receiving coil increases as the square of  $B_0$  due to two separate and independent factors. Firstly, the spin level population difference increases at higher magnetic fields and because the energy difference is very small compared with thermal energy, the population increases linearly with  $B_0$ , as does the size of the nuclear magnetic dipole moment (i.e. ‘longitudinal magnetization’). Secondly, the Larmor frequency at which the magnetic dipole moment precesses is proportional to  $B_0$ . Following Faraday’s law of electromagnetic induction, the signal produced by a magnetic dipole moment of constant amplitude will increase in proportion (i.e. linear) to the precession frequency. The combination of these two factors gives a  $B_0^2$  dependence of the signal intensity. However, the noise in high field NMR experiments also increases linearly with  $B_0$  (according to the same principles of electromagnetic induction), provided that the subject is the dominant noise source.

Including relaxation, the SNR for a simple, fully relaxed pulse-acquire experiment can be calculated by considering the amount of signal per unit time (proportional to  $TR/T_1$ ) acquired over a given spectral bandwidth [proportional to  $1/(\pi T_2)$  or  $1/(\pi T_2^*)$ ] and is given by:

$$SNR \propto B_0 \sqrt{\frac{T_2}{T_1}} \quad (3.25)$$

Using the extrapolated  $T_1$  and  $T_2$  relaxation curve of Figure 3.8, allows the calculation of the relative SNR as a function of magnetic field strength, as is shown in Figure 3.9. It follows that the SNR for singlet resonances in NMR spectroscopy increases less than linear with  $B_0$ , while the SNR for water starts to level off for magnetic fields beyond 7 T and possibly even shows a maximum around 20 T. It should be noted that these arguments are only valid for  $T_1$  and/or  $T_2$  based anatomical MRI acquisitions. For functional MRI,

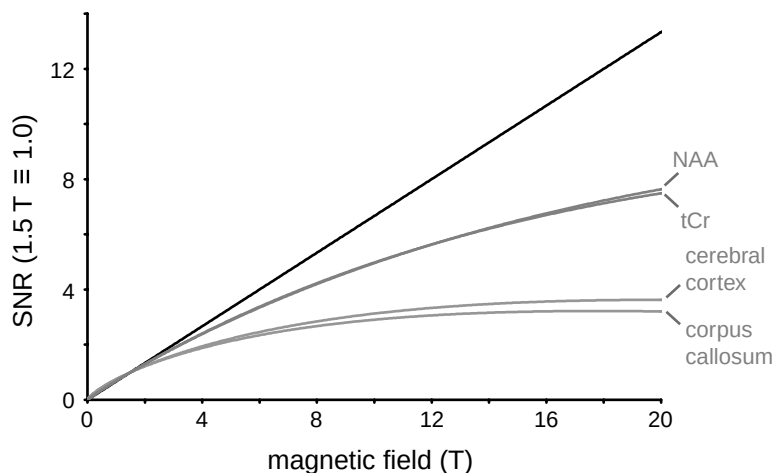


**Figure 3.9** Calculated magnetic field dependence of the SNR for water in gray and white matter and cerebral metabolites (NAA and tCr), as extrapolated from measured data at 4.0 T, 9.4 T and 11.7 T. The solid black line represents a linear increase in SNR with magnetic field strength. See text for more details.

and in particular BOLD fMRI, the situation is distinctly different. The BOLD effect scales linearly with magnetic field strength and more importantly the BOLD effect becomes spatially more specific since the contribution of nonspecific effects of draining veins become negligible at 7 T or higher (e.g. [68]). Therefore, despite the complications of increased  $B_0$  magnetic field inhomogeneity (see Chapter 10), it is desirable to perform BOLD fMRI studies of functional brain activation at the highest magnetic field. It should also be realized that while the SNR of metabolites increases less than linear with  $B_0$ , the quantification accuracy of metabolites (and especially lower concentration, scalar-coupled metabolites) may increase much better than linear with  $B_0$  due to reduced spectral overlap and simplified spectral patterns.

In combination with information on magnetic field inhomogeneity, the presented  $T_1$  and  $T_2$  relaxation times for metabolites can be used to predict the spectral resolution as a function of magnetic field strength [50]. It follows that the spectral line width converges to circa 0.015–0.020 ppm for  $B_0 > 9.4$  T. The spectral line width at low magnetic fields ( $B_0 < 2$  T) is relatively broad because at those fields the inherent  $T_2$  relaxation dominates the line width. Only at  $B_0 > 9.4$  T does (microscopic) magnetic field inhomogeneity become the dominant contribution to the observed spectral line width. Based on these results it is not expected that the spectral resolution for singlet resonances will dramatically increase beyond  $B_0 \sim 9.4$  T. However, significant improvements in spectral resolution for scalar-coupled spin systems like glutamate and glutamine is expected well beyond 9.4 T (see also Figure 2.13).

### 3.3 Magnetization Transfer

The determination of reaction rates and fluxes is important to characterize metabolic processes. NMR spectroscopy allows the noninvasive measurement of fluxes *in vivo*. The general principle of these measurements is to disturb the steady-state equilibrium of the system under investigation and to monitor the response to this perturbation. Some methods to achieve the perturbation are generally applicable, e.g. induction of concentration changes of a reactant. Other procedures are very specific for NMR, e.g. spin transfer techniques. The former approach is often invasive and usually does not allow the determination of reaction rates and fluxes under steady-state conditions. An exception to the rule in this category of experiments is where one disturbs the isotope enrichment of a reactant in an otherwise, steady-state situation. For NMR spectroscopy, infusion of isotope-enriched substrates allows the determination of metabolic pathway fluxes, for example from [1- $^{13}\text{C}$ ]glucose to glutamate, glutamine and GABA through the TCA cycle as will be discussed in detail in Section 3.5.

Here, the principles of magnetization or spin transfer techniques will be discussed. Rather than utilizing an exogenous perturbation, magnetization transfer (MT) methods exploit the magnetic properties of the endogenous reactants under investigation.

In general, a spin or magnetization transfer experiment consists of three periods, namely a preparation period in which the spins are allowed to return to the Boltzmann equilibrium situation, followed by a mixing period in which the reaction partners are allowed to exchange magnetization after which transverse magnetization is created and detected in the detection period. The methods presently available for magnetization transfer measurements

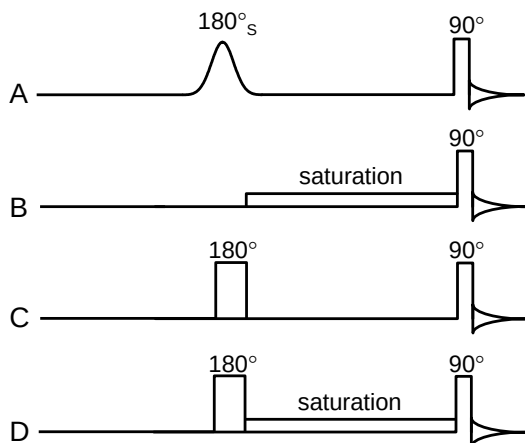


**Figure 3.10** Basic NMR pulse sequences used in the study of exchange reactions with magnetization transfer techniques. (A) Frequency-selective inversion recovery, or inversion transfer (IT), (B) saturation transfer (ST) and nonselective inversion recovery in (C) the absence and (D) the presence of saturation.

mainly differ in the manner in which the mixing is achieved. Figure 3.10 A/B shows two of the most commonly employed methods, namely inversion transfer (IT) and saturation transfer (ST). Occasionally two-dimensional exchange spectroscopy (2DES) is employed for *in vivo* reaction rate measurements. 2DES utilizes an additional delay for evolution, prior to the mixing period, inherent to two-dimensional (2D) NMR spectroscopy. Principles of 2D NMR are discussed in Chapter 8. In both the IT and ST methods one of the reaction partners, for example reactant A, is selectively perturbed, either by inversion or saturation. Following a mixing period during which the magnetization of the reaction partners is allowed to exchange, all magnetization is excited and detected. Intuitively, it is easy to understand that the longitudinal magnetization of the nonperturbed reactant, B, will be decreased due to the exchange of inverted or saturated magnetization coming from reactant A provided that the longitudinal magnetization of reactant A has not yet fully recovered due to  $T_1$  relaxation.

In order to quantitatively predict the effects of the different mixing techniques on a two-sided chemical equilibrium, the Bloch equations need to be modified to incorporate the effects of chemical exchange. Consider the equilibrium between reactants A and B:



where  $k_{AB}$  and  $k_{BA}$  are the unidirectional rate constants. The reaction flux is defined as  $k_{AB}[A]$  and  $k_{BA}[B]$ , where  $[ ]$  represents the concentration of the indicated reactant. Under steady-state conditions the reaction fluxes are equal, i.e.  $k_{AB}[A] = k_{BA}[B]$ . When the longitudinal equilibrium magnetization for reactants A and B is defined as  $M_{zA}^0$  and  $M_{zB}^0$  (these are directly proportional to the concentrations of A and B), the Bloch equations



incorporating chemical exchange [69, 70] are given by:

$$\frac{dM_{zA}(t)}{dt} = \frac{(M_{zA}^0 - M_{zA}(t))}{T_{1A}} - k_{AB}M_{zA}(t) + k_{BA}M_{zB}(t) \quad (3.27)$$

$$\frac{dM_{zB}(t)}{dt} = \frac{(M_{zB}^0 - M_{zB}(t))}{T_{1B}} + k_{AB}M_{zA}(t) - k_{BA}M_{zB}(t) \quad (3.28)$$

These differential equations can be solved analytically to provide the time dependence of the longitudinal magnetization in the presence of chemical exchange:

$$M_{zA}(t) = M_{zA}^0 + c_1 e^{a_1 t} + c_2 e^{a_2 t} \quad (3.29)$$

$$M_{zB}(t) = M_{zB}^0 + c_3 e^{a_1 t} + c_4 e^{a_2 t} \quad (3.30)$$

where

$$a_{1,2} = \frac{1}{2} [-(R_A + R_B) \pm [(R_A - R_B)^2 + 4k_{AB}k_{BA}]^{1/2}] \quad (3.31)$$

$$c_2 = \frac{[(M_{zA}(0) - M_{zA}^0)(a_1 + R_A) + k_{BA}(M_{zB}^0 - M_{zB}(0))]}{(a_1 - a_2)} \quad (3.32)$$

$$c_1 = M_{zA}(0) - M_{zA}^0 - c_2 \quad (3.33)$$

$$c_3 = c_1 \frac{(a_1 + R_A)}{k_{BA}} \quad (3.34)$$

$$c_4 = c_2 \frac{(a_2 + R_A)}{k_{BA}} \quad (3.35)$$

$R_A$  and  $R_B$  are the longitudinal relaxation rate constants for spins A and B, respectively, in the presence of chemical exchange, i.e.  $R_A$  and  $R_B$  are given by:

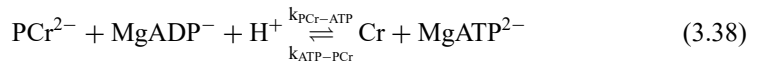
$$R_A = \frac{1}{T_{1A}} + k_{AB} \quad (3.36)$$

$$R_B = \frac{1}{T_{1B}} + k_{BA} \quad (3.37)$$

where  $T_{1A}$  and  $T_{1B}$  are the longitudinal relaxation times in the absence of chemical exchange. The different techniques for magnetization transfer essentially differ in the magnitude of  $M_{zA}(0)$  and  $M_{zB}(0)$  and to which extent further manipulations during the evolution period are performed.

### 3.3.1 Creatine Kinase

The equilibrium reaction most commonly studied with *in vivo* MT techniques is the exchange of a high-energy phosphate group between PCr and ATP, i.e.:



This reaction is catalyzed by creatine kinase (CK). There have been several physiological functions postulated for the CK/PCr system, although controversy about the exact role remains. The first, most generally accepted function is ‘temporal energy buffering’, i.e.

during a workload the levels of ATP remain constant through the conversion of PCr (present at relatively high concentrations) to ATP. Secondly, CK acts as an ‘energy transport system’ between sites of ATP synthesis (e.g. mitochondria) and sites of ATP utilization (e.g. myofibrilles). This is supported by the finding of several different CK isoenzymes, which are specifically located either in the mitochondria or in the cytoplasm [71]. Mitochondrial CK catalyzes the synthesis of PCr from ATP (which was formed through oxidative phosphorylation), after which PCr (being a much smaller molecule than ATP) diffuses to the site of energy utilization where the cytosolic CK isoenzyme converts PCr to ATP. Besides these main functions, it has been proposed that CK (1) also prevents a rise in intracellular free ADP, thus preventing an inactivation of cellular ATPases and a net loss of adenine nucleotides, (2) functions as a proton buffer keeping the intracellular pH at physiological levels during a workload and (3) generates an appropriate local ATP/ADP ratio at sites where CK is functionally coupled to ATP-consuming enzymes and processes. A high ATP/ADP level in the vicinity of ATPase increases the thermodynamic efficiency of ATP hydrolysis.

The reaction partners relevant for MT techniques are the phosphate group of PCr which is in chemical exchange with the third, or gamma, phosphate group of ATP.

### 3.3.2 Inversion Transfer

During an IT experiment [72, 73], the magnetization of one of the reactants is inverted with a selective inversion pulse such that, for instance,  $M_{zB}(0) = -M_{zB}^0$  and  $M_{zA}(0) = M_{zA}^0$ . These initial conditions do not simplify the evolution curves given by Equations (3.29)–(3.35). Figure 3.11 shows *in vivo*  $^{31}\text{P}$  NMR spectra from rat skeletal muscle obtained with an inversion transfer sequence in which the  $\gamma$ -ATP resonance was selectively inverted. Due to the short  $T_2$  relaxation time of  $\gamma$ -ATP, relaxation during the long selective inversion pulse prevents a complete inversion of the  $\gamma$ -ATP resonance. However, this can be accounted for by using the appropriate  $M_{zB}(0)$  in Equations (3.29) and (3.30). It can be seen from Figure 3.11A that while the  $\gamma$ -ATP resonance gradually recovers, the intensity of the PCr resonance initially decreases, after which it returns to its equilibrium value. Figure 3.11B shows the theoretical inversion transfer curves for  $k_{\text{PCr-ATP}} = 0.5 \text{ s}^{-1}$ ,  $k_{\text{ATP-PCr}} = 1.0 \text{ s}^{-1}$ ,  $T_{1,\text{PCr}} = 5.0 \text{ s}$ ,  $T_{1,\text{ATP}} = 2.0 \text{ s}$  and  $M_{z,\text{PCr}}^0/M_{z,\text{ATP}}^0 = 2.0$ . These are typical values for the CK equilibrium in brain *in vivo*.

The IT curves as shown in Figure 3.11 are therefore dependent on four kinetic parameters,  $k_{\text{PCr-ATP}}$ ,  $k_{\text{ATP-PCr}}$ ,  $T_{1,\text{PCr}}$  and  $T_{1,\text{ATP}}$ . Analysis of a dataset as shown in Figure 3.11A can provide all four parameters, although the acquisition of a second independent dataset in which the PCr resonance is inverted will probably provide more reliable estimates [73].

### 3.3.3 Saturation Transfer

In a ST experiment [69, 70, 73, 74], one of the reactants is selectively saturated, such that  $M_{zA}(0) = M_{zA}^0$ ,  $M_{zB}(0) = 0$  and  $M_{zB}(t) = 0$  (i.e.  $M_{zB}$  is saturated throughout the evolution period). The selective saturation of resonance B considerably simplifies Equations (3.29)–(3.35) to the form given by:

$$\frac{M_{zA}(t)}{M_{zA}^0} = \frac{1}{R_A T_{1A}} + \left[ 1 - \left( \frac{1}{R_A T_{1A}} \right) \right] e^{-R_A t} \quad (3.39)$$

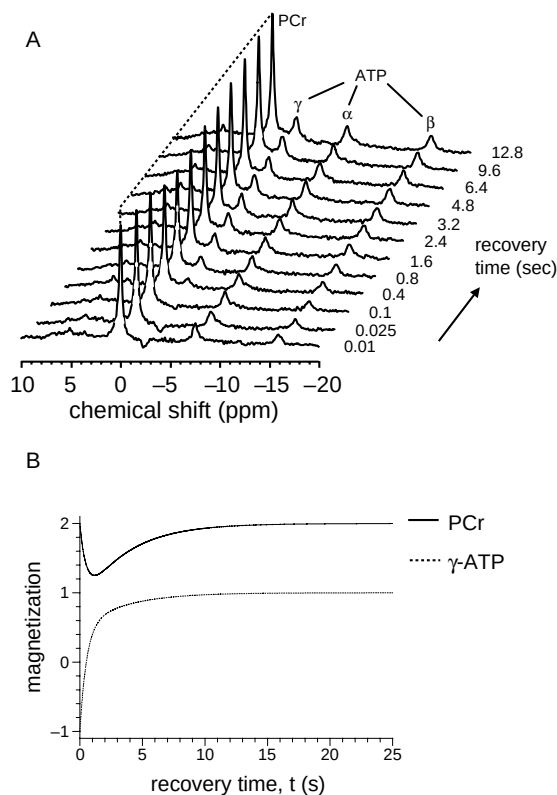


**Figure 3.11** (A) Inversion transfer dataset obtained from rat skeletal muscle in vivo at 4.7 T. Selective inversion of the  $\gamma$ -ATP resonance causes a decrease in intensity of the phosphocreatine resonance due to magnetization transfer. (B) Theoretical inversion transfer curves for a two-site equilibrium as expressed through Equations (3.29)–(3.30) with  $k_{AB} = 0.5 \text{ s}^{-1}$ ,  $k_{BA} = 1.0 \text{ s}^{-1}$ ,  $T_{1A} = 5.0 \text{ s}$ ,  $T_{1B} = 2.0 \text{ s}$  and  $M_{zA}^0/M_{zB}^0 = 2.0$ . Note that the unidirectional fluxes are identical.

Because the relaxation properties of nucleus B do not have any effect on resonance A, the evolution curve for resonance A will be a single-exponential curve with a time constant solely determined by the relaxation properties of nucleus A (i.e. by the inherent relaxation time  $T_{1A}$  and the exchange rate constant  $k_{AB}$ ). Figure 3.12A shows a typical saturation recovery dataset obtained from rat skeletal muscle in which the  $\gamma$ -ATP resonance is selectively saturated. For long saturation times, Equation (3.39) reduces to:

$$\frac{M_{zA}(\infty)}{M_{zA}^0} = \frac{1}{R_A T_{1A}} = \frac{1}{1 + k_{AB} T_{1A}} \quad (3.40)$$

If  $T_{1A}$  is known from a different experiment, the rate constant  $k_{AB}$  may be derived from a single experiment with a long saturation time, i.e. the so-called steady-state ST experiment. When the entire dataset is recorded the technique is often referred to as time-dependent saturation transfer. When  $T_{1A}$  is not known, the entire evolution curve described by Equation

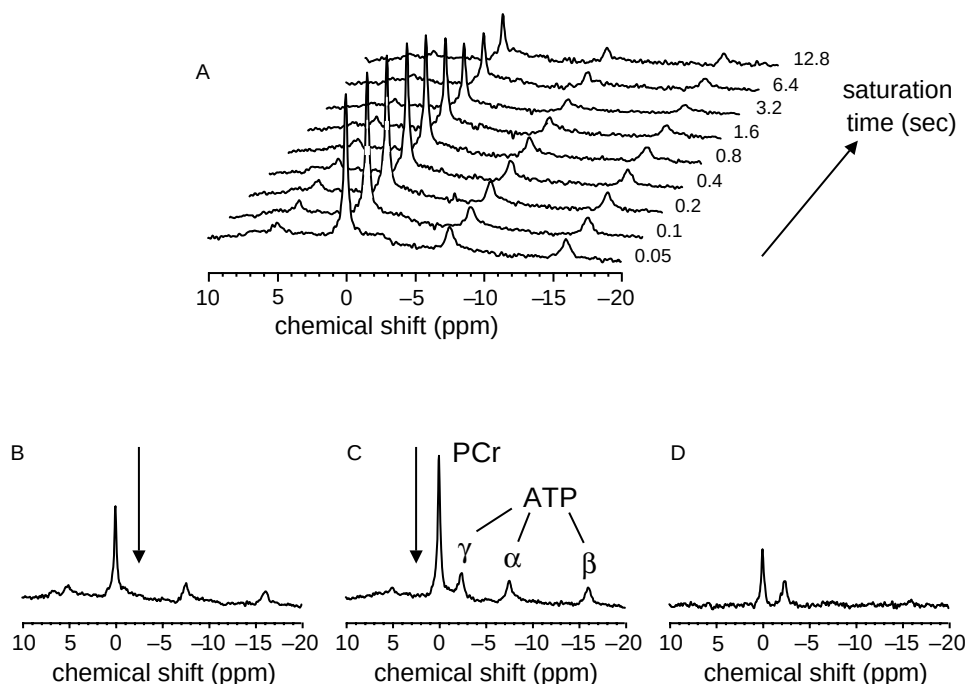


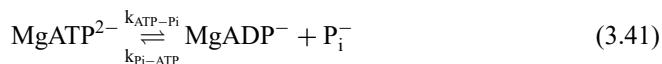
**Figure 3.12** (A) Saturation transfer dataset obtained from rat skeletal muscle in vivo at 4.7 T. The  $\gamma$ -ATP resonance was selectively saturated for a specific, but variable saturation time, resulting in a decrease of the PCr resonance intensity. (B) Saturation transfer spectrum with a 6.4 s saturation of the  $\gamma$ -ATP resonance. (C) Identical experiment as shown in (B), except that the saturation pulse was applied at a mirror position relative to PCr. (D) Subtraction of (B) from (C) gives a spectrum without effects of nonspecific saturation, but with effects due to magnetization transfer.

(3.39) needs to be sampled after which both  $k_{AB}$  and  $T_{1A}$  can be obtained by data fitting. Alternatively,  $T_{1A}$  can be measured in a separate experiment, for instance with an inversion recovery sequence (Figure 3.10D) combined with the selective saturation of reactant B. The saturation ensures that the recovery curve is single-exponential recovering with a  $R_A$  relaxation rate. The magnetization ( $M_{ZA}(\infty)/M_{ZA}^0$ ) at long inversion times equals  $1/(R_A T_{1A})$  and can be used to estimate  $T_{1A}$ .

Saturation is never perfectly selective, i.e. when saturating the  $\gamma$ -ATP resonance there will also be some saturation of the PCr resonance due to ‘RF bleedover’ (Figure 3.12B). If not compensated for, this unwanted saturation will lead to incorrect  $k_{AB}$  and  $T_{1A}$  values. Compensation can be achieved by performing a second experiment in which the saturation is performed at a resonance frequency ‘mirrored’ with respect to the PCr resonance frequency (Figure 3.12C). In this experiment, no ST effects will occur, but there will be RF bleedover effects on the PCr signal. Subtracting the experiment will give a difference spectrum representing the MT without any effects of RF bleedover (Figure 3.12D).

### 3.3.4 ATPases

Selective perturbation of the  $\gamma$ -ATP resonance in a  $^{31}\text{P}$  NMR spectrum allows the determination of the unidirectional rate constant from PCr to ATP, i.e.  $k_{\text{PCr-ATP}}$ . Determining the reverse rate constant, i.e.  $k_{\text{ATP-PCr}}$ , will result in the observation that  $k_{\text{PCr-ATP}}[\text{PCr}] \neq k_{\text{ATP-PCr}}[\text{ATP}]$ . This is because it was assumed that the PCr/ATP system can be described by a two-site chemical exchange reaction. However, for a complete description of the *in vivo* situation the exchange reaction given by Equation (3.38) needs to be extended with:



as catalyzed by ATPases and several other enzymes. Saturation of the PCr will lead to an underestimation of  $k_{\text{ATP-PCr}}$ , because the ATP is not only supplied with perturbed magnetization from PCr, but also with unperturbed, fully relaxed magnetization from inorganic phosphate,  $\text{P}_i$ . This problem can be alleviated, as described by Ugurbil [75], by incorporating an additional saturation field. By continuously saturating the  $\text{P}_i$  resonance, the ATP pool is not longer supplied by magnetization from the  $\text{P}_i$  pool. The three-site exchange has effectively been reduced to a two-site chemical exchange process.

Rather than being a complication, the exchange reaction given by Equation (3.41) also holds unique information on the ATP synthesis rate. Following selective saturation of the  $\gamma$ -ATP resonance, any decrease in the  $\text{P}_i$  resonance is proportional to the ATP synthesis rate. Utilizing the improved sensitivity of high magnetic fields, Lei *et al.* [76] were able to detect significant changes in the small  $\text{P}_i$  resonance and showed that the calculated ATP synthesis rate is in excellent agreement with previous measurements of glucose consumption rates.

So far most of the applications of  $^{31}\text{P}$  MT have been triggered by the quest to understand the fundamental energetic processes *in vivo*. Especially the abundant CK/PCr system has been extensively studied, because of its central role in energy metabolism. The CK substrates, PCr and ATP are readily observed by  $^{31}\text{P}$  NMR and have  $T_1$  relaxation rates that are in the same order as the unidirectional rate constants of the equilibrium reaction. These studies have experienced a new impulse by the development of transgenic mice which selectively miss one or more of the CK isoenzymes [77, 78]. The combination of biogenetics and *in vivo* NMR will undoubtedly find a wide range of applications in the near future. The combination of MT and the CK reaction *in vivo* can also be utilized to obtain dynamic information on energy metabolism *in vivo*. MT experiments have been performed during resting and stimulated conditions in several systems, like in animal skeletal muscle during muscle contraction [79, 80] and in the human cortex during visual stimulation [81]. The MT experiment has also been used to establish a clinically relevant relation between the cardiac post ischemic CK reaction velocity and the severity of ischemia in perfused hearts [82]. By combining MT with MRI techniques, Hsieh and Balaban [83] were able to spatially map the reaction velocity of CK. More recently, the MT experiment has been combined with  $^{13}\text{C}$  NMR to study the 2-oxoglutarate/glutamate exchange in rat brain *in vivo* [84, 85].

The described MT techniques are, in principle, all capable of measuring the kinetic parameters involved in an exchange process, but there are some specific advantages and disadvantages when they are applied *in vivo*. The IT and ST techniques are both very robust in obtaining the kinetic parameters. Both techniques take approximately the same

amount of measurement time ( $\sim 45$ – $90$  min), whereby IT has a larger dynamic range than ST. However, the IT dataset allows the fitting of all four kinetic parameters simultaneously, which is impossible for the ST technique. In some cases, a disadvantage of IT is that the MT depends on the concentration of the exchange partner. The steady-state ST technique is considerably faster ( $\sim 10$ – $20$  min), but can only be used when the intrinsic relaxation parameters are already known. 2DES is the recommended technique when studying unknown or multi-site exchange processes, since all exchange processes are detected simultaneously. Furthermore, the presence of a cross peak proves the presence of chemical exchange. However, the recording of 2D exchange spectra is time-consuming ( $\sim 90$ – $180$  min) and quantification is cumbersome, preventing widespread use *in vivo*.

The results obtained with different MT techniques are not always in agreement. While ST experiments have measured a greater forward than reverse rate for the CK reaction in muscle, brain and heart [79, 86, 87], IT and 2D spectroscopy have indicated equal rates. This discrepancy may be explained in terms of the type of perturbation employed. While ST continuously perturbs the labeled resonance, 2D NMR spectroscopy and IT are essentially pulse-labeling methods. This difference may lead to a different sensitivity of detecting small exchanging metabolite pools, since these will be continuously labeled in ST experiments but not in 2D NMR spectroscopy and IT experiments. This could also explain the detection of an apparent exchange between the  $\gamma$ - and  $\beta$ -ATP resonances with ST, while not observed with pulse-labeling techniques.

### 3.3.5 Fast Magnetization Transfer Methods

While MT techniques can provide unique information about metabolic fluxes noninvasively *in vivo*, animal and particular human applications have been rather limited due to long experimental acquisition times. The most straightforward MT technique, i.e. ST, requires the acquisition of two NMR spectra under fully relaxed conditions with selective saturation of one resonance (and a control saturation at a mirror position). These two experiments can become prohibitively long as a result of the characteristically long  $^{31}\text{P}$  longitudinal relaxation times. Furthermore, since  $T_{1A}$  is typically not known, a separate  $T_1$  relaxation measurement is required, making the measurements impractical for most clinical studies. The experimental duration can be decreased by acquiring nonlocalized  $^{31}\text{P}$  NMR spectra, utilizing the increased sensitivity at high magnetic fields [76] or acquiring the data under partial saturating conditions.

A novel technique that significantly increases the temporal resolution was proposed by Bottomley *et al.* [88]. It is well-known that the SNR of a NMR experiment is optimal when data can be acquired continuously, a condition which can be approximated experimentally by employing short repetition times TR and small nutation angles  $\alpha$ , equal to the Ernst angle (see also Section 1.11). Furthermore, the ST experiment essentially boils down to the measurement of three independent parameters,  $M_{ZA}(\infty)$ ,  $M_{ZA}^0$  and  $R_A$ , which can be achieved in four experiments; a two-point  $T_1$  measurement based on two different nutation angles (see Exercise 3.5) in the presence of saturation to obtain  $M_{ZA}(\infty)$  and  $R_A$  and a two-point  $T_1$  measurement in the absence of saturation to obtain  $M_{ZA}(0)$ . It has been shown that this four-angle saturation transfer (FAST) technique obtains identical values for  $k_{AB}$  as conventional ST in a small fraction of the time. FAST makes the assumption of single-exponential  $T_1$  signal recovery. While this is valid when selectively saturating one

resonance ( $T_{\text{observed}} = 1/R_A$ ), the magnetization recovers bi-exponentially during a regular inversion recovery experiment. Under most conditions this results in a small error in the calculated  $k_{AB}$  (<5 %), but it is an inherent assumption that one needs to be aware of. The conventional saturation recovery does not make this assumption since  $M_{ZA}(\infty)/M_{ZA}^0$  is measured at very long TR and the  $T_1$  is measured in the presence of saturation.

### 3.3.6 Off-resonance Magnetization Transfer

In the preceding sections, MT was described between compounds in acid–base exchange reactions (used for pH determination as detailed in Section 2.3.2) and enzyme-catalyzed exchange reactions. In 1989, Wolff and Balaban reported a new MT mechanism between mobile, NMR observable water and bound, NMR unobservable water [89]. As shown in Figure 3.7, tissue water exists in a number of environments where it has different rotational mobility. Water in the cytosol, extracellular fluid and CSF is highly mobile with a very short rotation correlation time. Dipolar relaxation theory (Figure 3.4) predicts that this ‘bulk’ water has a relatively long  $T_2$  relaxation time constant, hence giving rise to a narrow NMR resonance. Water in protein hydration layers is relatively immobile with longer rotation correlation times. The  $T_2$  relaxation of this ‘bound’ water can be so short that the corresponding NMR resonance spans 10–100 kHz. All tissue water combined thus gives a NMR spectrum consisting of a narrow resonance from the mobile water on top of a very broad (NMR unobservable) resonance from the immobile water (Figure 3.13A middle graph). However, this immobile water pool can be studied through changes in the signal from the mobile water pool using the technique of off-resonance magnetization transfer [89], in which a saturation pulse is applied at a frequency far enough off-resonance from the mobile spins such that the narrow resonance is not affected directly. In general, water in different compartments is in rapid exchange, either through proton exchange or cross-relaxation, such that saturation of the immobile spins lead to a signal decrease of the mobile spins (Figure 3.13A right graph). When the experiment is repeated for different saturation frequency offsets, a so-called Z-spectrum is obtained as shown in Figure 3.13A (left graph). When the saturation frequency is close to the mobile spins, the narrow resonance is directly affected according to:

$$\frac{M_s}{M_0} = \frac{1 + \Delta\omega^2 T_2^2}{1 + \Delta\omega^2 T_2^2 + \gamma^2 B_1^2 T_1 T_2} \quad (3.42)$$

However, the direct saturation effect becomes negligible beyond a few kHz, such that any signal decrease can be attributed to MT from the immobile to the mobile water pools. Using equations similar to Equations (3.27)–(3.35), the Z-spectrum can be quantitatively evaluated to obtain relaxation parameters of the immobile water protons, as well as the exchange rate between and the relative fractions of the pools.

Wolff and Balaban combined the off-resonance MT technique with MRI to obtain MT image contrast, different from conventional  $T_1$ ,  $T_2$  or proton density contrast. Figure 3.14A shows a single-slice spin-echo image of cat brain with experimental hydrocephalus in the absence of off-resonance saturation. In a second scan, the same experiment is performed, but now with a 3 s saturation pulse ( $\gamma B_1/2\pi = 350$  Hz) irradiating 10 kHz off-resonance. Figure 3.14C shows a calculated image of the ratio between saturation ( $M_s$ ) and no saturation ( $M_0$ ),

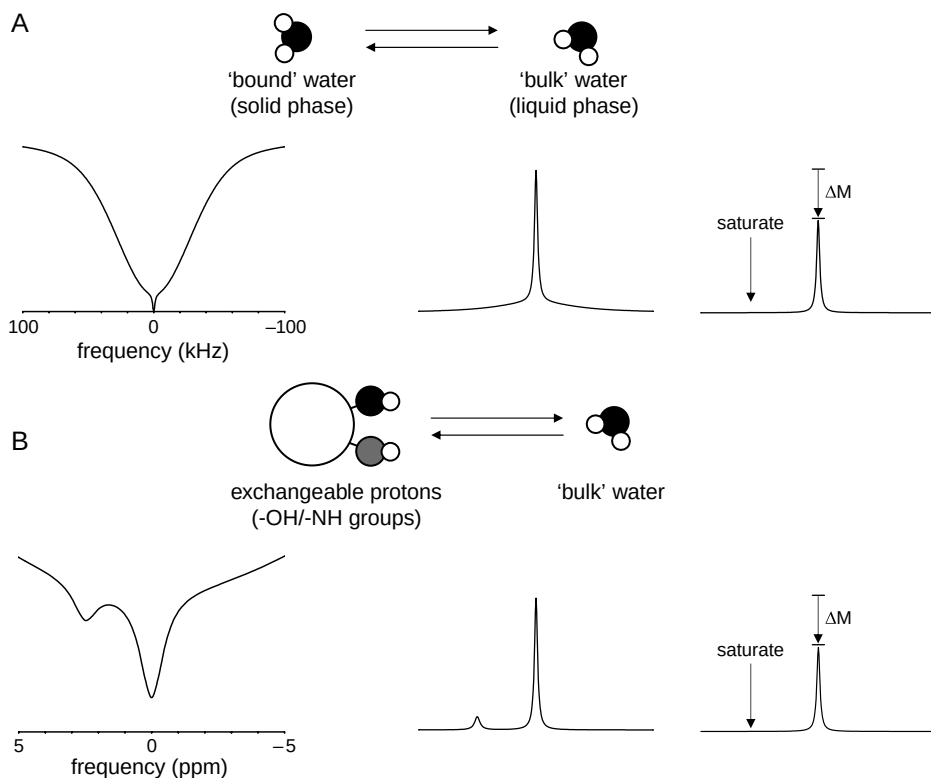


**Figure 3.13** Off-resonance MT experiments. (A) Off-resonance saturation of a system that entails exchange between mobile and immobile pools will generally lead to a signal-frequency curve (also called Z-spectrum) that is symmetrical and spans over 10–100 kHz. (B) Off-resonance saturation of a system that entails exchange between chemical groups, like –OH or –NH exchangeable protons, will lead to an asymmetrical Z-spectrum. Saturation at the chemical shift position of the exchangeable protons leads to a decrease of the water resonance and typically only spans several kHz. In addition to the chemical exchange saturation transfer (CEST) effect, off-resonance saturation also leads to the symmetrical MT effect between mobile and immobile protons.

also known as a magnetization transfer ratio (MTR) image. The CSF, having a low protein content, shows a ratio close to unity, whereas ratios in brain tissue vary between 0.4 and 0.9. A more quantitative representation is given by the rate constant of cross-relaxation,  $k$ , which can be calculated according to Equation (3.40), i.e.:

$$k = \frac{1}{T_{1,s}} \left( 1 - \frac{M_s}{M_0} \right) \quad (3.43)$$

where  $T_{1,s}$  is the  $T_1$  relaxation time in the presence of off-resonance saturation. Figure 3.14B shows a calculated  $T_{1,s}$  map obtained with an inversion recovery sequence in the presence of off-resonance saturation. Figure 3.14D shows the calculated  $k$  map. The CSF now exhibits



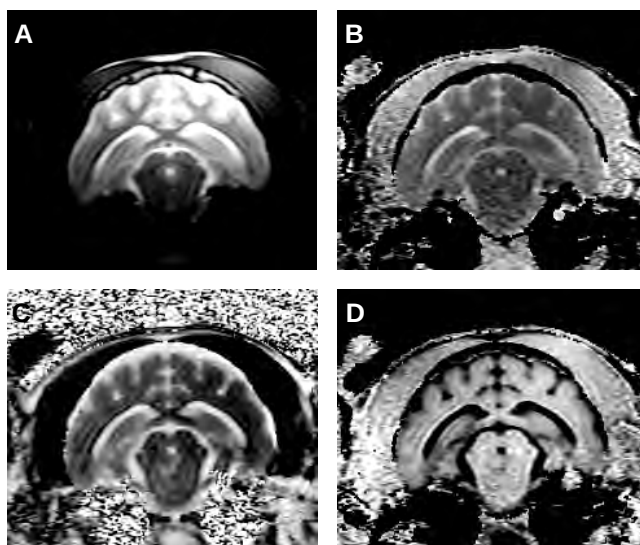


**Figure 3.14** MT contrast imaging on cat brain with kaolin-induced hydrocephalus. (A) Regular (adiabatic) spin-echo image ( $TR = 5000$  ms,  $TE = 50$  ms) obtained with a surface coil. (B)  $T_1$  relaxation time map, obtained by fitting five inversion recovery images (in the presence of off-resonance saturation) with varying recovery times. (C) Ratio image of the signal with and without 10 kHz off resonance saturation. (D) Rate constant map as calculated from (B) and (C) according to Equation (3.40). Rate constants vary between  $0.2\text{ s}^{-1}$  and  $0.8\text{ s}^{-1}$ . CSF exhibits a rate constant of almost zero. (Courtesy of K. P. J. Braun.)

a cross-relaxation rate constant close to zero, while  $k$  ranges from  $0.2$  to  $0.8\text{ s}^{-1}$  in brain tissue. The unique image contrast of MT imaging has found widespread applications in the clinical evaluation of neurological disorders, especially for multiple sclerosis and other white matter lesions and tumors (see [90] for review).

In 1994, Dreher *et al.* [91] demonstrated that the off-resonance MT effect can also be observed for total creatine. This observation has been confirmed and extended to other metabolites including lactate and glutamate [92–96]. Figure 3.15A shows an off-resonance MT dataset for lactate in a C6 glioma, implanted in rat brain [93]. The MT experiment was combined with spatial localization and spectral editing to eliminate signal from extracranial fat and mobile lipids in the tumor, respectively. Figure 3.15B shows the integrated lactate signal intensities together with the theoretical curve for direct saturation. This curve was calculated according to Equation (3.42) with  $(\gamma B_1/2\pi) = 450\text{ Hz}$ ,  $T_1 = 1730\text{ ms}$  and  $T_2 = 202\text{ ms}$ . A significant discrepancy between the theoretical curve for direct saturation and the experimentally measured data points indicates the presence of cross-relaxation between mobile and immobile (NMR invisible) metabolite pools. A fit of the experimental data (solid line) suggests a bound fraction of only  $0.1\%$  with an exchange rate  $3.5\text{ s}^{-1}$ . Therefore, a small bound fraction may still cause a significant MT effect. The bound fraction of  $0.1\%$  will not affect the quantification of the lactate resonance. However, the MT effect will have important consequences if the NMR experiment for metabolite quantification is

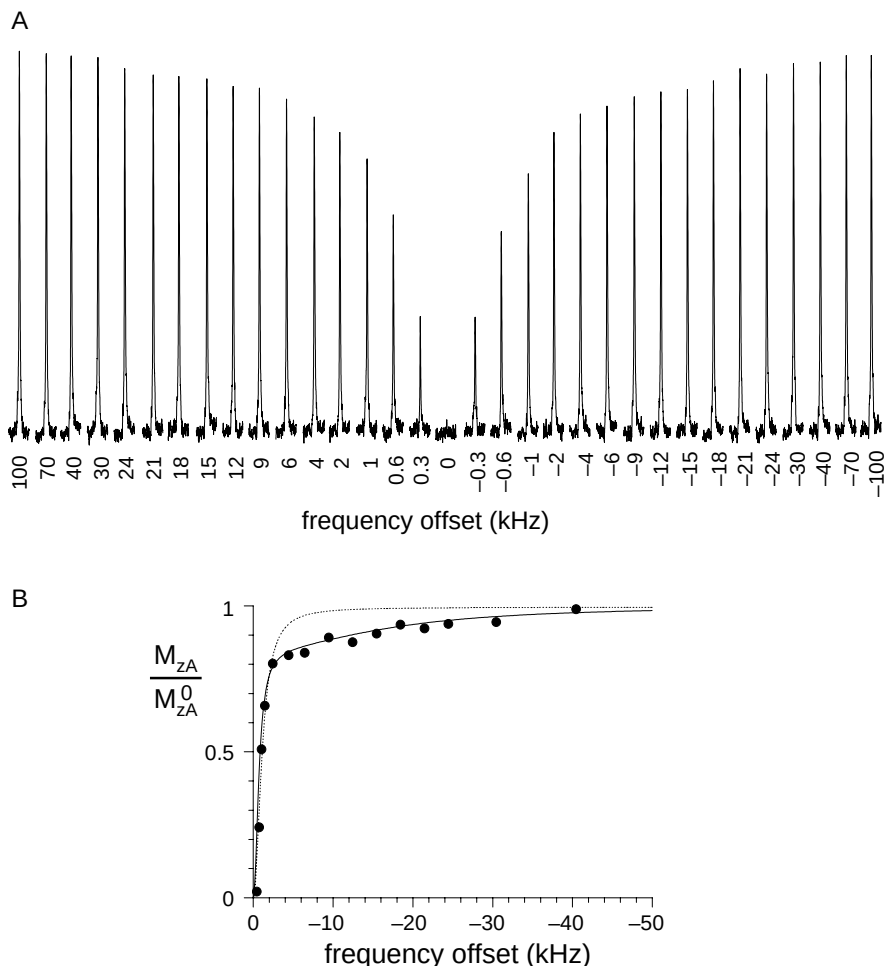


**Figure 3.15** (A) Off-resonance MT dataset of lactate obtained from a C6 glioma in rat brain in vivo. Each spectrum is the result of 32 accumulations with a repetition time of 6 s, using a 3 s off-resonance saturation RF field ( $\gamma B_1/2\pi = 450$  Hz). Unambiguous lactate detection was achieved by a combination of spatial localization and spectral editing. (B) Integrated intensities (dots) of the lactate resonance shown in (A) as a function of the frequency offset of the saturation RF field. The dotted line depicts the theoretical curve for direct saturation. The solid curve is the best fit of the experimental data (Courtesy of Y. Luo and M. Garwood.)

performed incorrectly. In the early *in vivo*  $^1\text{H}$  MRS experiments, water suppression was often performed with a long, low-power presaturation pulse on the water. In the presence of cross-relaxation as shown in Figure 3.15, this would lead to a significant reduction in the lactate resonance and, consequently to an underestimation of the lactate concentration, even though direct saturation effects are negligible.

Soon after the initial reports on metabolite MT effects, it became apparent that water is an important mediator in the observed signal modulations [94, 97–99]. Figure 3.16B

shows Z-spectra for NAA, total creatine, and total choline. When the low-power saturation pulse is on-resonance for a particular metabolite, the signal intensity reduces to zero due to direct saturation. For the total creatine resonance there is a small off-resonance MT effect visible (i.e. the signal intensity at +1 ppm is lower than at -1 ppm). However, in addition there is a strong effect on the total creatine resonance when the water resonance is saturated. A similar, but smaller effect is visible for NAA, but is completely absent for the total choline resonance. A likely explanation (Figure 3.16A) is that the exchangeable protons in the amide groups of creatine and phosphocreatine are in chemical exchange with the bulk water protons, which in turn experience MT effects from the bound water through cross-relaxation and chemical exchange. The effect on the amide protons is relayed to the creatine methyl group by intramolecular cross-relaxation. This observation was further confirmed by significant decreases in the total creatine and NAA resonance upon selective inversion of the water [94, 97, 98]. Therefore, even pulsed water suppression techniques

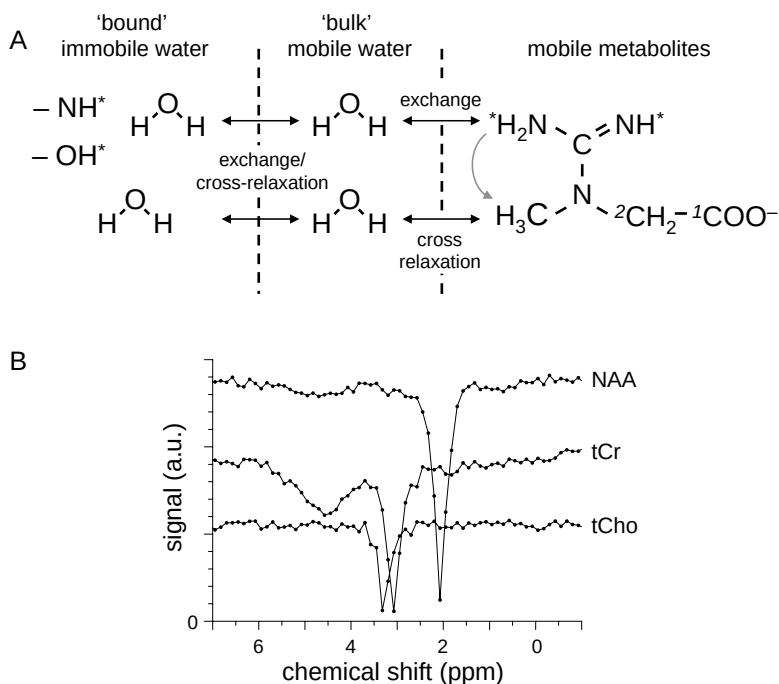


**Figure 3.16** (A) Water-mediated MT effects for total creatine (tCr) methyl protons. Saturation of the water protons leads through chemical exchange and cross-relaxation to a decrease in the NMR signal of the tCr methyl protons. (B) Off-resonance MT dataset of N-acetyl aspartate (NAA), tCr and total choline (tCho) obtained from rat brain in vivo. Each spectrum is the result of 64 accumulations with a repetition time of 6 s, using a 4.5 s off-resonance saturation RF field ( $\gamma B_1/2\pi = 22$  Hz). The tCr resonance shows a significant decrease in signal intensity upon saturation of the water, an effect absent for the NAA and tCho methyl resonances, possibly due to the absence of nearby exchangeable protons [Reproduced with permission from R. A. de Graaf et al., *Magn. Reson. Med.* **41**, 1136–1144 (1999), Copyright John Wiley & Sons, Inc.]

(see Chapter 6) can affect metabolite ratios *in vivo*, although the most commonly used CHESS method [100] was found to have minimal effects [94].

### 3.3.7 Chemical Exchange Dependent Saturation Transfer

In the early 1990s, Balaban and coworkers [101–103] demonstrated the detection of low-concentration metabolites with greatly enhanced sensitivity through a MT effect on the water which is now known as the chemical exchange dependent saturation transfer (CEST) effect. The CEST effect is similar to that described in the previous section and Figure 3.16A. The chemical exchange between a compound and water is referred to as slow when  $\Delta\omega\tau \gg 1$ , where  $\Delta\omega$  is the chemical shift difference between the two exchanging compounds and  $\tau$  is the ‘lifetime’ at either exchange site. The lifetime inversely proportional to the exchange rate between the compounds. Slowly exchanging compounds have two distinct chemical shifts, as opposed to a single averaged chemical shift for fast exchanging compounds as observed for  $P_i$  (see Chapter 2). Saturating at the chemical shift position of the compound of interest will lead to a signal decrease of the water (Figure 3.13B). This has been used to detect low-concentration macromolecular signals with the high-sensitivity of water [104, 105]. In addition to the chemical shift specific CEST effect, the water will also experience the regular off-resonance MT effect as described in Section 3.3.6. However, the two effects are readily separated because (1) the CEST effect only spans a frequency range of several kHz (as opposed to  $>50$  kHz for regular MT) and (2) it is asymmetric around the water frequency (whereas the regular MT effect is largely symmetric).

The CEST effect can be enhanced by increasing the  $\Delta\omega\tau$  product. This has been achieved through the use of paramagnetic compounds, specifically designed to bind specific substrates or pools. The techniques are collectively known as paraCEST [106–108]. For extensive reviews on CEST and related phenomena the reader is referred to the literature [105].

## 3.4 Diffusion

### 3.4.1 Principles of Diffusion

Diffusion is the random translational (or Brownian) motion of molecules or ions that is driven by internal thermal energy. In 1855 Fick devised two differential equations that quantitatively described diffusion of molecules through ultra-thin membranes [109, 110]. According to Fick’s first law of diffusion, the flux of particles  $\mathbf{J}$  (i.e. the number of particles that moves through a 2D plane per unit of time), at spatial position  $\mathbf{r}$  and time  $t$ , is directly proportional to the concentration gradient  $\nabla c$  according to:

$$\mathbf{J}(\mathbf{r}, t) = -D\nabla c(\mathbf{r}, t) \quad (3.44)$$

where  $D$  is the diffusion coefficient and  $c(\mathbf{r}, t)$  is the concentration of particles. Conservation of mass is expressed by the equation of continuity:

$$\frac{\partial c(\mathbf{r}, t)}{\partial t} = -\nabla \cdot \mathbf{J}(\mathbf{r}, t) \quad (3.45)$$

Combining Equations (3.44) and (3.45) then leads to Fick's second law of diffusion:

$$\frac{\partial c(\mathbf{r}, t)}{\partial t} = D \nabla^2 c(\mathbf{r}, t) \quad (3.46)$$

where it is assumed that  $D$  is independent of the concentration. Up to this point, the derivations have assumed a net flux by diffusion over a concentration gradient. To describe diffusion in the absence of internal concentration gradients, like diffusion in pure water, it is convenient to introduce a probability function  $P(\mathbf{r}_0|\mathbf{r}, t)$  which gives the probability of a particle having moved from position  $\mathbf{r}_0$  to position  $\mathbf{r}$  over time  $t$ . For isotropic diffusion,  $P(\mathbf{r}_0|\mathbf{r}, t)$  also obeys Fick's second law of diffusion according to:

$$\frac{\partial P(\mathbf{r}_0|\mathbf{r}, t)}{\partial t} = D \nabla^2 P(\mathbf{r}_0|\mathbf{r}, t) \quad (3.47)$$

Given the starting condition  $P(\mathbf{r}_0|\mathbf{r}, 0) = \delta(\mathbf{r}_0 - \mathbf{r})$ , where  $\delta$  is Dirac's delta function, and the boundary condition  $P(\mathbf{r}_0|\mathbf{r}, 0) \rightarrow 0$  for  $r \rightarrow \infty$ , the solution to Equation (3.47) for unbounded, isotropic diffusion yields a Gaussian dependence on the displacement:

$$P(\mathbf{r}_0|\mathbf{r}, t) = (4\pi Dt)^{-3/2} \exp[-(\mathbf{r} - \mathbf{r}_0)^2/4Dt] \quad (3.48)$$

Note that the probability  $P(\mathbf{r}_0|\mathbf{r}, t)$  depends on displacement  $(\mathbf{r} - \mathbf{r}_0)$ , but not on the initial position  $\mathbf{r}_0$ . Since diffusion is a random process and the displacements are equally probable in all directions, the net (or mean) displacement  $\langle(\mathbf{r} - \mathbf{r}_0)\rangle$  is zero. Therefore, the molecular displacement associated with 3D diffusion is calculated as the average square displacement  $\lambda^2 = \langle(\mathbf{r} - \mathbf{r}_0)^2\rangle$  according to the Einstein–Smoluchowski equation:

$$\lambda^2 = \langle(\mathbf{r} - \mathbf{r}_0)^2\rangle = 6Dt \quad (3.49)$$

Equation (3.49) demonstrates that displacement resulting from diffusion is simply related to the diffusion coefficient and that, for the case of unrestricted diffusion, the average square displacement increases linearly with the diffusion time  $t$  (Figure 3.17). The average square displacements for diffusion in 1D and 2D spaces are given by  $2Dt$  and  $4Dt$ , respectively.

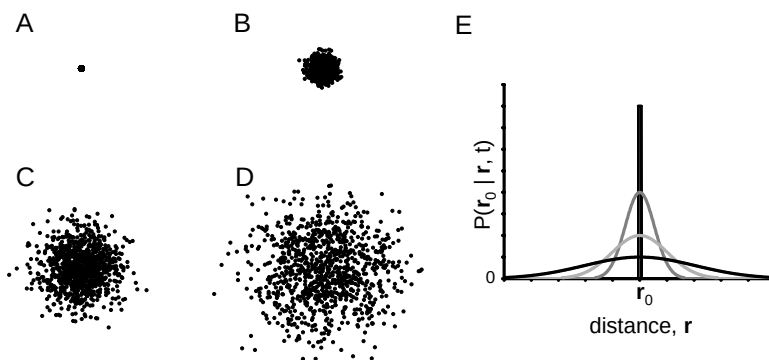


**Figure 3.17** Diffusion in two spatial dimensions. Starting from a single point in space (A) the mean square displacement of diffusing particles increases linearly with time (B–D). Diffusion can be described quantitatively by a probability function  $P(\mathbf{r}_0|\mathbf{r}, t)$ , see Equation (3.48), as is graphically depicted in (E).

Deviations from the Gaussian distribution function of Equation (3.48) will arise if the translational displacements are restricted by geometrical constraints (see Section 3.4.4).

### 3.4.2 Diffusion and NMR

Early on it was recognized that NMR is able to accurately measure the self-diffusion of molecules in isotropic liquids. In the classical paper on spin-echoes, Hahn [111] outlined the measurement of diffusion in the presence of a constant background gradient, which was subsequently modified and further developed by Carr and Purcell [112] and others [113]. However, it was not until the development of a pulsed-gradient spin-echo method by Stejskal and Tanner [114] that NMR diffusion measurements became a routine technique to measure molecular self-diffusion coefficients.

Diffusion measurements by NMR rely on the principle of signal loss through diffusion dependent phase dispersal as is qualitatively illustrated in Figure 3.18. Figure 3.18A shows the transverse magnetization of three point samples in the XZ plane at the end of the positive gradient lobe applied in the z direction. As expected, the spins acquire a position-dependent phase  $\phi_1$  according to  $\phi_1 = \gamma r G \delta$ , where  $r$  is the position of the point sample and  $G$  and  $\delta$  the magnetic field gradient strength and duration, respectively. A negative gradient of equal duration but opposite amplitude, applied following a delay  $\Delta$ , induces as position-dependent phase  $\phi_2$ . When all spins are stationary (Figure 3.18B),  $\phi_1 = -\phi_2$ , such that at the time of data acquisition all spins have zero phase ( $\phi_1 + \phi_2 = 0$ ). Since the NMR signal represents the sum of all spins (or in this case point samples) in the object under investigation, the absence of phase dispersal guarantees maximum signal (ignoring  $T_1$ ,  $T_2$  and magnetic field homogeneity effects).

However, when the spins change spatial position during the delay  $\Delta$ , the phase induced by the second magnetic field gradient no longer cancels the phase induced by the first magnetic field gradient, i.e.  $\phi_1 + \phi_2 \neq 0$  (Figure 3.18C). Therefore, application of magnetic field gradients in the presence of diffusion leads to phase dispersal across the sample, which in turn will lead to phase cancellation and hence signal loss when signal is acquired from the entire sample. While the exact functional form for the diffusion dependent signal loss will be derived next, it is straightforward to see that the function will be dependent on the diffusion coefficient, the area of the magnetic field gradient (i.e. amplitude and duration) as well as on the separation between the magnetic field gradients. For example, increasing the delay  $\Delta$  increases the diffusion time, which according to Equation (3.49) increases the mean square displacement. As the spins move further away from the starting position, the phase reversal becomes worse, leading to more phase cancellation and hence more signal loss.

It should be noted that macroscopic motion does not lead to signal loss *per se* (Figure 3.18D). In the presence of macroscopic motion (subject motion or flow) during the delay  $\Delta$ , all spins move by the same spatial distance. As a result, the spins will not have zero phase at the end of the second magnetic field gradient, but because the spatial displacement was constant (as opposed to random for diffusion), all spins have acquired the same (nonzero) phase (i.e.  $\phi_1 + \phi_2 = \Delta\phi = \text{constant}$ ). Since there is no phase dispersal across the sample, there will not be any macroscopic signal loss. However, when the motion changes from scan to scan, the net phase  $\Delta\phi$  will also change. During signal averaging in MRS this scan-to-scan phase variation can lead to signal loss. For MRI applications any

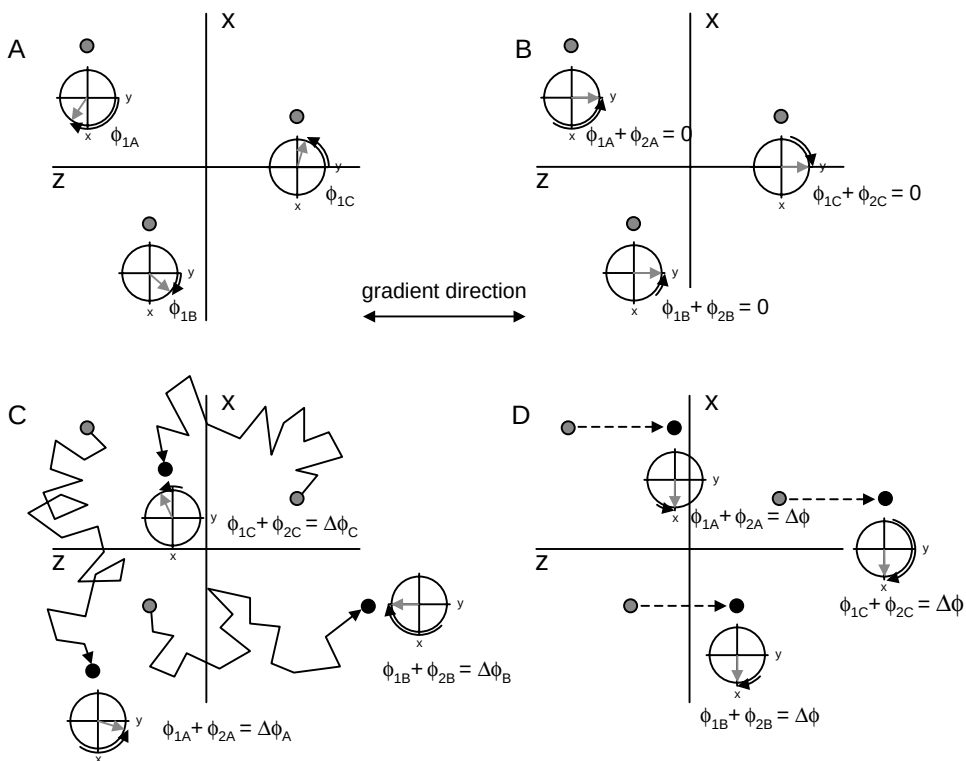


**Figure 3.18** Schematic representation of the relation between Brownian motion and the acquired phase shift of a magnetic field gradient in the z direction. (A) Application of a magnetic field gradient leads to a position-dependent phase shift. (B) For stationary spins, the acquired phase can be perfectly refocused with a second magnetic field gradient of equal, but opposite amplitude. (C) For mobile, diffusing spins the spatial position changes over time, such that the second magnetic field gradient applied at a later time does not perfectly refocus the phase, leading to a randomly distributed net phase and consequently signal loss over a macroscopic volume. (D) When all spins change their spatial position coherently, for example due to macroscopic motion of flow, the net phase is constant across a macroscopic volume.

scan-to-scan phase instability will lead to image artifacts (ghosting) in the phase-encoding direction.

In order to quantitatively describe diffusion in the presence of time-varying magnetic field gradients, it is most convenient to follow the formalism outlined by Torrey [115], which was subsequently used by Stejskal and Tanner [114]. The Bloch equations (see Section 1.6) extended to include the effects of diffusion are given by:

$$\frac{\partial \mathbf{M}(\mathbf{r}, t)}{\partial t} = \gamma \mathbf{M} \times \mathbf{B}(\mathbf{r}, t) - \frac{M_x \hat{x} + M_y \hat{y}}{T_2} - \frac{(M_z - M_0) \hat{z}}{T_1} + D \nabla^2 \mathbf{M} \quad (3.50)$$

where the macroscopic magnetization  $\mathbf{M}$  is the vector sum of  $M_x$ ,  $M_y$  and  $M_z$  along the unit axes  $\hat{x}$ ,  $\hat{y}$  and  $\hat{z}$ , respectively. In case of anisotropic diffusion the last term of

Equation (3.50) should be replaced with  $\nabla \mathbf{D} \cdot \nabla \mathbf{M}$ . Note the similarity of the diffusion part of Equation (3.50) with Fick's second law of diffusion given by Equation (3.46). Equation (3.50) can be simplified by defining a complex magnetization  $M_{xy} = M_x + iM_y$  and by assuming that the magnetic field  $B_0$  and magnetic field gradient  $\mathbf{G}$  are along the  $z$  axis, so that  $B_x = B_y = 0$  and  $\mathbf{B}_z = B_0 \hat{\mathbf{z}} + \mathbf{rG}$ :

$$\frac{\partial M_{xy}}{\partial t} = i\omega_0 M_{xy} - \frac{M_{xy}}{T_2} - i\gamma \mathbf{rG} M_{xy} + D \nabla^2 M_{xy} \quad (3.51)$$

The part not directly related to diffusion can be eliminated from Equation (3.51) by means of the substitution:

$$M_{xy} = M_{xy}^* e^{i\omega_0 t - t/T_2} \quad (3.52)$$

to yield the equation:

$$\frac{\partial M_{xy}^*}{\partial t} = -i\gamma \mathbf{rG} M_{xy}^* + D \nabla^2 M_{xy}^* \quad (3.53)$$

The solution to Equation (3.53) in the presence of diffusion and time-varying gradients is given by:

$$M_{xy}^* = e^{-D \int_0^t F^2(t') dt'} \quad (3.54)$$

where

$$F(t') = \int_0^{t'} \gamma G(t'') dt'' \quad (3.55)$$

With Equation (3.54) the effect of diffusion on the detected NMR signal from any NMR sequence with any magnetic field gradient combination can be quantitatively calculated. First consider the basic Hahn spin-echo method (Figure 3.19A) in the presence of a constant background gradient  $G_0$ . Between the  $90^\circ$  excitation and the  $180^\circ$  refocusing pulses (i.e. during the first half of the echo time TE), Equation (3.55) evaluates to:

$$F(t) = \int_0^t \gamma G_0 dt \quad \text{for } 0 \leq t \leq TE/2 \quad (3.56)$$

The  $180^\circ$  refocusing pulse causes any acquired phase shift to be reset by twice the amount, such that Equation (3.55) during the second half of the echo-time evaluates to:

$$F(t) = -F(TE/2) + \int_{TE/2}^t \gamma G_0 dt \quad \text{for } TE/2 \leq t \leq TE \quad (3.57)$$

Substituting Equations (3.56) and (3.57) into Equation (3.54) gives the signal attenuation due to diffusion at echo time TE:

$$M_{xy}^* = e^{-\frac{1}{12} D \gamma^2 G_0^2 TE^3} \quad (3.58)$$



The well-known  $TE^3$  dependence of the spin-echo signal amplitude in the presence of magnetic field inhomogeneity and diffusion can lead to a significant underestimation of measured  $T_2$  relaxation times when a Hahn spin-echo is used for refocusing.

When the calculation as outlined for a regular spin-echo is repeated for a CPMG spin-echo pulse train with  $n$   $180^\circ$  refocusing pulses, such that  $TE = nTE_{\text{CPMG}}$ , the signal attenuation at the top of the spin-echo equals:

$$M_{xy}^* = e^{-\frac{1}{12n^2} D \gamma^2 G_0^2 TE^3} \quad (3.59)$$

Therefore the sensitivity of a CPMG spin-echo towards diffusion is greatly reduced and approaches zero when  $n \rightarrow \infty$  (or  $TE_{\text{CPMG}} \rightarrow 0$ ). This feature has been used by Michaeli *et al.* [66] to demonstrate that the decrease in observed  $T_2$  relaxation times with increasing magnetic fields is largely due to diffusion through increased microscopic magnetic field gradients.

The use of a constant magnetic field gradient for diffusion weighting has several disadvantages. For example, since the background gradient is on during the RF pulses, the RF/gradient combination will lead to slice selection and subsequently lower acquired signal. Increasing the sensitivity towards diffusion can be achieved by increasing the gradient amplitude or duration, both mechanisms leading to signal loss due to a narrower slice and a longer echo-time, respectively. These problems were recognized and solved by Stejskal and Tanner [114, 116] with the introduction of pulsed-gradient methods for the measurement of diffusion.

Figure 3.19B–D shows three commonly used pulsed-gradient methods with constant, halfsine and trapezoidal shaped gradients for the measurement of diffusion as incorporated in a Hahn spin-echo sequence. The amount of diffusion sensitivity introduced by the pulsed magnetic field gradients is often expressed as a so-called  $b$ -value (in  $\text{ms } \mu\text{m}^{-2}$ ), such that the detected signal in the presence of diffusion gradients,  $S(b)$ , relative to the signal without diffusion gradient  $S(0)$  is given by:

$$\ln \left( \frac{S(b)}{S(0)} \right) = -bD \quad (3.60)$$

where the  $b$ -value is given by:

$$b = \int_0^{TE} (F(t))^2 dt \quad (3.61)$$

where  $F(t)$  is given by Equation (3.55). When the phase reversal effect of the  $180^\circ$  refocusing pulse is explicitly taken into account, the spin-echo specific form of Equation (3.61) becomes [114]:

$$b = \int_0^{TE} (F(t))^2 dt - 4f \int_{TE/2}^{TE} F(t) dt + 2f^2 TE \quad (3.62)$$

where  $f$  equals  $F(TE/2)$ . Using Equation (3.61) or (3.62) allows the derivation of the analytical expression for the  $b$ -value for the particular combinations of gradient pairs shown in Figure 3.19. For the constant amplitude gradient pair of duration  $\delta$  and amplitude

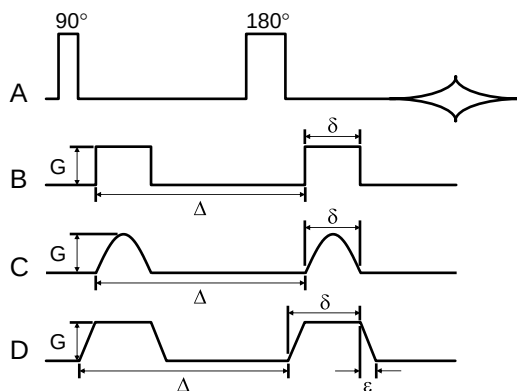


**Figure 3.19** Diffusion sensitized spin-echo experiments. (A) The  $90^\circ$  excitation and  $180^\circ$  refocusing pulses form a spin-echo at TE. (B–D) The pulse sequence can be sensitized for diffusion by the applications of a pair of pulsed gradients surrounding the  $180^\circ$  pulse. Most commonly used gradient wave forms are (B) constant, (C) halfsine and (D) trapezoidal. The exact definition of the pulse duration  $\delta$  and the time between gradient pulses  $\Delta$  are indicated.  $\varepsilon$  is the rise time of the trapezoidal ramp. Quantitative expressions for the corresponding b-values are given by Equations (3.63)–(3.65).

G, separated by a delay  $\Delta$  (Figure 3.19B), the b-value is given by:

$$b = \gamma^2 G^2 \delta^2 \left( \Delta - \frac{\delta}{3} \right) \quad (3.63)$$

Although this b-value is often given in the literature, it can never represent the real value, since it implies an infinitely short gradient risetime. More realistic (i.e. practically feasible) gradient waveforms are halfsine or trapezoidal shaped gradients (Figure 3.19C and D). The b-values of the halfsine and trapezoidal gradient pairs are given by:

$$b = \frac{4}{\pi^2} \gamma^2 G^2 \delta^2 \left( \Delta - \frac{\delta}{4} \right) \quad (3.64)$$

and

$$b = \gamma^2 G^2 \left[ \delta^2 \left( \Delta - \frac{\delta}{3} \right) + \frac{\varepsilon^3}{30} - \frac{\delta \varepsilon^2}{6} \right] \quad (3.65)$$

respectively.  $\varepsilon$  is the gradient rise-time for the trapezoidal gradient waveform. Knowing the exact b-value for a given gradient pair combination allows the determination of the diffusion coefficient. Usually, the diffusion experiment is repeated with different b-values (either by changing G,  $\delta$ , or  $\Delta$ ) to achieve a reasonable span of signal intensities (100 %–30 %) after which the data are fitted to Equation (3.54) [or Equation (3.60)] as shown in Figure 3.20B and C. For the majority of diffusion experiments, the b-value is incremented by changing the gradient amplitude, while keeping the duration and separation identical. The primary reasons for this choice are that for constant  $\delta$  and  $\Delta$ , (1) the echo time can remain constant and (2) the diffusion time remains constant, which is especially important when observing restricted diffusion (see Section 3.4.4).

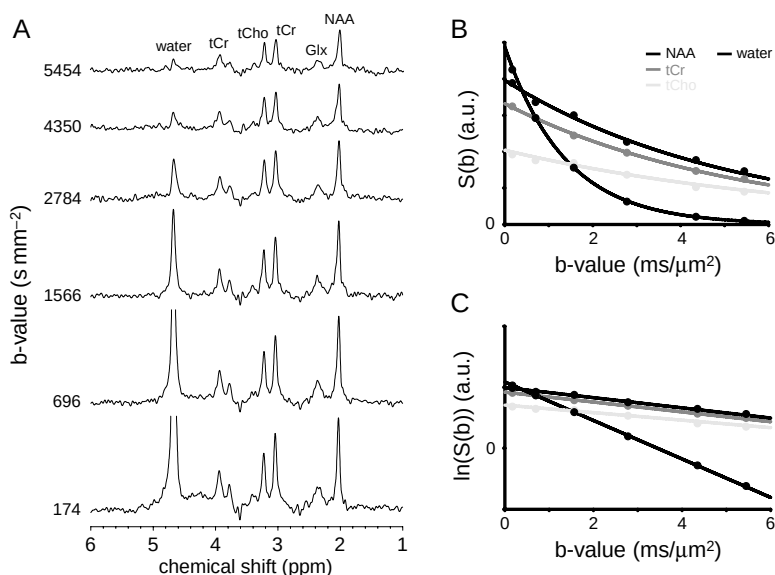


**Figure 3.20** Diffusion-weighted  $^1\text{H}$  NMR spectra of rat brain in vivo acquired at 4.7 T. The spectra were obtained with a diffusion-sensitized PRESS localization sequence ( $TR = 3000$  ms,  $TE = 144$  ms). Water suppression was achieved with the CHESS technique (see Chapter 6). The  $b$ -factors are 174, 696, 1566, 2784, 4350, 5454  $\text{s mm}^{-2}$  ( $\delta = 6$  ms and  $\Delta = 25$  ms) with diffusion weighting increasing from bottom to top. Observed resonances have been assigned to water, total creatine (tCr), choline-containing compounds (tCho), glutamate/glutamine (Glx) and N-acetyl aspartate (NAA). Note that the water signal decreases substantially faster than the metabolites indicating a higher diffusion coefficient. All spectra were subject to the same phase correction. (B) Signal intensity of the major resonances shown in (A) and the exponential fit according to Equation (3.54). (C) Natural logarithm of the signal intensities. The diffusion coefficient is given by the slope according to Equation (3.60).

Figure 3.20A shows a series of diffusion-weighted  $^1\text{H}$  NMR spectra from normal rat brain. Spatial localization was achieved with the PRESS technique (see Chapter 6). Diffusion-sensitizing gradient pairs were positioned around each of the two  $180^\circ$  refocusing pulses. Increasing the diffusion weighting (by increasing the gradient amplitude) leads to increased signal attenuation. The diffusion-weighting increases from bottom to top. From the unsuppressed, residual water signal it can be seen that the diffusion of water is considerably faster than that of the metabolites, as expected. Fitting the experimental signals to Equation (3.60) gives apparent diffusion constants (ADCs) for the major metabolites in the range of  $0.15\text{--}0.17 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$ , while the water ADC amounts to  $0.71 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$  (Figure 3.20B and C).

For accurate diffusion measurements, precise knowledge of the attenuation factor (i.e.  $b$ -value) is required. For simple, nonlocalized diffusion measurements the attenuation factor is given by Equations (3.63)–(3.65), or related expressions. However, for localized measurements (either imaging or localized spectroscopy) an accurate determination of the attenuation factor is more difficult, since all gradients should be taken into account.

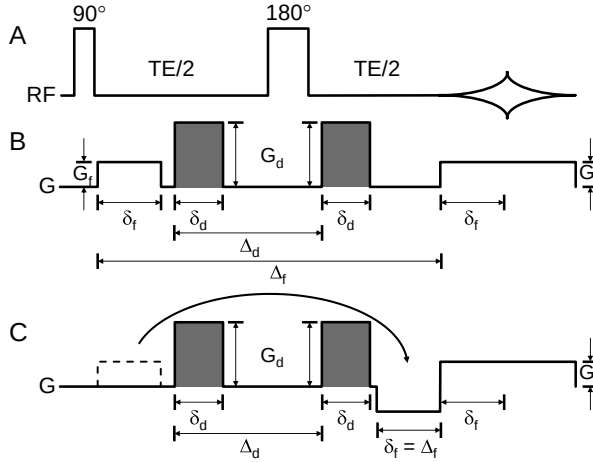


**Figure 3.21** (A) Standard spin-echo MRI sequence using a combination of (B/C) frequency-encoding (white) and diffusion-sensitizing (gray) magnetic field gradients. The imaging gradients are characterized by a duration  $\delta_f$ , amplitude  $G_f$  and separation  $\Delta_f$ , while the diffusion-sensitizing gradients are described by duration  $\delta_d$ , amplitude  $G_d$  and gradient separation  $\Delta_d$ . The weak imaging gradients by themselves do not have a significant effect on the  $b$ -value, because the diffusion gradients are deliberately set to high values for sufficient diffusion weighting. However, temporal mixing of the imaging and diffusion gradients as shown in (B) will lead to significant cross-terms as described in the text. (C) Keeping imaging and diffusion gradient separated in time completely eliminates cross-terms.

Figure 3.21 shows a spin-echo pulse sequence incorporated with a pair of diffusion gradients (of duration  $\delta_d$ , amplitude  $G_d$  and separated by a delay  $\Delta_d$ ). Furthermore, the sequence has two additional gradients for frequency-encoding to produce a 1D image (see Chapter 4 for details on MRI). These frequency-encoding gradients are of duration  $\delta_f$ , amplitude  $G_f$  and separated by a delay  $\Delta_f$ . For the gradient combination shown in Figure 3.21B, the  $b$ -value can be calculated as:

$$b = \gamma^2 G_f^2 \delta_f^2 \left( \Delta_f - \frac{\delta_f}{3} \right) + \gamma^2 G_d^2 \delta_d^2 \left( \Delta_d - \frac{\delta_d}{3} \right) + 2\gamma^2 G_d G_f \delta_d \delta_f \Delta_d \quad (3.66)$$

The total  $b$ -value is the sum of three components. The first term represents the attenuation term due to the MRI frequency-encoding gradients, while the second term is due to the diffusion gradients. The last term is a so-called ‘cross-term’ between the imaging and diffusion gradients. If not accounted for, the cross-terms can induce a significant error in the calculation of the diffusion coefficients. It is almost always possible to redesign a pulse sequence such that cross-terms are minimized or even completely cancelled. The simple gradient order modification as shown in Figure 3.21C leads to a  $b$ -value given by:

$$b = \gamma^2 G_f^2 \delta_f^2 \left( \Delta_f - \frac{\delta_f}{3} \right) + \gamma^2 G_d^2 \delta_d^2 \left( \Delta_d - \frac{\delta_d}{3} \right) \quad (3.67)$$

While the  $b$ -value is still influenced by the frequency-encoding gradients, it does not affect the calculation of the diffusion coefficient. Most commonly, the diffusion gradient

amplitude  $G_d$  is incremented in subsequent experiments, after which the diffusion coefficient is calculated by fitting the  $\ln(\text{signal})/b$ -value curve according to Equation (3.60). The  $b$ -value introduced by the MRI gradients is constant for all  $G_d$  increments and therefore simply shifts the  $\ln(\text{signal})/b$ -value curve, but does not change the slope. In the case of Figure 3.21B the cross-term increases with the  $G_d$  increments which will lead to an over-estimation of the diffusion coefficient, unless the cross-terms are quantitatively taken into account. Qualitatively, the presence of cross-terms can be determined when in between the time of dephasing and rephasing of one gradient pair combination, another gradient along the same or an orthogonal axis is applied.

Up to this point, the factors affecting the  $b$ -value have been described, since accurate knowledge of this value is essential for a proper interpretation of the diffusion experiment. It should be realized that the parameter of interest, i.e. the diffusion constant  $D$ , is also affected by several factors, especially when measurements in intact tissue are performed. The dependence of the diffusion constant of an isotropic medium (or a solute dissolved in an isotropic medium) on parameters like viscosity and temperature is described by:

$$D = \frac{kT}{6\pi\eta R_H} \quad (3.68)$$

which is known as the Stokes–Einstein relation for translational diffusion. In Equation (3.68),  $k$  is Boltzmann's constant,  $T$  is the absolute temperature,  $\eta$  is the viscosity and  $R_H$  is the hydrodynamic radius. The hydrodynamic radius is a measure of the radius of a solute, including the surrounding hydration layers. Therefore  $R_H$  can be much larger than would be expected on basis of the physicochemical structure of the solute. Note, that the viscosity in Equation (3.68) itself also has a strong temperature dependence whereby the diffusion constant is not linear [as suggested by Equation (3.68)] but exponentially dependent on the temperature:

$$D = D_\infty e^{-E_a/kT} \quad (3.69)$$

in which  $E_a$  is the activation energy associated with translational diffusion. For water,  $E_a$  equals  $\sim 0.18$  eV, corresponding to the energy required to break hydrogen bonds. Figure 3.22 shows the temperature dependence of the diffusion constant of water [117].

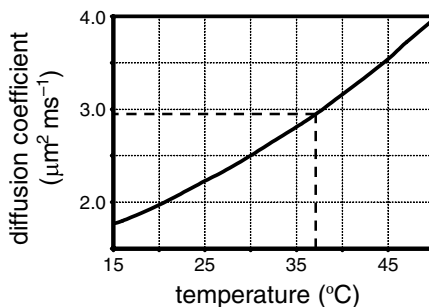


**Figure 3.22** Temperature dependence of the diffusion coefficient of water. In the physiological range, the diffusion coefficient is almost linearly dependent on the temperature.

Because of the noninvasive character of NMR, diffusion measurements can also be performed under *in vivo* conditions. The molecular displacement can be affected by a wide range of factors like the presence of cellular membranes, high concentrations of proteins and the binding to macromolecules. Consequently, diffusion in the intracellular compartment may not be adequately described by the Stokes–Einstein relation [Equation (3.68)]. Furthermore, in the case of restricted diffusion (e.g. due to cellular compartmentalization), the measured diffusion constant may depend on the orientation of the diffusion-sensitizing gradients and on the specific timing parameters (i.e.  $\delta$  and  $\Delta$ ) used. For these reasons, the molecular displacements measured *in vivo* do not represent pure molecular self-diffusion. To discriminate between molecular self diffusion in isotropic media and the diffusive processes measured under *in vivo* conditions, the latter are described by ADCs. The measurement of water ADC values by imaging techniques is now frequently employed to discriminate ischemic brain lesions from normal tissue and to access the anisotropy of tissue architecture.

Metabolite diffusion measurements are more demanding than water diffusion measurements. Besides being present at lower concentrations, metabolites also have substantially lower diffusion constants. This requires higher gradients and/or longer diffusion times for sufficient signal attenuation due to diffusion (i.e.  $b$ -values exceeding  $10\,000\text{ s mm}^{-2}$  are required). This is especially true for measurements involving nuclei with low gyromagnetic ratios. For example, for  $^{31}\text{P}$  the gradients should be  $(\gamma_{\text{H}}/\gamma_{\text{P}}) \sim 2.5$  times stronger than for  $^1\text{H}$  to obtain a similar signal attenuation [118–121].

Several experimental imperfections can influence the obtained ADC values. In general, gradient performance should be excellent in that the diffusion gradient pairs are well balanced and induce minimal eddy currents (i.e. time-varying magnetic fields following a gradient pulse). Both effects are easily verified by diffusion measurements on an *in vitro* sample. In the case of unbalanced gradient pairs or eddy currents, the phase and the shape of the resonance lines will change with increasing  $b$ -values, respectively. In Chapter 10, methods to minimize time-varying magnetic fields, like active screening and pre-emphasis, are described. The constant linewidths and phases of the spectra shown in Figure 3.20A are a good indication that residual (time-varying) gradient effects are minimal.

Macroscopic motion and flow can also affect *in vivo* ADC measurements. A net displacement of spins (i.e. macroscopic motion) in the presence of a linear magnetic field gradient will cause a change in the phase of the entire NMR resonance. This is principally different from diffusion where the net displacement is zero, such that the ensemble average phase shift is also zero. However, macroscopic motion may also lead to signal attenuation on top of diffusion-related signal loss. For linear, macroscopic motion, the phase shift  $\phi$  is identical for every position along the gradient direction and is given by:

$$\phi = \gamma \delta \Delta \mathbf{G} \cdot \mathbf{v} = \gamma \delta \Delta G v \cos \theta \quad (3.70)$$

where  $v$  is the linear velocity and  $\theta$  is the angle between the gradient direction and the direction of motion. Equation (3.70) is the basis for phase contrast MR angiography to obtain quantitative information about flow. From Equation (3.70) it follows that coherent translational motion during a diffusion experiment does not have to lead to signal loss *per se*. However, during signal averaging (which will always be employed for metabolite ADC measurements), the phase shift given by Equation (3.70) may appear incoherent between acquisitions, thereby leading to signal loss. More complex motional patterns like

turbulence lead to phase errors which are not refocused in individual acquisitions, such that signal loss can occur without signal averaging. In general, macroscopic motion-related signal losses *in vivo* are superimposed on diffusion-related signal losses, leading to an overestimation of the diffusion coefficients.

Several methods are available to either minimize or compensate for macroscopic motion and flow [122–125]. Appropriate immobilization of the object under investigation (and particularly anaesthetized laboratory animals) can minimize macroscopic motion-related signal loss to a large extent. Further, physiological motion like for instance cardiac cycle related CSF pulsations or respiration may be minimized by a combination of cardiac and respiratory triggering. Besides minimizing the motion-induced signal losses via a physiological approach, like immobilization or triggering, effects of motion can also be minimized by proper sequence programming. In principle, it is possible to utilize gradient moment nulling techniques to compensate for linear or higher order (i.e. accelerated) types of motion [122]. However, this reduces the maximum attainable b-value for a given echo-time.

Any residual effects of macroscopic motion can be minimized by methods which compensate the acquired phase shift post-acquisition. Although not used for spectroscopy applications, the use of navigator echoes is one of the most popular methods in MRI to compensate motion-related artifacts [125]. In this technique, an additional nonphase encoded echo is acquired at each phase-encoding increment in a MRI sequence (for details of phase-encoding and image reconstruction see Chapter 4). Because the phase of these so-called navigator echoes should be identical in the absence of motion, deviations from a constant phase can be used to correct the image echoes for linear movements of the object. For spectroscopy, Posse *et al.* [123] have proposed a similar but slightly different method. By storing each acquired FID or echo separately, the FIDs can have an individual phase correction, after which they can be coherently summed as part of signal averaging without any motion-related signal loss.

### 3.4.3 Anisotropic Diffusion

In the preceding discussion, the diffusion has been described as a scalar 1D process. This is a valid approximation if diffusion in an isotropic solution is considered, i.e. when the diffusion has no directional preference. However, *in vivo* the presence of physical barriers (e.g. cell membranes, muscle fibers), may lead to anisotropic diffusion, i.e. the measured diffusion has a dependence on the direction of diffusion sensitization. This phenomenon has been observed in diffusion measurements of the spinal cord [126], brain white matter [127, 128], heart wall muscle [129–131] and skeletal muscle [118–121, 132].

For a more quantitative description of anisotropic diffusion, the classical Stejskal–Tanner relationship given by Equation (3.60) no longer holds. The scalar diffusion coefficient  $D$  should be replaced by a rank two,  $3 \times 3$  diffusion tensor (or matrix)  $\mathbf{D}$ , given by:

$$\mathbf{D} = \begin{pmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{pmatrix} \quad (3.71)$$

The diagonal elements represent the diffusion along the x, y, and z axes in the laboratory frame, i.e. the frame in which the magnetic field gradients are applied. The off-diagonal

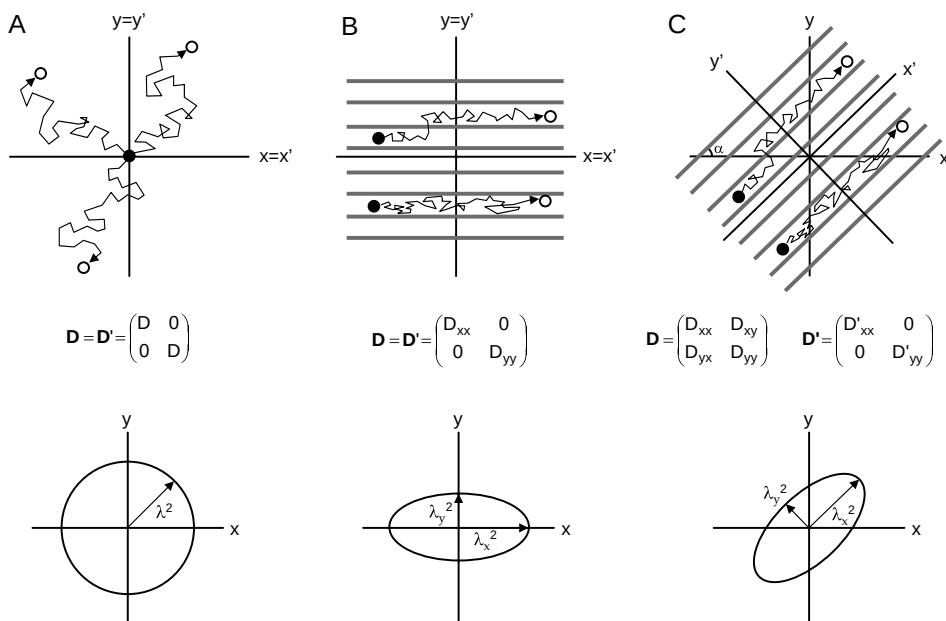


**Figure 3.23** 2D diffusion in the gradient and cell frames of reference. (A) For isotropic, unrestricted diffusion, the diffusion coefficient  $D$  and mean square displacement  $\lambda^2$  are the same in both directions leading to a 2D diffusion tensor with all off-diagonal elements equal to zero. The 2D diffusion ellipsoid (bottom) will be circular since  $\lambda_x^2 = \lambda_y^2 = \lambda^2$ . (B) For anisotropic, restricted diffusion the diffusion coefficients in the  $x$  and  $y$  directions are not equal, leading to an elliptical displacement profile (bottom). However, since the gradient and cell frames of reference coincide, the off-diagonal elements of the 2D diffusion tensor are still zero. (C) In the more general case where the gradient and cell frames of reference do not coincide, the off-diagonal elements become nonzero. However, the eigenvalues of the diffusion tensor provide the principle, orthogonal diffusivities, leading to a rotated, elliptical displacement profile (bottom).

elements represent the correlation between the diffusion in perpendicular directions. For isotropic diffusion, there is no correlation between diffusion in orthogonal directions and the off diagonal elements are zero (see also Figure 3.23A for 2D diffusion). Furthermore,  $D_{xx} = D_{yy} = D_{zz} = D$ . For anisotropic diffusion one has to consider how the laboratory frame relates to the principle axes, i.e. the axes which coincide with the three main orthogonal directions of diffusion, of the cell (or tissue) frame of reference. In the principle axes system of the cell frame of reference, the diffusion tensor  $\mathbf{D}'$  is given by:

$$\mathbf{D}' = \begin{pmatrix} D'_{xx} & 0 & 0 \\ 0 & D'_{yy} & 0 \\ 0 & 0 & D'_{zz} \end{pmatrix} \quad (3.72)$$

as is also indicated for 2D diffusion in Figure 3.23B. However, the cell frame of reference almost never coincides with the laboratory frame. The diffusion tensor  $\mathbf{D}$  in the laboratory



frame is linked to  $\mathbf{D}'$  by the relation:

$$\mathbf{D} = \mathbf{R}^{-1} \mathbf{D}' \mathbf{R} \quad (3.73)$$

in which  $\mathbf{R}$  is a rotation matrix (see Appendix A1). When the cell frame of reference deviates from the laboratory frame, the off-diagonal elements in  $\mathbf{D}$  are no longer zero and have to be taken into account for a complete description of anisotropic diffusion (Figure 3.23C). For uncharged particles, as water, the diffusion tensor  $\mathbf{D}'$  (or  $\mathbf{D}$ ) is symmetric. Using the formalism of the diffusion tensor, the Stejskal–Tanner relationship of Equation (3.60) can be rewritten as:

$$\ln \left( \frac{S(\mathbf{b})}{S(0)} \right) = - \sum_{i=1}^3 \sum_{j=1}^3 b_{ij} D_{ij} \quad (3.74)$$

where  $b_{ij}$  is a component of the  $\mathbf{b}$  matrix and  $D_{ij}$  is a component of the diffusion tensor  $\mathbf{D}$ . The  $b_{ij}$  terms can be analytically calculated using Equation (3.61) or (3.62). Note that cross-terms between perpendicular gradients are now appearing in  $b_{ij}$  components for which  $i \neq j$ . In order to quantitatively describe anisotropic diffusion at least seven experiments need to be performed [to obtain  $D_{xx}$ ,  $D_{xy}$ ,  $D_{xz}$ ,  $D_{yy}$ ,  $D_{yz}$ ,  $D_{zz}$  and  $S(0)$ ], in which diffusion gradients are applied in various oblique directions. Note that for isotropic diffusion, i.e.  $D_{ij} = 0$  for  $i \neq j$  and  $D_{ij} = D$  for  $i = j$ , Equation (3.74) reduces to the classical Stejskal–Tanner equation Equation (3.60) with  $b = (b_{xx} + b_{yy} + b_{zz})/3$ , showing that isotropic diffusion is simply a special case of a more general formulation of diffusion given by Equation (3.74). In practice the diffusion tensor cannot be calculated sufficiently accurate with only seven measurements, for instance due to noise in the echo intensity. Therefore, one normally performs many experiments after which  $\mathbf{D}$  is estimated with multivariate linear regression. Figure 3.24A–F show the full diffusion tensor [Equation (3.71)] for excised rat brain at 11.75 T as calculated from 13 diffusion-weighted images along different oblique spatial

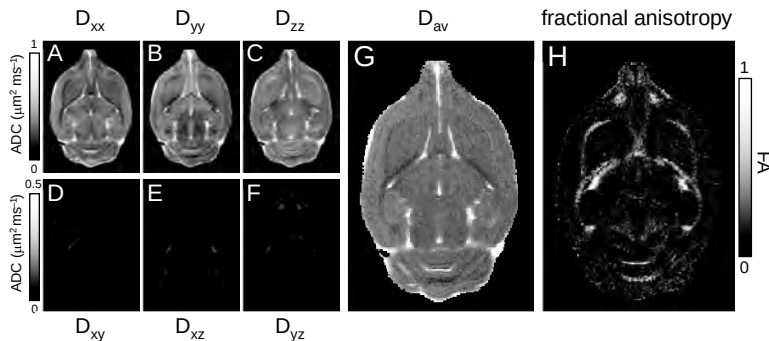


**Figure 3.24** (A)–(F) ADC maps from excised rat brain calculated from 13 diffusion-weighted images, thereby making up the complete 3D diffusion tensor. In order to reduce the features of diffusion anisotropy, an average or trace diffusion map  $D_{av} = (D_{xx} + D_{yy} + D_{zz})/3$  can be calculated (G), whereas diffusion anisotropy can be emphasized by calculating the fractional anisotropy (H) according to Equation (3.76). Note that the image contrast in (G) is solely due to differences in ADC between free ventricular water and restricted brain water.

axes and one nondiffusion-weighted image (i.e.  $b \sim 0 \text{ ms } \mu\text{m}^{-2}$ ). It follows that relatively isotropic tissues like certain cerebral gray matter areas and CSF have similar  $D_{xx}$ ,  $D_{yy}$  and  $D_{zz}$  values and small  $D_{xy}$ ,  $D_{xz}$  and  $D_{yz}$  values. However, in anisotropic tissues, like cerebral white matter,  $D_{xx}$ ,  $D_{yy}$  and  $D_{zz}$  are different and dependent on the orientation of the object relative to the magnetic field gradient direction. This observation of diffusion anisotropy in cerebral white matter is further confirmed by significant correlation between diffusion in orthogonal directions as described by  $D_{xy}$ ,  $D_{xz}$  and  $D_{yz}$ .

Although knowledge of the complete diffusion tensor provides insight in tissue orientation, for many applications the orientational dependence of the diffusion upon the gradient direction is of no interest and knowledge of the scalar diffusion coefficient suffices. For these applications one can use the average diffusion constant of three orthogonal directions, i.e. the trace of the diffusion tensor, since the trace of a matrix is invariant under rotations.

$$\text{Tr}(\mathbf{D}') = D'_{xx} + D'_{yy} + D'_{zz} = D_{xx} + D_{yy} + D_{zz} = \text{Tr}(\mathbf{D}) = 3D_{av} \quad (3.75)$$

Figure 3.24G shows a diffusion trace ( $D_{av}$ ) image. All orientation dependent contrast visible in the six diffusion maps of Figures 3.24A–F has disappeared and the remaining image contrast is largely due to diffusion coefficient differences between fast diffusing water in CSF and slower diffusing water in cerebral tissues. Note that there are several composite parameters that are rotationally invariant, of which the trace of the diffusion tensor is the most relevant and most commonly used.

For many other applications, like the study of cerebral white matter, diffusion anisotropy should be emphasized, rather than suppressed. Among several useful parameters [133] the fractional anisotropy (FA) is commonly used and is defined as:

$$\text{FA} = \frac{\sqrt{3[(D'_{xx} - D_{av})^2 + (D'_{yy} - D_{av})^2 + (D'_{zz} - D_{av})^2]}}{\sqrt{2(D_{xx}^2 + D_{yy}^2 + D_{zz}^2)}} \quad (3.76)$$

where  $D'_{xx}$ ,  $D'_{yy}$  and  $D'_{zz}$  are the diffusion coefficients along the principal axes in the cell frame of reference and can be calculated as the eigenvalues of the diffusion tensor in the laboratory frame, i.e. Equation (3.71). Figure 3.24H shows the FA map for rat brain as calculated from the diffusion tensor images shown in Figure 3.24A–F. Rather than eliminating the effects of diffusion anisotropy as was achieved for the diffusion trace (Figure 3.24G), the fractional anisotropy map clearly enhances those tissues that exhibit strong anisotropic diffusion behavior, i.e. white matter tracts.

As it is difficult to display diffusion tensor data with regular 2D images (at least six images would be required as shown in Figure 3.24A–F), the concept of diffusion ellipsoid images has been proposed [134, 135]. A diffusion ellipsoid is a 3D representation of the diffusion distance covered in space by molecules in a given diffusion time,  $t$ . Diffusion ellipsoids are easily constructed from the eigenvalues of the diffusion tensor (which correspond to  $D'_{xx}$ ,  $D'_{yy}$  and  $D'_{zz}$ ) according to:

$$\frac{x'^2}{2D'_{xx}t} + \frac{y'^2}{2D'_{yy}t} + \frac{z'^2}{2D'_{zz}t} = \left(\frac{x'}{\lambda_{x'}}\right)^2 + \left(\frac{y'}{\lambda_{y'}}\right)^2 + \left(\frac{z'}{\lambda_{z'}}\right)^2 = 1 \quad (3.77)$$

where  $\lambda$  corresponds to the 1D root mean square displacement along the principal diffusion axis [Equation (3.49)]. Figure 3.25 shows a coronal diffusion ellipsoid image of the

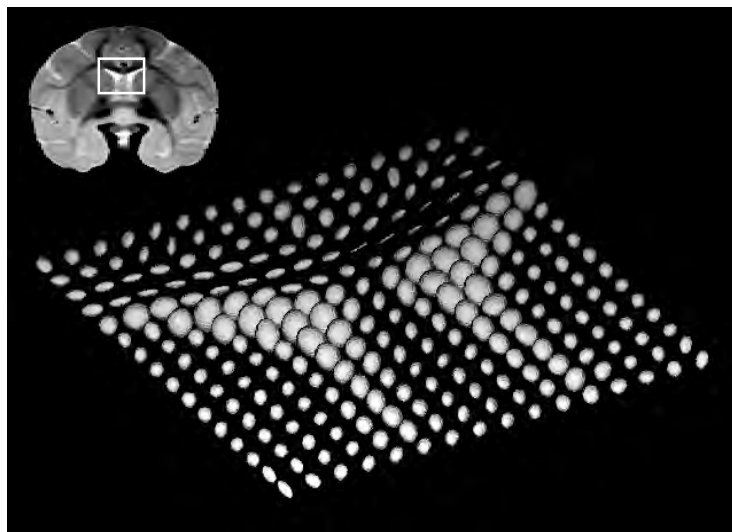


**Figure 3.25** Diffusion ellipsoid image of monkey brain. Diffusion ellipsoids represent surfaces of constant-mean-squared translational displacement after a constant time following the release of the spins at the center of each voxel. The ellipsoids were calculated for each pixel within the rectangular region of interest as shown on the  $T_2$ -weighted image. The shape of the diffusion ellipsoids is intrinsically related to the diffusion anisotropy. [Reproduced with permission from C. Pierpaoli and P. J. Basser, *Magn. Reson. Med.* **36**, 893–906 (1996), Copyright John Wiley & Sons, Inc.]

ventricular region in the monkey brain. The degree of anisotropy is now embodied in the amount of eccentricity of the diffusion ellipsoid, whereas the bulk mobility of the diffusing water is related to the size of the ellipsoid (see also Figure 3.23). The preferred direction of diffusion is indicated by the orientation of the main axis of the diffusion ellipsoid. Therefore, in isotropic tissues, like gray matter and CSF, diffusion is similar in all directions and the diffusion ellipsoids are largely spherical. Since diffusion in CSF is faster than in gray matter, the diffusion spheroids in CSF are larger than in gray matter. In white matter, the diffusion ellipsoids are nonspherical with the preferred diffusion direction indicated by the most elongated ellipsoid dimension. Linking and tracing the preferred diffusion direction in adjacent ellipsoids (i.e. pixels) allows for so-called white matter fiber tracking [136–141].

### 3.4.4 Restricted Diffusion

Since most membranes are semi-permeable to water, diffusion anisotropy in diffusion tensor imaging (DTI) is largely caused by diffusion hindrance. Diffusion anisotropy has also been observed for metabolites in animal and human brain [142] and skeletal muscle [118–121, 142]. However, unlike water, most metabolites are confined to the intracellular space on the timescale relevant for diffusion measurements. Therefore, in addition to diffusion hindrance due to intracellular organelles, metabolite diffusion is also affected by restricted diffusion caused by the boundaries of the cellular compartment.

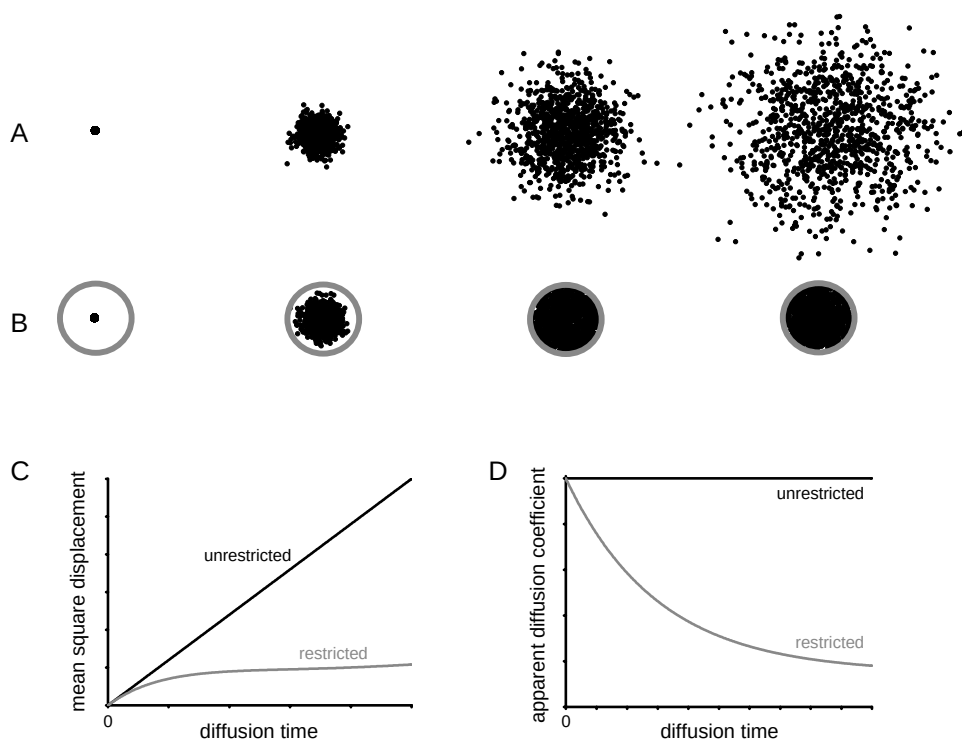


**Figure 3.26** Effects of boundaries on the measurement of diffusion. (A) For free, unrestricted diffusion (i.e. in the absence of physical boundaries) the mean square displacement increases linearly with time (C), which according to Equation (3.49) leads to a constant diffusion coefficient independent of the diffusion time (D). (B) In the presence of physical boundaries the diffusion may appear free and unrestricted for short diffusion times. However, for longer diffusion times an increasing number of particles encounter the physical barrier, thereby leading to a decreased mean square displacement, which for a spherical compartment, approaches the dimensions of the compartment. The restricted mean square displacement (C) leads to an increasingly smaller diffusion coefficient with increasing diffusion times (D), in accordance with Equation (3.49).

Figure 3.26 shows the effect of restricted diffusion on the measured, average diffusion coefficient,  $D_{av}$ . In analogy to diffusion tensor imaging, the orientational dependence of the measured diffusion coefficient can be eliminated by calculating the trace of the diffusion tensor, i.e.  $D_{av} = (D_{xx} + D_{yy} + D_{zz})/3$ . For unrestricted diffusion, the mean square displacement increases linearly with the diffusion time and as a result the measured diffusion coefficient is independent of the diffusion time. For restricted diffusion however, the root mean square displacement increases linearly with diffusion time only up to the point that the mean square displacement becomes comparable with the size of the restricting compartment. For longer diffusion times, the mean square displacement levels off to a constant value (for a spherical restricting compartment) as the molecules bounce off the compartment walls. As a result, the measured ADC [Equation (3.49)] decreases with

increasing diffusion time. When performing metabolite diffusion measurements *in vivo* it is therefore crucial to state the diffusion time in order to allow comparison with other measurements. However, besides being an additional complication to *in vivo* diffusion measurements, the dependence of the ADC on the diffusion time can also be used to obtain information about the size of the restricting compartment [120, 121]. Following experiments on excised tissues, Moonen *et al.* [118] were the first to demonstrate restricted diffusion of PCr in skeletal muscle. Quantitative studies on restricted metabolite diffusion were subsequently performed by van Gelderen *et al.* [120] and de Graaf *et al.* [121]. Figure 3.27 shows *in vivo* diffusion measurements of PCr and ATP on rat skeletal muscle at 4.7 T. Clear diffusion anisotropy is visible when the magnetic field gradient orientation is changed relative to the muscle fibers. Diffusion-weighting along the muscle fibers (Figure 3.27A) leads to rapid signal decline for PCr and ATP with increasing b-value, indicating fast diffusion. Applying diffusion gradients perpendicular to the muscle fibers (Figure 3.27B) leads to greatly reduced apparent diffusion due to restriction effects. Repeating the experiment at increasing diffusion times leads to a significant decline in the average (trace) diffusion coefficients (Figure 3.27D). Under the assumption of a cylindrical compartment, the curve in Figure 3.27D can be fitted with an analytical expression as given by Neumann [143]. Besides the size of the restricting compartment, the fitting results also indicated that the *in vivo* diffusion of PCr and ATP for diffusion time = 0 is close to that for free PCr and ATP in solution, indicating that *in vivo* diffusion is not fundamentally different from *in vitro* diffusion. *In vivo* diffusion is merely more complicated with related effects from diffusion hindrance, diffusion anisotropy and restriction.

### 3.5 Dynamic Carbon-13 NMR Spectroscopy

In Section 3.3 MT experiments were described in which endogenous compounds, like ATP and 2-oxoglutarate, were magnetically labeled by saturation or inversion. In the presence of a chemical exchange reaction or cross-relaxation, the magnetic label perturbs the reaction partner (e.g. PCr and glutamate) in a manner that is dependent on the exchange reaction rate, as well as the intrinsic  $T_1$  relaxation parameters. While these experiments are completely noninvasive and provide valuable information on enzyme kinetics *in vivo*, they are limited to exchange reactions that are fast relative to  $T_1$  relaxation, i.e.  $k_{AB} \gg 1/T_{1A,B}$ . If this condition can not be met,  $T_1$  relaxation has already destroyed the magnetic label before it can reach the exchange partner. This therefore excludes the measurement of important, but relatively slow, metabolic pathways like glycolysis.

The infusion of an exogenous compound that achieves a permanent magnetic perturbation offers an alternative to the previously described methods. Since the perturbation is permanent (with respect to  $T_1$  relaxation), it allows the measurements of much slower reaction rates and metabolic fluxes. The primary example of these types of experiments is given by the intravenous infusion of  $^{13}\text{C}$ -enriched substrates, like  $[1-^{13}\text{C}]\text{glucose}$  or  $[2-^{13}\text{C}]\text{acetate}$ . Carbon-12 is the common isotope and is present at a natural abundance of 98.89 %. However, since carbon-12 has no net nuclear spin it can not be detected by NMR. Carbon-13 is the second most abundant, stable and nonradioactive isotope of carbon with a natural abundance of 1.11 %. It has a nuclear spin 1/2 and is thus NMR-visible (see Table 1.1). When 99 %  $^{13}\text{C}$ -enriched  $[1-^{13}\text{C}]\text{glucose}$  is intravenously administered to the subject,

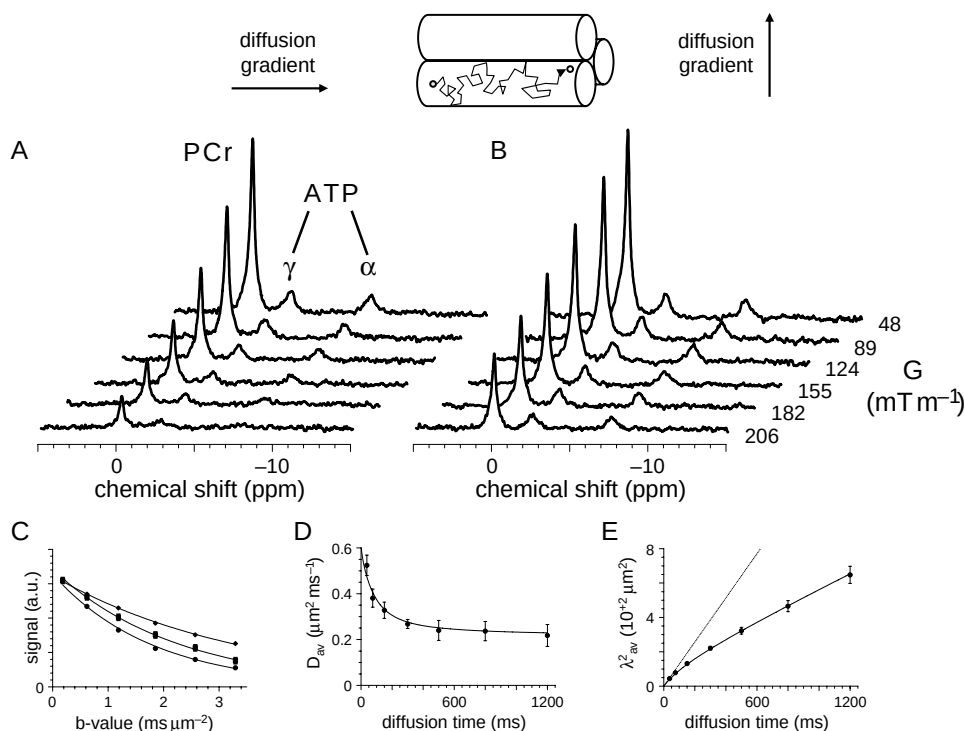


**Figure 3.27** In vivo diffusion-weighted  $^{31}\text{P}$  NMR spectra of rat skeletal hindleg muscle. The spectra were obtained with a 2.0 cm diameter surface coil and a diffusion-sensitized (selective) stimulated echo sequence ( $TR = 5.0$  s,  $TE = 20$  ms,  $TM = 60$  ms) executed with adiabatic RF pulses (see Chapters 5 and 6). The  $b$ -factors are 164, 563, 1093, 1707, 2354 and 3015  $\text{s mm}^{-2}$  ( $\delta = 9$  ms and  $\Delta = 73$  ms) with increasing diffusion-weighting from top to bottom. Observed resonances have been assigned to PCr, and  $\gamma$ - and  $\alpha$ -ATP. The  $\beta$ -ATP resonance is not visible due to the frequency-selective nature of the pulse sequence employed. All spectra were subject to the same phase correction. Diffusion weighting along the direction of the main muscle fibers (left) leads to a significantly larger decrease in all signals as compared with when the diffusion-weighting is applied perpendicular to the main muscle fibers (right). (C) Integrated signal intensities for PCr as a function of the  $b$ -value (constant diffusion time = 75 ms). The curves were fitted with a mono-exponential function to estimate the ADC. Diffusion anisotropy is evident from the curves for the different gradient directions. (D) Trace diffusion coefficient  $D_{\text{av}}$  for PCr as a function of the diffusion time. The solid line represents the best fit to the experimental data according to a theoretical model describing diffusion in infinitely long cylinders. (E) Trace mean square displacement  $\lambda^2_{\text{av}}$  for PCr as a function of the diffusion time, as calculated with Equation (3.49). The dotted line indicates the mean square displacement for unrestricted diffusion. The difference between the dotted and solid lines is again indicative of diffusion restriction. [Reproduced with permission from R. A. de Graaf et al. Biophys. J. **78**, 1657–1664 (2000), Copyright Biophysical Society.]

human or animal, the  $[1-^{13}\text{C}]$ glucose is taken up by the brain as if it was regular glucose. This is a fundamental advantage of NMR over similar experiments done with 2-deoxy-glucose and 2-fluoro-deoxy-glucose in autoradiography and PET [144–146]. The addition of a single neutron to carbon-12 has a negligible effect on the biochemical properties (i.e. membrane transport, metabolic rates) of carbon-13. In contrast the modifications required for PET necessitates the use of correction terms to take differences between glucose and deoxy-glucose membrane transport into account. Another advantage of NMR is that while deoxy-glucose is to a first approximation metabolically inert,  $[1-^{13}\text{C}]$ glucose enters the normal metabolic pathways like glycolysis and the TCA cycle. The  $^{13}\text{C}$ -label of  $[1-^{13}\text{C}]$ glucose is then transferred to molecules further downstream, like  $[3-^{13}\text{C}]$ pyruvate and lactate in glycolysis and  $[4-^{13}\text{C}]$ glutamate in the TCA cycle. The next section will detail the flow of  $^{13}\text{C}$  label from glucose to metabolites, but for now it should be clear that following the flow of  $^{13}\text{C}$  label from  $[1-^{13}\text{C}]$ glucose to other metabolites allows the study of metabolic pathways *in vivo*.

In general the detection of  $^{13}\text{C}$ -label turnover involves a number of considerations and decisions that determine the exact information content of the experiment. These considerations/decisions can be divided into three groups:

1. Choice of  $^{13}\text{C}$  label. While most studies are still performed with  $[1-^{13}\text{C}]$ glucose, several other substrates are available that provide complimentary information. In particular,  $[2-^{13}\text{C}]$ acetate,  $[2-^{13}\text{C}]$ glucose and  $[1,6-^{13}\text{C}_2]$ glucose/ $[\text{U}-^{13}\text{C}_6]$ glucose are alternative substrates. The relative merits of these substrates are discussed in Section 3.5.3.
2. Direct  $^{13}\text{C}$  versus indirect  $^1\text{H}$ - $[^{13}\text{C}]$  detection.  $^{13}\text{C}$ -enriched compounds can be detected directly with  $^{13}\text{C}$  NMR, or alternatively the protons attached to the  $^{13}\text{C}$  nuclei can be detected with  $^1\text{H}$ - $[^{13}\text{C}]$  NMR. While  $^1\text{H}$ - $[^{13}\text{C}]$  NMR provides a higher sensitivity, direct  $^{13}\text{C}$  NMR typically provides a higher information content, including the detection of isotopomer resonances.
3. Exact metabolic model. The obtained  $^{13}\text{C}$  turnover data must be fitted with a mathematical model in order to obtain information about the biochemical pathways. The complexity of the metabolic model (e.g. one, two or three compartments) as well as the underlying assumptions (e.g. certain metabolic pools and fluxes are unknown and must be assumed) can have significant effects on the final metabolic flux values. The considerations involved with metabolic modeling are discussed in Section 3.5.2.

### 3.5.1 General Set-up

Before the experimental details are discussed, the general set-up of a  $^{13}\text{C}$  NMR turnover study is described (Figure 3.28). While the description will be focused on animal experiments, the set-up is similar for human studies. Noticeable differences will be mentioned wherever applicable. Following the commonly employed preparations (general anaesthesia and tracheotomy or intubation) two small lines are placed in the femoral artery and vein or tail vein for periodic sampling of arterial blood and intravenous infusion of substrates, respectively. In human studies, the two lines are typically placed in the antecubital vein in each arm.

The infusion protocol is typically designed to quickly reach a high and stable isotopic enrichment of the substrate in the blood. Figure 3.28B shows a practical glucose infusion

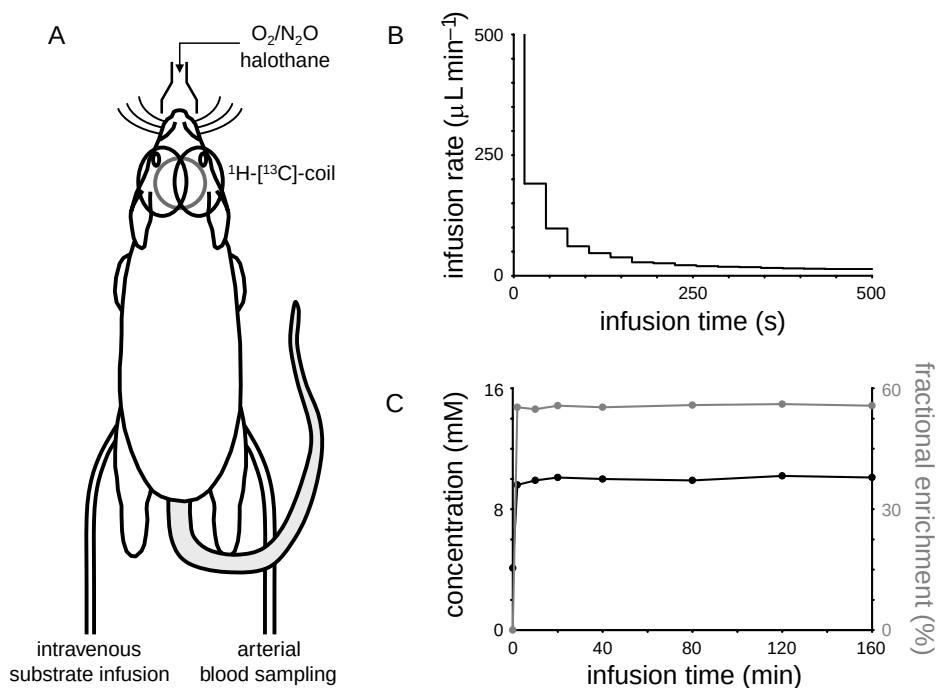


**Figure 3.28** (A) Experimental set-up for a  $^1H$ - $^{13}C$  NMR experiment of  $^{13}C$ -labeled substrate turnover in rat brain in vivo. The set-up for  $^{13}C$ - $^1H$  NMR is identical with the exception of the RF coil (see also Chapter 10). (B) Infusion protocol for intravenous administration of a 0.75 M glucose solution in order to raise the blood glucose level to circa 10 mM in an adult rat. (C) Measured blood glucose concentration (black line) and  $^{13}C$  fractional enrichment (gray line) in an adult rat (200 g) during the infusion protocol shown in (B).

protocol for an adult rat that is designed to increase the intravenous glucose concentration from euglycemic levels (circa 4 mM following an overnight fast) to hyperglycemic levels (10–12 mM), while reaching a 50–70 % fractional enrichment of the glucose within 1 min. However, exact knowledge of the time course of substrate concentration and isotopic enrichment is critical for a quantitative evaluation of  $^{13}C$  labeling time courses. Without exact knowledge of the input function, the rate of  $^{13}C$  label appearance in metabolites like glutamate and glutamine cannot be interpreted since the entry rate of the  $^{13}C$ -labeled substrate is unknown. The substrate concentration and isotopic enrichment time courses can be obtained through periodic sampling of arterial blood followed by quantitative analysis with a combination of methods, like glucosometer analysis, gas chromatography-mass spectrometry and high-resolution NMR spectroscopy. Figure 3.28C shows typical blood glucose time courses.

In response to the elevated glucose levels, endogenous insulin is secreted from the pancreas in order to stimulate glucose uptake in the liver. In order to suppress this effect and reduce the experimental costs of the expensive  $^{13}C$ -labeled glucose, the glucose infusion in human studies is frequently combined with an intravenous infusion of somatostatin, a hormone that inhibits insulin release. Human  $^{13}C$  turnover studies can be greatly simplified



by oral administration of the substrate [147]. While this gives comparable metabolic rates as the studies using administration through intravenous infusion, the uncertainties on the metabolic rates are increased.

In concert with the onset of infusion, NMR data acquisition (either direct or indirect) is started. The NMR spectra are typically acquired in 2–10 min blocks in order to achieve sufficient SNR per spectrum, while still allowing the detection of dynamic changes in the metabolite  $^{13}\text{C}$  fraction. Figure 3.29A shows a typical  $^{13}\text{C}$  NMR turnover dataset in rat brain following the intravenous infusion of  $[1,6-^{13}\text{C}_2]\text{glucose}$ . Prior to infusion the  $^{13}\text{C}$  NMR spectrum is, besides the 1.11 % natural abundance, devoid of resonances. Immediately following the onset of infusion, the glucose resonances rapidly increase and remain relatively constant throughout the time course. Over time the  $^{13}\text{C}$  label can be observed in  $[4-^{13}\text{C}]\text{glutamate}$ ,  $[4-^{13}\text{C}]\text{glutamine}$  and several other metabolites. When the  $^{13}\text{C}$  label has completed one revolution of the TCA cycle it appears in the C2 and C3 positions of the mentioned metabolites. The principles of  $^{13}\text{C}$  and  $^1\text{H}-[^{13}\text{C}]$  NMR methods are discussed in Chapter 8.

Following data acquisition, the NMR resonances must be converted to absolute concentrations and fractional enrichments  $[^{13}\text{C}/(^{12}\text{C}+^{13}\text{C})]$ . For  $^1\text{H}-[^{13}\text{C}]$  NMR the calculation of fractional enrichments is typically more straightforward than for  $^{13}\text{C}$  NMR, as  $^1\text{H}-[^{13}\text{C}]$  NMR methods inherently detect the  $^{13}\text{C}$  fraction, as well as the total pool of a given metabolite. For  $^{13}\text{C}$  NMR the concentrations can be made relative to the 1.11 % natural abundance signals or an assumed metabolite pool. The details on metabolite quantification are discussed in Chapter 9. Following this step, absolute metabolite turnover curves are obtained, as shown for  $[4-^{13}\text{C}]\text{glutamate}$  and  $[4-^{13}\text{C}]\text{glutamine}$  in Figure 3.29B, which will serve as input for the metabolic model.

### 3.5.2 Metabolic Modeling

Metabolic modeling is a set of procedures which express a collection of metabolic pathways as mathematical differential equations in order to obtain absolute fluxes through these pathways. Some of the principles of metabolic modeling will first be demonstrated for a simple example, after which the complete metabolic model for cerebral energy metabolism and neurotransmitter cycling will be summarized.

Consider the simple three-step chemical reaction of a substrate S being converted to a product P which is subsequently further metabolized to other products F:



The reaction rate  $k_{\text{S} \rightarrow \text{P}}$  (in  $\text{s}^{-1}$ ) describes how fast a S molecule is converted to a P molecule and is determined by the enzyme characteristics, as well as thermodynamics. In this example it is assumed that the reaction is unidirectional, i.e. the reverse reaction ( $\text{P} \rightarrow \text{S}$ ) does not occur. Reaction rates are very difficult to measure directly *in vivo* and do not give direct information about metabolism. Metabolic fluxes,  $V$  (in  $\text{mM s}^{-1}$  or  $\text{mmol kg}^{-1} \text{s}^{-1}$ ), are more relevant in the context of *in vivo* metabolic pathways and are defined as the amount of S that is converted to P per unit time, i.e.:

$$V_{\text{S} \rightarrow \text{P}} = k_{\text{S} \rightarrow \text{P}}[\text{S}] \quad (3.79)$$

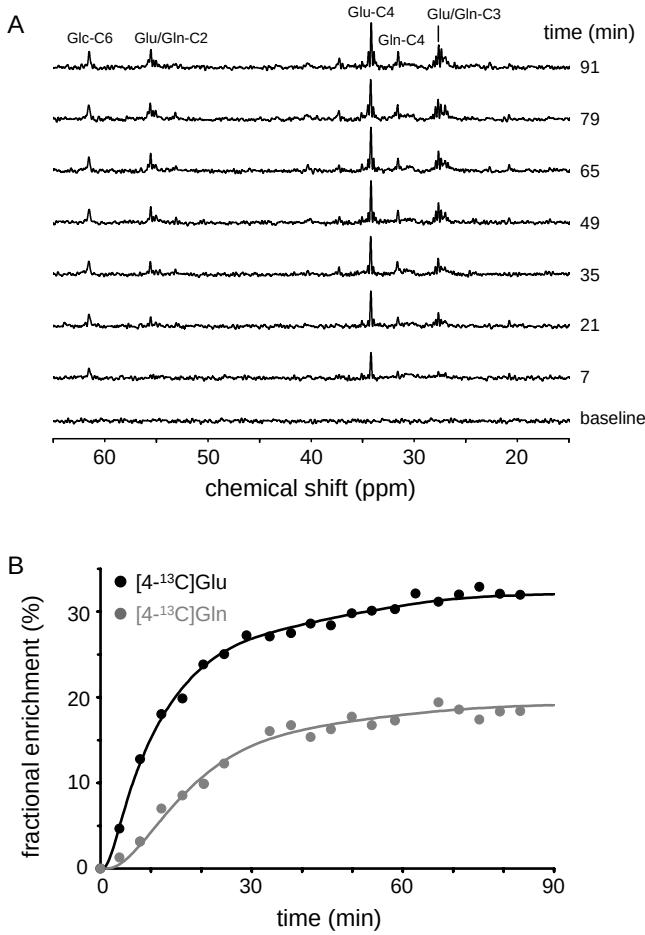


**Figure 3.29** (A) Time resolved  $^1\text{H}$ -decoupled,  $^{13}\text{C}$  NMR spectra from rat brain in vivo following the intravenous infusion of  $[1,6\text{-}^{13}\text{C}_2]\text{glucose}$ . Spectra are acquired with an adiabatic INEPT sequence ( $TR = 4000\text{ ms}$ ,  $200\text{ }\mu\text{l}$ , see also Chapter 8) at  $7.05\text{ T}$ . (B) Turnover curves of  $[4\text{-}^{13}\text{C}]\text{glutamate}$  and  $[4\text{-}^{13}\text{C}]\text{glutamine}$ . Dots represent measured fractional enrichments as obtained through LC model spectral quantification (see Chapter 9) of the data shown in (A), whereas the solid line represents the best mathematical fit to a three-compartment (blood, glutamatergic neurons and astroglia) metabolic model (see also Figure 3.30).

where  $[\text{S}]$  is the concentration of substrate  $\text{S}$  (in  $\text{mM}$  or  $\text{mmol kg}^{-1}$ ). The amount of product  $\text{P}$  changes over time according to the amount of  $\text{S}$  being converted to  $\text{P}$  minus the amount of  $\text{P}$  being converted to  $\text{F}$ . In other words, the size of the metabolic pool  $\text{P}$  changes according to the amount of  $\text{S}$  flowing to  $\text{P}$  (i.e. the ‘inflow’) minus the flow of  $\text{P}$  to  $\text{F}$  (i.e. the ‘outflow’). Mathematically this can be expressed as a differential equation according to:

$$\frac{d[\text{P}(t)]}{dt} = V_{\text{S} \rightarrow \text{P}} - V_{\text{P} \rightarrow \text{F}} = V_{\text{in}} - V_{\text{out}} \quad (3.80)$$

This type of equation is also referred to as a mass-balance equation since it balances ('tracks') all mass of compound P over time and over all possible reactions involving P. In many situations the metabolic pool (concentration) of compound P does not change with time, making  $dP(t)/dt = 0$ , which is referred to as a steady-state condition. It is now immediately clear that measuring the steady-state concentration of compound P does not give any insight in the formation and degradation (i.e. the 'turnover') of compound P. The metabolic fluxes  $V_{in}$  and  $V_{out}$  can change dramatically, but as long as  $V_{in} = V_{out}$  the concentration of compound P will remain constant.

The dynamic behavior of P can be studied in detail by introducing a marker in substrate S which can be followed over time when it flows to product P. Several techniques exist that introduce radioactive tracers to monitor metabolic pathways, including autoradiography, positron emission tomography (PET) and single photon emission computed tomography (SPECT). NMR spectroscopy is a unique technique to study metabolic pathways noninvasively *in vivo* since it utilizes nonradioactive substrates that are metabolized in the exact same manner as regular, nonlabeled substrates. Carbon-13 is most frequently used as a NMR label for a number of reasons. Firstly, whereas carbon-12 does not have a nuclear magnetic moment, carbon-13 is a spin-1/2 nucleus and can therefore be detected by NMR spectroscopy. Secondly, most metabolically active compounds have a carbon backbone, making the label generally applicable. Thirdly, replacing the common  $^{12}\text{C}$  isotope (98.89 % natural abundance) with the  $^{13}\text{C}$  isotope has negligible effects on metabolism.

Now reconsider the simple, three-step chemical reaction of Equation (3.78). Introduction of  $^{13}\text{C}$ -labeled substrate  $S^*$  will lead to the formation of  $^{13}\text{C}$ -labeled product  $P^*$ , which can be detected with  $^{13}\text{C}$  or  $^1\text{H}$ - $^{13}\text{C}$  NMR spectroscopy. Mathematically, the formation of  $^{13}\text{C}$ -labeled product  $P^*$  can be described by:

$$\frac{d[P^*(t)]}{dt} = \frac{[S^*(t)]}{[S]} V_{in} - \frac{[P^*(t)]}{[P]} V_{out} \quad (3.81)$$

This type of equation is referred to as an isotope-balance equation since it balances ('tracks') the isotope of compound P over time and over all possible reactions involving P. Together with the mass-balance Equation (3.80) it gives a complete description of the total P and  $^{13}\text{C}$ -labeled  $P^*$  metabolic pools. The ratio  $[P^*]/[P]$  is typically referred to as the fractional enrichment FE ( $0 \leq \text{FE} \leq 1$ ) of compound P.

When the fractional enrichment of substrate S is constant over time, i.e.  $[S^*(t)] = [S^*(0)]$ , the time dependence of  $[P^*]$  under steady-state conditions (i.e.  $V_{in} = V_{out}$ ) can be analytically determined as:

$$[P^*(t)] = [P] \left( \frac{[S^*]}{[S]} \right) (1 - e^{-V_{in}t/[P]}) \quad (3.82)$$

It follows that  $[P^*(t)]$  builds up as an exponential function to a final fractional enrichment equal to  $([S^*]/[S])$  with a rate  $(V_{in}/[P])$ . Large metabolic pools will therefore turn over slower than small metabolic pools. When the substrate inflow can not be described by a step function, Equation (3.81) is more difficult to solve analytically and often a numerical approach is employed instead. This is mandatory for the description of metabolic pathways *in vivo*, where multiple pathways can flow into a single pool. Figure 3.30 schematically shows the metabolic pathways involved in cerebral glucose metabolism and glutamatergic and GABAergic neurotransmitter cycling. The time dependence of each metabolic pool

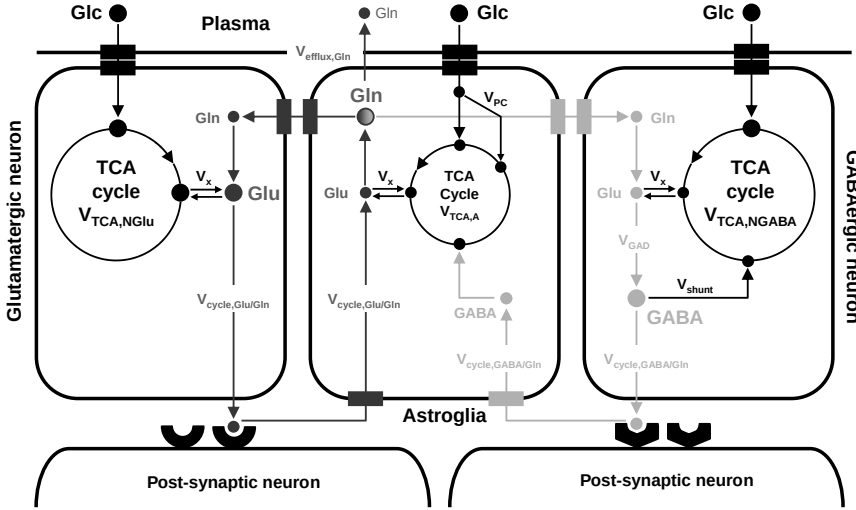


**Figure 3.30** Four-compartment metabolic model comprising the blood/plasma, glutamatergic neurons, GABAergic neurons and astroglia. Glucose enters the brain with the aid of glucose transporter in the blood–brain barrier. In the glycolytic pathway, glucose is broken down into two pyruvate molecules which enter the tricarboxylic acid (TCA) cycle. One of the TCA cycle intermediates, 2-oxoglutarate (2OG), is in rapid exchange with a large glutamate pool that can be observed with NMR. The TCA cycle flux can be obtained from the glutamate turnover and is denoted  $V_{TCA}$ . Due to compartmental localization of specific enzymes, the fate of glutamate differs in each of the three cellular compartments. In glutamatergic neurons, glutamate acts as an excitatory neurotransmitter and is released into the synaptic cleft in response to an action potential. Following interaction with post-synaptic receptors, the glutamate is taken up by the astroglia and converted to glutamine. Glutamine is ultimately transported back to the glutamatergic neuron, where it is converted to glutamate, thereby completing the so-called glutamate–glutamine neurotransmitter cycle. The flux through this cycle,  $V_{cycle,Glu/Gln}$ , can be obtained by following the glutamine turnover. In the GABAergic neuron, the glutamate is first converted to GABA which is the primary inhibitory neurotransmitter. Similar to the glutamatergic neuron, a GABA–glutamine neurotransmitter cycle exists between GABAergic neurons and astroglia. A metabolic pathway specific to astroglia is the carboxylation of pyruvate catalyzed by the astroglia-specific enzyme pyruvate carboxylase (PC). Note that the letter size roughly corresponds to the metabolic pool size of the corresponding metabolite (e.g. a large glutamine pool resides in astroglia).

can be described by a differential equation. For example, the mass-balance equation for the neuronal glutamate [ $Glu_N$ ] and GABA [ $GABA_N$ ] pools are given by:

$$\frac{d[Glu_N]}{dt} = V_{cycle,Glu/Gln} + V_{x,2OG/Glu} - (V_{cycle,Glu/Gln} + V_{x,Glu/2OG}) = 0 \quad (3.83)$$

$$\frac{d[GABA_N]}{dt} = V_{GAD} - (V_{shunt} + V_{cycle,GABA/Gln}) = 0 \quad (3.84)$$

The different fluxes are defined as shown in Figure 3.30.

Similar equations can be constructed for all other metabolic pools. For the isotope-balance equations one also has to consider the position of the carbon atom within a molecule, since different carbon positions will label at different rates depending on the substrate and metabolic pathways. For the C4 position of neuronal glutamate [ $\text{Glu}_N4^*$ ] and the C2 position of neuronal GABA [ $\text{GABA}_N2^*$ ] the isotope-balance equations are given by:

$$\begin{aligned} \frac{d[\text{Glu}_N4^*]}{dt} = & \frac{[\text{G ln}_N 4^*]}{[\text{G ln}_N]} V_{\text{cycle, Glu/G ln}} + \frac{[2\text{OG}_N 4^*]}{[2\text{OG}_N]} V_{x, 2\text{OG/Glu}} \\ & - \frac{[\text{Glu}_N 4^*]}{[\text{Glu}_N]} (V_{\text{cycle, Glu/G ln}} + V_{x, \text{Glu/2OG}}) \end{aligned} \quad (3.85)$$

$$\frac{d[\text{GABA}_N 2^*]}{dt} = \frac{[\text{Glu}_N 4^*]}{[\text{Glu}_N]} V_{\text{GAD}} - \frac{[\text{GABA}_N 2^*]}{[\text{GABA}_N]} (V_{\text{shunt}} + V_{\text{cycle, GABA/G ln}}) \quad (3.86)$$

One noticeable exception to the relatively simple, linear metabolic fluxes is the transport of glucose across the blood–brain barrier. Glucose is transported from the blood into the brain by GLUT-1 transporters and can be described by Michaelis–Menten kinetics [148–151]:

$$\begin{aligned} \frac{d[\text{Glc}_b(t)]}{dt} = & \frac{V_{\text{max}}[\text{Glc}_p(t)]}{K_m + [\text{Glc}_b(t)]/V_d + [\text{Glc}_p(t)]} \\ & - \frac{V_{\text{max}}[\text{Glc}_b(t)]}{V_d(K_m + [\text{Glc}_p(t)]) + [\text{Glc}_b(t)]} - \text{CMR}_{\text{Glc}} \end{aligned} \quad (3.87)$$

where  $K_m$  is the Michaelis–Menten constant,  $V_{\text{max}}$  is the maximum glucose transport rate and  $\text{CMR}_{\text{Glc}}$  is the cerebral metabolic rate of glucose consumption.  $V_d$  is the volume fraction of glucose ( $V_d = 0.77 \text{ mL/g}$ ) under the assumption that glucose distributes uniformly throughout the brain water space. It should be noted that at higher magnetic fields, brain glucose levels and fractional enrichments can be measured directly in  $^1\text{H}$  NMR spectra [152, 153], such that any assumptions concerning glucose transport can be avoided.

Mass- and isotope-balance equations similar to Equations (3.83)–(3.86) can be constructed for all metabolic pools, thereby making up the metabolic model. The model describing the metabolic pathways shown in Figure 3.30 is referred to as a four-compartment model, as it is composed of a blood compartment, and compartments for the glutamatergic neuron, GABAergic neuron and astroglia. Using the blood or brain glucose levels and fractional enrichments, the metabolic pool sizes and the measured fractional enrichment curves allows the calculation, through nonlinear least-squares optimization, of a number of important fluxes like the neuronal and astroglial TCA cycles and the glutamate–glutamine and GABA–glutamine neurotransmitter cycles. For more details on metabolic models and turnover data analysis the reader is referred to the literature [154–156].

### 3.5.3 Substrates

*[1- $^{13}\text{C}$ ]Glucose and [1,6- $^{13}\text{C}_2$ ]Glucose.* The majority of  $^{13}\text{C}$  NMR studies *in vivo* have used [1- $^{13}\text{C}$ ]glucose as the substrate. Besides the economic consideration that [1- $^{13}\text{C}$ ]glucose is the least expensive  $^{13}\text{C}$ -labeled form of glucose, the choice for [1- $^{13}\text{C}$ ]glucose is governed by the fact that the transfer of  $^{13}\text{C}$  label to [4- $^{13}\text{C}$ ]glutamate is indicative of the most active energy-producing metabolic pathways, namely glycolysis and the neuronal TCA cycle (see Figure 3.30). While the labeling pattern of metabolites from [1,6- $^{13}\text{C}_2$ ]glucose is nearly identical to that from [1- $^{13}\text{C}$ ]glucose, the fractional enrichment

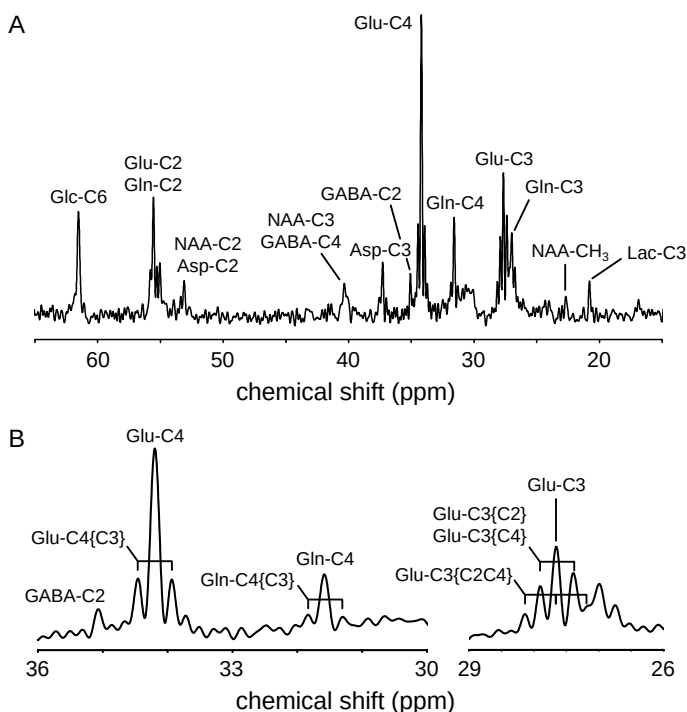


**Figure 3.31** (A)  $^1\text{H}$ -decoupled,  $^{13}\text{C}$  NMR spectrum of rat brain in vivo obtained between 120 min and 150 min following the onset of intravenous infusion of  $[1,6\text{-}^{13}\text{C}_2]\text{glucose}$ . Besides the singlet resonances, the  $^{13}\text{C}$  NMR spectrum (B) is characterized by doublet and triplet resonances arising from isotopomers. In particular glutamate and glutamine exhibit several isotopomers of which the C3-C4 combination is most abundant.

of pyruvate and hence of all subsequent metabolic pools will be twice as high, leading to an improved detection sensitivity.  $[1,6\text{-}^{13}\text{C}_2]\text{Glucose}$  is more expensive than  $[1\text{-}^{13}\text{C}]\text{glucose}$ , but this is only a minor consideration when considering the small amounts of material used to study cerebral metabolism in rat brain. For human experiments,  $[\text{U-}^{13}\text{C}_6]\text{glucose}$  is a less expensive alternative when performing  $^1\text{H}\text{-}[^{13}\text{C}]$  NMR. For direct  $^{13}\text{C}$  NMR detection,  $[\text{U-}^{13}\text{C}_6]\text{glucose}$  is not recommended as the NMR spectrum will be complicated by the multiple homonuclear  $^{13}\text{C}\text{-}^{13}\text{C}$  scalar couplings, giving rise to isotopomer resonances. It follows that label transfer from  $[1,6\text{-}^{13}\text{C}_2]\text{glucose}$  to  $[4\text{-}^{13}\text{C}]\text{glutamate}$  is detectable within minutes following the start of glucose infusion. Provided that the exchange between the mitochondrial 2-oxoglutarate and cytosolic glutamate pools is rapid, the label transfer is indicative of the TCA cycle rate. Note that through simultaneous measurement of  $[3\text{-}^{13}\text{C}]\text{glutamate}$  the TCA cycle rate can be determined even if the mitochondrial/cytosolic exchange rate is slow [155,157]. Figure 3.29 further shows that, with a small time lag, label is transferred to  $[4\text{-}^{13}\text{C}]\text{glutamine}$ . This has been interpreted as reflecting a glutamate–glutamine neurotransmitter cycle, and has subsequently been confirmed in many studies [155,157–162]. Following 90 min of glucose infusion, a wide range of metabolites is labeled, including  $[\text{n-}^{13}\text{C}]\text{glutamate}$  and glutamine ( $n = 2, 3$  or  $4$ ),  $[2\text{-}^{13}\text{C}]\text{aspartate}$ ,  $[3\text{-}^{13}\text{C}]\text{aspartate}$ ,  $[1,6\text{-}^{13}\text{C}_2]\text{glucose}$  and  $[\text{n-}^{13}\text{C}]\text{GABA}$  ( $n = 2, 3$  or  $4$ ). Besides the singlet  $^{13}\text{C}$

NMR resonances, the NMR spectrum also contains several doublet and triplet resonances (Figure 3.31). These so-called isotopomer resonances are due to homonuclear  $^{13}\text{C}$ - $^{13}\text{C}$  scalar coupling in molecules where two  $^{13}\text{C}$  nuclei are immediately adjacent to each other, like in  $[3,4\text{-}^{13}\text{C}_2]\text{glutamate}$ . This situation occurs when  $[4\text{-}^{13}\text{C}]\text{glutamate}$  labeled in the first TCA cycle is labeled again at the C3 position in the second TCA cycle. The chance that this occurs increases with the duration of the infusion. The infusion for regular  $^{13}\text{C}$  turnover studies is between 90 min and 150 min, leading to several isotopomers, in particular  $[2,3\text{-}^{13}\text{C}_2]$ ,  $[3,4\text{-}^{13}\text{C}_2]$  and  $[2,3,4\text{-}^{13}\text{C}_3]\text{glutamate}$  and glutamine (Figure 3.31). However, for much longer infusion times, as required to detect  $[^{13}\text{C}]\text{glycogen}$  turnover [163–165], the  $^{13}\text{C}$  NMR spectrum is dominated by isotopomer resonances [166]. Analysis of the exact isotopomer patterns can provide complementary information to the dynamic  $^{13}\text{C}$  turnover curves as shown in Figure 3.29. Table 3.4 summarizes the values of metabolic rates that have been measured in resting human [147, 155, 167–173] and anaesthetized rat brain [154, 157, 158, 161, 174–177].

$[2\text{-}^{13}\text{C}]\text{Glucose}$ . In order to determine the rate of the glutamate–glutamine cycle from the glutamine synthesis rate, one must distinguish glutamine labeling via the glutamate–glutamine cycle from other metabolic pathways that may contribute to the flow through glutamine synthetase. The glutamate–glutamine cycle and anaplerosis are the only two pathways that provide carbon skeletons for glutamine synthesis. Glutamine efflux is the primary pathway of nitrogen removal from the brain. At steady state the concentration of glutamine remains constant. Therefore any loss of glutamine by efflux to the blood must be compensated by synthesis *de novo* of glutamine through anaplerosis. For synthesis of glutamine *de novo* by anaplerosis pyruvate derived from glucose is converted to oxaloacetate by carbon dioxide fixation ( $V_{\text{PC}}$ , see Figure 3.30), a reaction catalyzed by the astroglial enzyme pyruvate carboxylase. Through the action of the TCA cycle, oxaloacetate is converted to 2-oxoglutarate, which is converted to glutamate. Astroglial glutamate is then converted to glutamine by glutamine synthetase. A limitation of using  $[1\text{-}^{13}\text{C}]\text{glucose}$  as a labeled precursor to measure the glutamate–glutamine cycle flux is that  $^{13}\text{C}$  label entering glutamine by this pathway can not be easily distinguished from  $^{13}\text{C}$  label that enters glutamine from the anaplerotic pathway or the astroglial TCA cycle.  $[1\text{-}^{13}\text{C}]\text{Glucose}$  labels  $[4\text{-}^{13}\text{C}]\text{glutamate}$  through the action of pyruvate dehydrogenase and labels  $[2\text{-}^{13}\text{C}]\text{glutamate}$  through pyruvate carboxylase. However, the carbon label of  $[4\text{-}^{13}\text{C}]\text{glutamate}$  is quickly transferred to  $[2\text{-}^{13}\text{C}]\text{glutamate}$  in subsequent turns of the overwhelming neuronal TCA cycle, thereby obscuring  $[2\text{-}^{13}\text{C}]\text{glutamate}$  labeling by astroglial anaplerosis. Using  $[2\text{-}^{13}\text{C}]\text{glucose}$  as a label precursor offers a highly sensitive alternative to measuring the fluxes through anaplerosis and the astroglial TCA cycle.  $[2\text{-}^{13}\text{C}]\text{Glucose}$  labels astroglial  $[3\text{-}^{13}\text{C}]\text{glutamate/glutamine}$  through pyruvate carboxylase and labels  $[5\text{-}^{13}\text{C}]\text{glutamate/glutamine}$  through the action of pyruvate dehydrogenase. However, the C5 position of TCA cycle intermediates is ultimately lost as carbon dioxide in subsequent turns of the TCA cycle. Therefore, the accumulation of (astroglial)  $[3\text{-}^{13}\text{C}]\text{glutamine}$  is a direct indication of anaplerotic activity, without contamination by neuronal TCA cycle metabolism. Under most conditions the anaplerotic pathway constitutes only a small fraction of the TCA cycle flux (Table 3.4). Figure 3.32C shows a  $^{13}\text{C}$  NMR spectrum acquired from human brain, 60 min following the onset of intravenous  $[2\text{-}^{13}\text{C}]\text{glucose}$  infusion [173]. Compared with the data acquired during  $[1\text{-}^{13}\text{C}]\text{glucose}$  infusion (Figure 3.22A), most of the  $^{13}\text{C}$  NMR resonances have increased only slightly above natural

**Table 3.4** Ranges of metabolic rates (in  $\mu\text{mol min}^{-1} \text{g}^{-1}$ ) in rat and human brain as measured by  $^{13}\text{C}$  or  $^1\text{H}$ - $^{13}\text{C}$  NMR spectroscopy.<sup>a</sup>

| Species          | $^{\text{N}}V_{\text{TCA}}$ | $^{\text{A}}V_{\text{TCA}}$ | $\text{T}V_{\text{TCA}}$ | $V_{\text{Gln}}$ | $V_{\text{PC}}$ | $V_{\text{cycle}}$       |
|------------------|-----------------------------|-----------------------------|--------------------------|------------------|-----------------|--------------------------|
| Rat <sup>b</sup> | 0.52–0.69                   | 0.02                        | 0.46–0.90                | 0.21–0.30        | 0.05–0.10       | 0.14–0.25                |
|                  |                             |                             | 0.62–0.90 (gray matter)  |                  |                 | 0.25–0.38 (gray matter)  |
|                  |                             |                             | 0.12–0.31 (white matter) |                  |                 | 0.01–0.06 (white matter) |
| Human            | 0.57–0.71                   | 0.06–0.14                   | 0.52–0.88                | 0.23–0.36        | 0.02–0.09       | 0.17–0.32                |
|                  |                             |                             | 0.80–0.88 (gray matter)  |                  |                 |                          |
|                  |                             |                             | 0.17–0.28 (white matter) |                  |                 |                          |

<sup>a</sup> $^{\text{N}}V_{\text{TCA}}$ , rate of neuronal TCA cycle;  $^{\text{A}}V_{\text{TCA}}$ , rate of astroglial TCA cycle;  $\text{T}V_{\text{TCA}}$ , rate of total TCA cycle ( $= ^{\text{N}}V_{\text{TCA}} + ^{\text{A}}V_{\text{TCA}}$ );  $V_{\text{Gln}}$ , rate of glutamine synthesis;  $V_{\text{PC}}$ , rate of anaplerotic flux through pyruvate carboxylase (PC);  $V_{\text{cycle}}$ , rate of glutamate–glutamine neurotransmitter cycle.

<sup>b</sup>Measured under different forms of anaesthesia (halothane,  $\alpha$ -chloralose).



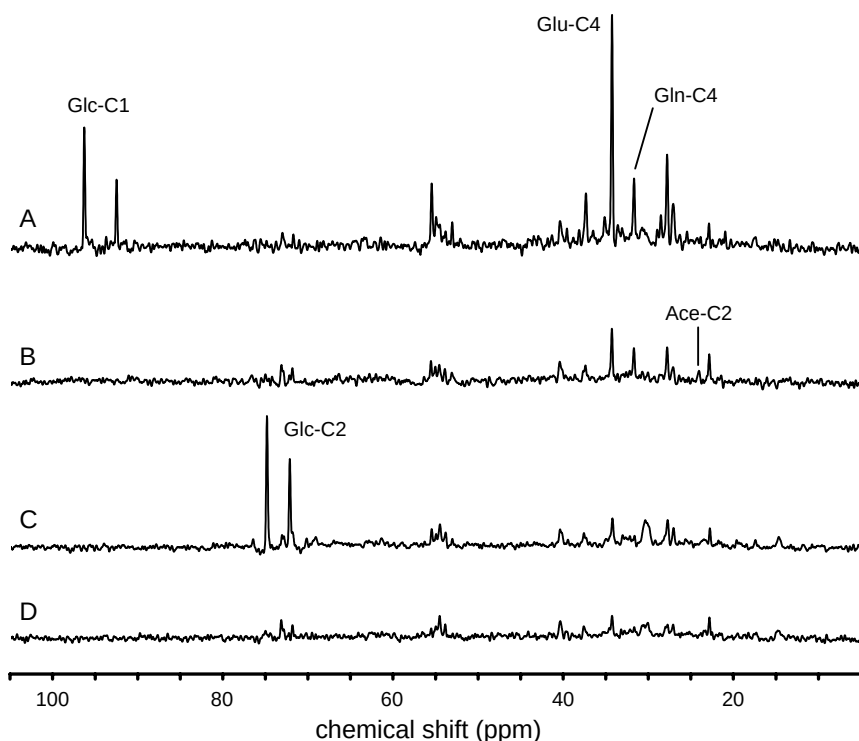


**Figure 3.32** Localized  $^1\text{H}$ -decoupled,  $^{13}\text{C}$  NMR spectra acquired from human brain at 2.1 T circa 60 min following the intravenous infusion of (A)  $[1\text{-}^{13}\text{C}]\text{glucose}$ , (B)  $[2\text{-}^{13}\text{C}]\text{acetate}$  or (C)  $[2\text{-}^{13}\text{C}]\text{glucose}$ . All  $^{13}\text{C}$  NMR spectra were acquired from the occipital lobe (144 ml) with an adiabatic INEPT sequence (see Chapter 8). (D) Natural abundance  $^{13}\text{C}$  NMR spectra acquired in the absence of intravenous substrate infusion. [A, C, and D Reproduced with permission from G. F. Mason et al., *J. Neurochem.* **100**, 73–86 (2007), Copyright Blackwell Publishing and (B) G. F. Mason et al., *Diabetes* **55**, 929–934 (2006) Copyright American Diabetes Association.]

abundance levels (compare with the natural abundance spectrum in Figure 3.22D). Even without metabolic modeling this indicates that the anaplerotic pathway through pyruvate carboxylase represents only a minor flux when compared to the neuronal TCA cycle.

**$[2\text{-}^{13}\text{C}]\text{Acetate}$ .** Since glucose is transported and metabolized in both neurons and astroglia, extensive mixing of the label occurs through the glutamate–glutamine cycle. Therefore, the TCA cycle fluxes in the neuronal and astroglial compartments are convolved. The formation of  $[4\text{-}^{13}\text{C}]\text{glutamate}$  will be heavily weighted by the neuronal TCA cycle, making the glucose experiment relatively insensitive to the smaller astroglial TCA cycle flux. Neuronal and astroglial metabolism can be more directly distinguished by using acetate as a substrate. It has been shown that acetate is almost exclusively metabolized in astroglia due to a far greater transport capacity [178]. Therefore,  $[2\text{-}^{13}\text{C}]\text{acetate}$  will be selectively transported into the astroglial compartment and converted to  $[2\text{-}^{13}\text{C}]\text{acetylCoA}$ , which

labels the astroglial TCA cycle intermediates. Next, the small (0.5–1.0 mM) astroglial glutamate pool is labeled after which the  $^{13}\text{C}$  label arrives in the large glutamine pool. The large neuronal glutamate pool is subsequently labeled from astroglial  $[4-^{13}\text{C}]\text{glutamine}$  that is transported to the neuronal compartment as part of the glutamate–glutamine neurotransmitter cycle.  $[2-^{13}\text{C}]\text{Acetate}$  experiments on human and animal brain have confirmed earlier observations made through the use of  $[1-^{13}\text{C}]\text{glucose}$  and have determined that the astroglial TCA cycle is only a small fraction of the neuronal TCA cycle (Table 3.4). Figure 3.32B shows a  $^{13}\text{C}$  NMR spectrum acquired from human brain 60 min following the onset of  $[2-^{13}\text{C}]\text{acetate}$  infusion [179]. Most noticeably is the different ratio between  $[4-^{13}\text{C}]\text{glutamate}$  and  $[4-^{13}\text{C}]\text{glutamine}$  when compared with Figure 3.32A. This can be understood from the fact that during  $[2-^{13}\text{C}]\text{acetate}$  infusion the large astroglial glutamine pool is labeled first, while during  $[1-^{13}\text{C}]\text{glucose}$  infusion the large neuronal glutamate pool is labeled first.

### 3.5.4 Applications

While the exact formulation of metabolic models and the assumptions associated with them remain a point of discussion, the calculated metabolic fluxes obtained by different groups and with different substrates are remarkably similar (Table 3.4). Rather than focusing on the relatively minor differences, the section on dynamic  $^{13}\text{C}$  turnover is concluded with some of the novel findings and applications that have been reported.

One of the most interesting and significant findings obtained with dynamic  $^{13}\text{C}$  NMR spectroscopy is that the glutamate–glutamine neurotransmitter cycle flux is linearly related to the TCA cycle flux [158, 159, 180] over a wide range of cerebral activity. In essence the  $^{13}\text{C}$  NMR results support the notion that for every glutamate molecule that is released and recycled during neurotransmission a glucose molecule is oxidized in the TCA cycle. This mechanism provides a valuable link between function (neurotransmission) and energetics (glucose oxidation). Most of the commonly used high-resolution imaging modalities, like BOLD, CBF or CBV MRI or PET detect signal that is directly or indirectly related to energetics. With the metabolic link provided by  $^{13}\text{C}$  NMR these high-resolution imaging results can potentially provide unique information on brain function. The relation between neurotransmitter cycling and glucose oxidation has since been extended to intense activation (seizures) [162, 181], the separation of multiple cerebral tissue types [157] and the separation between excitatory and inhibitory neurotransmission [177]. Furthermore, important links between neurotransmitter cycling, glucose oxidation and the underlying neuronal firing have been obtained [182]. Besides  $^{13}\text{C}$  NMR studies, dynamic metabolic pathways can also be studied with other nuclei, in particular nitrogen-15 [160, 183, 184] and oxygen-17 [185–188].

### 3.6 Hyperpolarization

The low sensitivity of NMR originates from the low magnetic energy of nuclear spins, compared to the thermal energy at room temperature, leading to an extraordinarily low spin polarization. The most straightforward, but arguably also the most expensive method to increase the spin polarization and hence the sensitivity is to increase the external magnetic

field. However, even at a high magnetic field of 9.4 T, the spin-polarization is only 31 ppm. Improved RF coil design, like phased-array receivers and cooled coils, increases the detection sensitivity and decreases the noise, but do not change the fundamental spin polarization. Therefore the sensitivity potential of NMR, which will be reached when full polarization is achieved, remains largely dormant.

A number of methods have been proposed to enhance the nuclear polarization of spins to a significant fraction of unity. These methods, which are collectively referred to as hyperpolarization techniques, have recently begun to show potential for *in vivo* applications. They include a ‘brute force’ approach [189, 190], optical pumping of noble gases [191–193], para-hydrogen-induced polarization (PHIP) [194, 195], and dynamic nuclear polarization (DNP) [196, 197]. This section will briefly describe the principles of some of the hyperpolarization techniques. For more complete reviews the reader is referred to the literature.

### 3.6.1 ‘Brute Force’ Hyperpolarization

From Equation (1.27) it follows that the thermal equilibrium polarization increases with increasing magnetic field strength  $B_0$  and with decreasing temperature  $T$ . A relatively straightforward ‘brute force’ approach to increase the polarization in a sample is therefore to place it in a strong magnetic field at a temperature close to 0 K. Note that Equation (1.27) was derived under the assumption that  $(h\nu/kT) \ll 1$ , which is no longer valid when the temperature approaches absolute zero (see also Exercise 1.1). In this case, the spin polarization is given by:

$$P = \frac{n_\alpha - n_\beta}{n_\alpha + n_\beta} = \tanh\left(\frac{h\nu}{2kT}\right) \quad (3.88)$$

Figure 3.33 shows polarization curves for protons, carbon-13 nuclei and electrons as a function of temperature in a magnetic field of 11.75 T (500 MHz for protons). Decreasing the temperature from body (310 K) to liquid helium (4 K) increases the polarization for protons circa 75 times to circa 0.3 %. Even though this is a highly significant increase, the

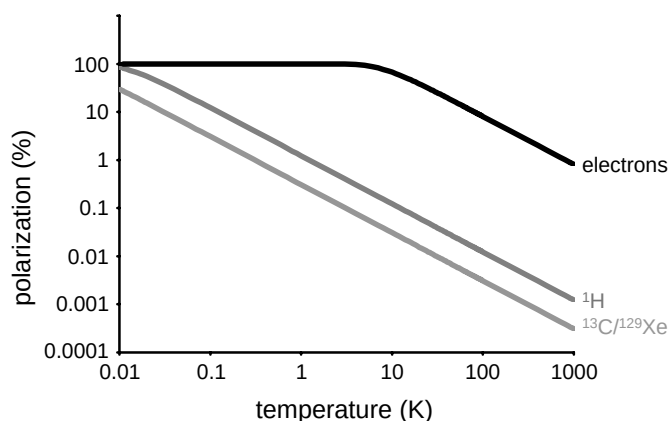


**Figure 3.33** Spin polarization for  $^1\text{H}$ ,  $^{13}\text{C}/^{129}\text{Xe}$  and unpaired electrons as a function of temperature at a magnetic field of 11.75 T.

bulk of the spins do not contribute to the polarization until the temperature is decreased to the milli-Kelvin range close to absolute zero temperature. While working at liquid helium temperature is trivial, achieving temperatures close to absolute zero is challenging and in many cases impractical. Note that electrons have >95 % polarization at 4 K due to their much larger gyromagnetic ratio ( $\gamma_e = 658.21\gamma_H$ ).

While ‘brute force’ approaches are relatively straightforward they are not used for *in vivo* NMR applications due to the extremely long  $T_1$  relaxation time constants (hours to weeks) at milli Kelvin temperatures [198]. Furthermore, ‘brute force’ methods have currently only been demonstrated on noble gases and no successful transition to room temperature has been reported. This latter point is of crucial importance for any application *in vivo*.

### 3.6.2 Optical Pumping of Noble Gases

The term optical pumping refers to the excitation of resonant atomic transitions wherein the rate of light absorption depends on the electronic spin state of the atom (see [191–193] for reviews). For the case of hyperpolarized gas, the most successful methods make use of simple atoms with a 2S or 3S valence, like  $^3\text{He}$  or alkali metals. When circularly polarized light is tuned to the lowest  $S \rightarrow P$  energy transition and the source is sufficiently narrow to avoid excitation of other states then the S state is, over time, depopulated by the light, leaving a net electron polarization. The electron polarization is ultimately transferred to the desired nuclear polarization using one of two methods: spin-exchange optical pumping or metastability exchange optical pumping. In the following, the theoretical basis of optical pumping will be briefly discussed. For more extensive treatments the reader is referred to the literature [191–193].

*Metastability Exchange Optical Pumping (MEOP).* During the metastability exchange optimal pumping procedure a sample of  $^3\text{He}$  is placed in a small (mT range), but relatively homogeneous magnetic field and excited by an electric discharge such that a small fraction ( $\sim 1$  ppm) of  $^3\text{He}$  is converted to a metastable  $2^3\text{S}_1$  state (the metastable  $^3\text{He}^*$  can be considered a plasma, i.e. an ionized gas). A metastable state is an excited state with a lifetime longer than a regular excited state, but with a shorter lifetime than the lowest, often most stable ground state (Figure 3.34). A metastable state often functions as a temporary ‘energy trap’. Circularly polarized light with a wave length specific for the  $2^3\text{S}_1 \rightarrow 2^3\text{P}_0$  electronic spin transition ( $\lambda = 1083$  nm) optically pumps the valence electron to the  $2^3\text{P}_0$  spin state. A large equilibrium population for the metastable electrons is achieved in microseconds. Through the hyperfine interaction between the electronic and nuclear spin wave functions, the electronic polarization is quickly transferred to the nucleus in the same metastable  $^3\text{He}^*$  atom. The rest of the nonexcited (ground state)  $^3\text{He}$  becomes polarized through so-called metastability exchange, during which the nuclear polarization of metastable  $^3\text{He}^*$  is transferred to ground state  $^3\text{He}$ . The depolarized  $^3\text{He}^*$  atoms regain their polarization again through the continuous optical pumping process (Figure 3.34).

The efficiency of this method is ultimately determined by the amount and lifetime of the metastable  $^3\text{He}^*$  atoms. The presence of high levels of  $^3\text{He}^*$  will lead to depolarization of other metastable states, causing inefficient transfer to the  $^3\text{He}$  ground-state. Optimal conditions are achieved with a helium pressure of circa 1 mbar. The low number of optically active atoms necessitates the use of fairly long (several meters) polarization cells

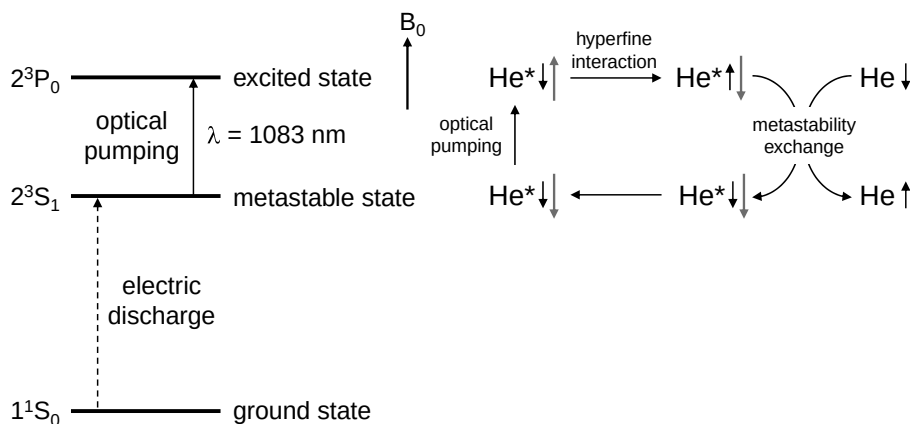


**Figure 3.34** MEOP polarization method.  $^3\text{He}$  atoms at low pressure are excited into a metastable triplet state, denoted  $^3\text{He}^*$ , by a weak electric discharge. Optical pumping of the metastable helium proceeds most efficiently via absorption of 1083 nm light on the  $2^3S_1 \rightarrow 2^3P_0$  transition. The electron spin of  $^3\text{He}^*$  is transferred to the nucleus by hyperfine interactions, after which metastability exchange collisions result in a ground-state polarized  $^3\text{He}$  atom.

over which a homogeneous magnetic field and an intense optical laser must be maintained. The metastability exchange optical pumping process is an attractive method to generate relatively large quantities of hyperpolarized helium gas (10–30 L day<sup>-1</sup> at 1 bar and 50 % polarization). However, the low gas pressure necessitates the use of compression systems and cryogenic liquefiers that ideally do not affect the spin polarization, before the hyperpolarized gas can be administered to the subject.

**Spin-Exchange Optical Pumping (SEOP).** The principle of SEOP is similar to that of MEOP, with the essential difference that instead of using endogenous metastable atoms, SEOP uses an alkali metal vapor, most often rubidium. Furthermore, SEOP can take place at higher pressures (of several bars) without sacrificing polarization. SEOP is a two-step process which starts with optical pumping of a dense alkali-metal vapor, in most cases rubidium, with circularly polarized laser light tuned to the rubidium D1 line ( $\lambda = 795$  nm), which corresponds to the energy difference between the  $5^2S_{1/2}$  ground state and the  $5^2P_{1/2}$  excited state (Figure 3.35). The left-circularly polarized light excites electrons from the spin-down sub-level ( $m_S = -1/2$ ) in the ground state to the spin-up sub-level ( $m_P = +1/2$ ) in the excited state. Collisions with the noble gas rapidly equalize the sub-levels of the excited state. Then quenching collisions with nitrogen atoms (present at circa 0.1 bar) repopulate the ground state sub-levels with almost equal probability. Since the rubidium atoms have  $-1/2$  units of electron-spin angular momentum before absorbing the photons and 0 units of electron-spin angular momentum after being quenched from the excited state, on average each absorbed photon deposits  $1/2$  unit of spin angular momentum in the rubidium vapor, the remainder being lost to translational motion. The result of the combined optical pumping, collisional mixing and  $\text{N}_2$ -mediated quenching is a nearly 100 % spin polarization of the rubidium valence electron since the relaxation rate of  $\sim 10^3 \text{ s}^{-1}$

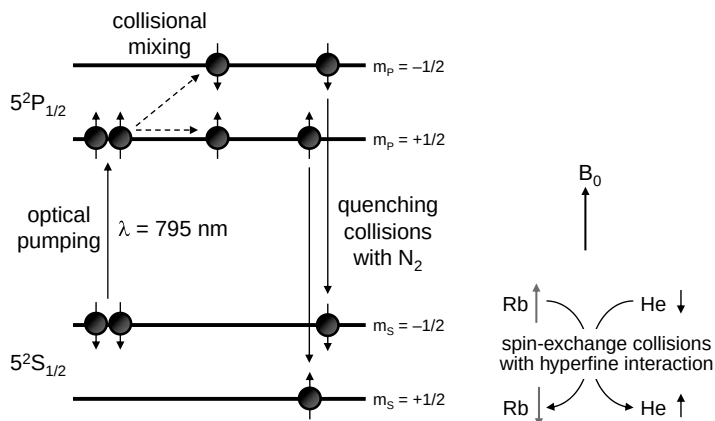


**Figure 3.35** SEOP polarization method. Optical pumping of rubidium vapor with circularly polarized 795 nm laser light on the unresolved  $5^2S_{1/2} \rightarrow 5^2P_{1/2}$  transition creates a large electronic-spin polarization in Rb atoms which is in turn transferred to  $^3\text{He}$  nuclei via spin-exchange collisions in combination with hyperfine interactions. See text for more details.

is much smaller than the photon-rubidium spin-exchange rate of  $10^6 \text{ s}^{-1}$ . In principle any alkali-metal vapor can be polarized in this manner, but rubidium is particularly convenient due to the commercial availability of appropriate diodes and lasers. Furthermore, the similarity of other energy level differences in the lighter alkali metals has prevented efficient SEOP in lithium, sodium and potassium.

During the second step the rubidium polarization is transferred to the  $^3\text{He}$  through spin-exchange collisions (Figure 3.35). During any Rb –  $^3\text{He}$  binary collision there is a small probability that the wave function of the rubidium valence electron will penetrate through the  $^3\text{He}$  atom's electron cloud to the  $^3\text{He}$  nucleus. The hyperfine interaction between the  $^3\text{He}$  nucleus and the Rb valence electron can then induce both species to flip their spins, thereby transferring angular momentum to the  $^3\text{He}$  nucleus from the Rb valence electron. Unfortunately, the probability for this interaction is very small, making the spin-exchange process very slow. Even though the typical time constant for the build-up of  $^3\text{He}$  nuclear polarization is between 5 h and 20 h, high density  $^3\text{He}$  gas (up to several bars) with up to 60 % polarization can be obtained at sub-liter quantities.

### 3.6.3 Para-hydrogen-induced Polarization (PHIP)

In 1986 Bowers and Weitekamp [194] theoretically predicted that the hydrogenation of small organic molecules with para-hydrogen can lead to strong nonequilibrium polarizations of the protons in the hydrogenated compound. They confirmed their predictions experimentally in 1987 [195]. The novel polarization techniques were initially dubbed PASADENA and ALTADENA, but are now commonly known under their collective name of para-hydrogen-induced polarization (PHIP).

Molecular hydrogen exists in two spin isomer states, ortho-hydrogen and para-hydrogen (Figure 3.36A). Para-hydrogen is the lowest energy state, a nuclear spin singlet, where the two proton spins are antiparallel to one another. Ortho-hydrogen, a nuclear spin triplet,

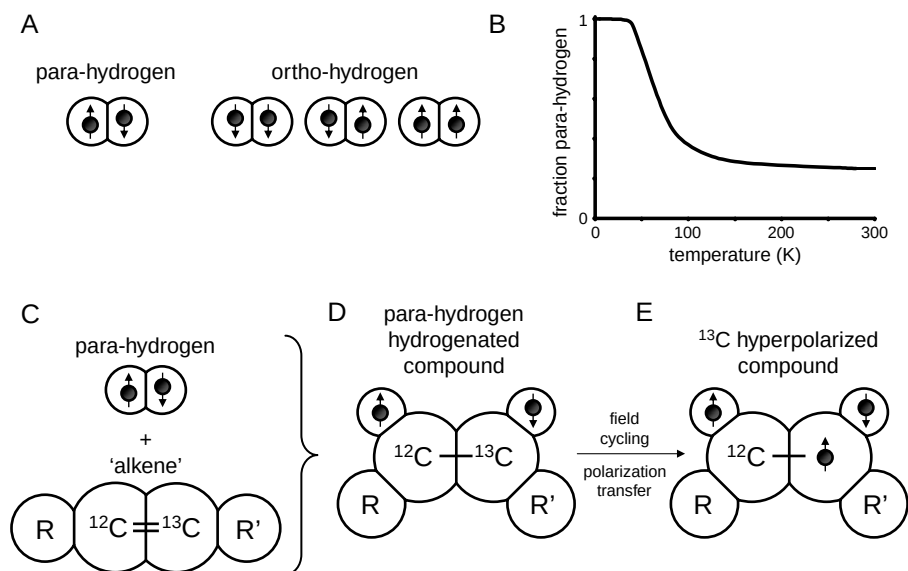


**Figure 3.36** (A) Four possible spin states of the hydrogen  $H_2$  molecule, giving rise to 25 % para-hydrogen and 75 % ortho-hydrogen at room temperature. (B) The fraction of para-hydrogen can, with the aid of a catalyst, be increased to >99 % when the temperature is lowered to <20 K. Following removal of the catalyst the system can be brought to room temperature where the para/ortho-hydrogen distribution returns only very slowly to the 25 %/75 % room temperature equilibrium. (C) When para-hydrogen is combined with a compound containing double bonds, like alkenes, (D) a para-hydrogen hydrogenated compound is formed in which the symmetry of the hydrogen nuclei is broken, thus leading to a greatly increased spin-order. (E) In-phase signals with a longer  $T_1$  relaxation time can be created by transferring the nonequilibrium proton spin order to a carbon-13 atom by field cycling or polarization transfer methods.

exists in a higher energy state. As the isomers have different energies, the energy level populations are temperature dependent with a circa 25 % mole fraction of para-hydrogen at room temperature. Lowering the temperature to 20 K increases the mole fraction of para-hydrogen to >99 % (Figure 3.36B). However, the interconversion between ortho- and para-hydrogen is normally very slow due to violation of symmetry selection rules. Fortunately, the addition of a paramagnetic catalyst can circumvent this selection rule, leading to the production of large quantities of para-hydrogen in a matter of hours. Upon removal of the catalyst, and returning to ambient temperatures, the ortho-/para-hydrogen system only very slowly re-establishes thermodynamic equilibrium, thereby greatly facilitating storage and transport of para-hydrogen.

When para-hydrogen hydrogenates another molecule through a chemical reaction, the spin correlation between the two protons will initially be retained (Figure 3.36C and D). However, the symmetry of the hydrogen molecule will be broken due to scalar and dipolar couplings with other spins, as well as differences in chemical environment. Technically, the nuclei are not polarized, but are in a state of increased spin order. Nevertheless, the

ordered spin state of each proton will give a highly enhanced NMR spectrum, consisting of characteristic antiphase resonances.

While direct proton NMR observation of the PHIP effect is common in hydrogenation reactions in transition metal chemistry [199], for many *in vivo* applications it is desirable to transfer the hydrogen spin-state to an adjacent heteronucleus, like carbon-13. Firstly, heteronuclei often have long  $T_1$  relaxation time constants of 30 s or more, providing a greater window for efficient transfer of the compound and subsequent injection into the subject. Secondly, the natural abundance of carbon-13 is very low, such that injection of a hyperpolarized  $^{13}\text{C}$  compound can be detected with minimal interference of ‘background’ signals. As one of the first *in vivo* applications of hyperpolarization,  $^{13}\text{C}$  MR angiography has provided images with excellent contrast-to-noise ratios [200–202]. Thirdly, the ordered spin-state of hydrogen does not provide a net polarization, resulting in anti-phase resonances. While this is not a problem for MR spectroscopy, it does lead to severe signal loss for MRI applications. Transfer to a heteronucleus can be accompanied by the generation of a net polarization on the carbon-13 nucleus (Figure 3.36E). Two commonly employed methods for transferring spin-order to heteronuclear spins are polarization transfer methods and so-called diabatic–adiabatic field cycling. The former methods are typically based on the INEPT sequence (see Chapter 8), which is commonly used for conventional  $^{13}\text{C}$  NMR spectroscopy and will be discussed in Chapter 8. Diabatic–adiabatic field cycling rapidly (i.e. nonadiabatically) lowers the magnetic field to the range of  $\mu\text{T}$ , such that the heteronuclear  $^1\text{H}$ – $^{13}\text{C}$  spin system is strongly coupled (i.e. has mixed spinstates) and dominated by the scalar coupling. The magnetic field is subsequently increased adiabatically, finally resulting in a net polarization of the carbon-13 nucleus.

### 3.6.4 Dynamic Nuclear Polarization

The method of dynamic nuclear polarization (DNP) as described in this section is based on the ‘solid effect’ originally discovered by Abragam and Proctor [203]. Inside a diamagnetic insulator with paramagnetic impurities, they observed a polarization much larger than the thermal equilibrium nuclear polarization when the insulator was exposed to microwave irradiation. A similar effect was predicted by Overhauser [10] for metals 5 years earlier.

To illustrate the principle of DNP consider a group of  $N_N$  nuclei of spin-1/2 resonating at Larmor frequency  $\omega_N$  and a group of  $N_e$  electron spins with Larmor frequency  $\omega_e$  positioned in an external magnetic field  $B_0$ . Figure 3.37 shows the energy levels. The lowest energy state corresponds to an antiparallel electron spin and a parallel nuclear spin with respect to the external magnetic field. When the nuclei and electrons would be completely separated, nuclear transitions can be achieved in the RF range at  $\omega_N$ . Electronic transitions are, due to the much larger energy level difference, achieved in the microwave range at frequency  $\omega_e$ . However, when the electron generates a finite magnetic field at the nuclei, the two become dipolar-coupled. This so-called hyperfine interaction will lead to a mixing of the four pure states, which in turn will lead to a small, but nonvanishing probability of simultaneous electron and nuclear spin transitions or flips.

Now suppose that the system is irradiated at the microwave frequency ( $\omega_e - \omega_N$ ) and the electronic line width  $\Delta\omega_e$  is small enough so that the transition ( $\omega_e + \omega_N$ ) does not occur. Besides a flip of the electron spin, the nuclear spin flips from an antiparallel to a parallel orientation (Figure 3.37B). The very fast electron relaxation time ( $T_{1e} \sim 1$



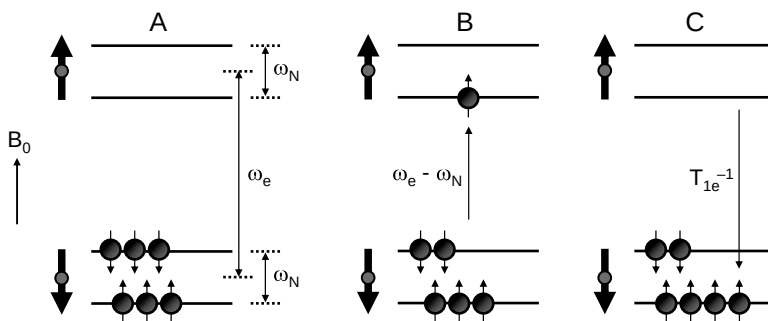


**Figure 3.37** Principle of dynamic nuclear polarization. (A) Energy level diagram for a two-spin-system consisting of a nuclear spin (small arrows) with resonance frequency  $\omega_N$  and electron spins (large arrows) with resonance frequency  $\omega_e$ . At thermal equilibrium all spins are approximately equally distributed over the two lower energy levels. (B) During selective irradiation at the microwave frequency ( $\omega_e - \omega_N$ ), without affecting the transition ( $\omega_e + \omega_N$ ), the electron spin is transferred to the higher energy level. (C) However, due to fast electron  $T_1$  relaxation the spin quickly returns to the lowest energy level, thereby slightly increasing the nuclear polarization. Continued microwave irradiation can quickly lead to a large nonequilibrium nuclear polarization.

ms) quickly flips the electron spin orientation (Figure 3.37C). Therefore, the combined effect of microwave irradiation and fast electron relaxation has led to a slight increase in polarization, as a single nuclear spin has been transferred from an antiparallel to a parallel orientation. Continuing this process will lead to a complete depletion of the antiparallel state, giving complete or 100 % nuclear polarization.

It is easy to see that if a microwave frequency ( $\omega_e + \omega_N$ ) was used, all the nuclear spins would end up in an antiparallel state, giving a complete, but inverted nuclear polarization. In the example of Figure 3.37 it was assumed that the electron spin polarization  $P_e$  was complete, which is realistic at low temperatures (see Figure 3.33). However, when  $P_e$  is not complete, the final nuclear spin polarization  $P_N$  will be equal to  $\pm P_e$ .

Typically the electron concentration is low ( $\sim 0.001$  electron spins per nuclear spin), so that one electron has to polarize not only the nuclear spins in the immediate vicinity, but also those that are further away. Resonant mutual flips or transitions between two neighboring nuclear spins, also referred to as spin diffusion, is the effective transport mechanism that supports this requirement. On average the  $N_e$  electron spins must polarize  $(N_N/N_e)$  nuclear spins. Furthermore, after each forced microwave-induced transition the electron spin must relax back into its thermal equilibrium before any of the  $(N_N/N_e)$  nuclear spins in its sphere of influence relax through a nuclear relaxation mechanism. This leads to the condition that:

$$\left(\frac{N_N}{N_e}\right) \left(\frac{T_{1e}}{T_{1N}}\right) \ll 1 \quad (3.89)$$

In other words, the total rate of spontaneous electron spin flips ( $N_e/T_{1e}$ ) should considerably exceed the rate of spontaneous nuclear spin flips ( $N_N/T_{1N}$ ). If the nuclear relaxation is dominated by the hyperfine interaction with the electrons, then Equation (3.89) will automatically hold. However, if other nuclear relaxation mechanisms are present, the

above condition may be violated and the nuclear polarization  $P_N$  could be much smaller than  $P_e$ .

Techniques for introducing the necessary amounts of unpaired electrons into the sample, consist either of mixing a small amount of a stable chemical radical to the liquid state or of exposing the solid state to ionizing radiation. DNP has been used as a polarization technique for organic molecules in the solid state. However, *in vivo* applications require the polarized material in the liquid state for injection into the subject. Ardenkjaer-Larsen *et al.* [197] have offered a solution to this challenging problem. The polarized solid material is brought into the liquid state by dissolution in an appropriate solvent, while preserving the large nuclear polarization.

While hyperpolarized *in vivo* NMR is only in its infancy, it is apparent that it holds great promise for a wide range of applications. Lung imaging with hyperpolarized noble gases is quickly becoming a routinely applied technique [204–206]. Hyperpolarized  $^{13}\text{C}$  MR angiography has been shown to yield excellent high contrast-to-noise images. And even though the utility of hyperpolarization for *in vivo* NMR spectroscopy remains to be demonstrated, there is no doubt that it will provide unique new information about fast metabolic processes.

### 3.7 Exercises

- 3.1 On a  $[2\text{-}^{13}\text{C}]$ acetate sample at 7.05 T the proton frequency and carbon-13 frequencies were measured at 300.015623 MHz and 75.229674 MHz, respectively. In an *in vivo*  $^1\text{H}$  NMR spectrum acquired from human brain at 4.0 T the proton frequency of glutamate-H4 was measured at 170.455322 MHz. Calculate the *in vivo* carbon-13 frequency of glutamate-C4 at 4.0 T.
- 3.2 The 2-oxoglutarate/glutamate chemical exchange as catalyzed by glutamate dehydrogenase is characterized by a rate constant of  $0.4\text{ s}^{-1}$ . Given the  $^{13}\text{C}$   $T_1$  relaxation times of 1.5 and 1.7 s for 2-oxoglutarate and glutamate measured in the absence of saturation, calculate the signal decrease in glutamate upon continuous saturation of 2-oxoglutarate.
- 3.3 **A** Given a longitudinal relaxation time constant  $T_1$  of 4.0 s for free water ( $\tau_c = 10^{-11}\text{ s}$ ) at 7.05 T, calculate the  $T_1$  for bone ( $\tau_c = 10^{-6}\text{ s}$ ) under the condition of pure dipolar relaxation. Assume equal dipolar distances  $r$  for all compounds.  
**B** Calculate the minimum  $T_1$  relaxation time constant at 7.05 T as a result of pure dipolar relaxation.  
**C** Calculate the transverse relaxation time constants  $T_2$  for water and bone at 7.05 T.  
**D** Calculate the  $T_1$  and  $T_2$ s for water and bone at 17.625 T.
- 3.4 A NMR spectroscopist measures a sample of 100 mM  $\text{H}_2\text{SO}_4$  and 100 mM glutathione in water.  
**A** Describe the expected pulse-acquire  $^{33}\text{S}$  NMR spectrum.  
**B** Describe the expected pulse-acquire  $^1\text{H}$  NMR spectrum.
- 3.5 Inversion recovery, as discussed in Section 1.11 is arguably the most robust method to measure the longitudinal relaxation time constant  $T_1$ . However, since inversion recovery is a relatively slow method, many alternative methods have been developed.

Consider a pulse-acquire sequence executed with a repetition time TR. The  $T_1$  relaxation time constant can be estimated after performing two steady-state experiments with nutation angles  $\alpha_1$  and  $\alpha_2$  during which signals  $M_1$  and  $M_2$  are acquired.

- A Derive an expression for  $T_1$  as a function of  $\alpha_1$ ,  $\alpha_2$ ,  $M_1$ ,  $M_2$  and TR.
  - B Calculate  $T_1$  when  $\alpha_1 = 30^\circ$ ,  $\alpha_2 = 60^\circ$ ,  $M_1 = 100$ ,  $M_2 = 150$  and TR = 500 ms.
  - C Discuss the pro's and con's of this method against an inversion recovery method and discuss how the performance of the two-point method can be optimized for a known  $T_1$  range.
- 3.6** A Verify that Equations (3.29) and (3.30) are solutions to the Bloch equations extended to accounted for chemical exchange [i.e. Equations (3.27) and (3.28)].
- B Derive Equation (3.39) from (3.27).
  - C Derive Equation (3.40) from (3.39).
- 3.7** Derive the principle of mass conservation [Equation (3.45)].
- 3.8** A Show that Equation (3.62) is the spin-echo-specific form of the more general form given by Equation (3.61).
- B Using Equation (3.62) [or Equation (3.61)] derive the analytical expression for the b-value of a pair of square diffusion gradients of amplitude G, duration  $\delta$  and separation  $\Delta$  as incorporated in a Hahn spin-echo (see also Figure 3.19B).
  - C For an echo-time TE of 20 ms and infinitely short RF pulses, calculate the maximum b-value achievable on a system with a maximum gradient strength of  $40 \text{ mT m}^{-1}$  per direction. Assume infinitely fast gradient ramping.
  - D Recalculate the maximum b-value for the more realistic case in which the magnetic field gradients have a finite ramp time of 500  $\mu\text{s}$  to maximum amplitude.
- 3.9** A Derive Equation (3.58).
- B For a well-shimmed sample  $G_0 = 1.0 \text{ Hz mm}^{-1}$ . Calculate the echo-time by which the water signal ( $D = 0.7 \mu\text{m}^2 \text{ ms}^{-1}$ ) has decreased by 5 % as a result of diffusion-related signal loss.
  - C Given a transverse relaxation time constant  $T_2 = 70 \text{ ms}$ , calculate the overall signal loss.
  - D In order to reduce the overall signal loss,  $G_0$  is increased such that the echo-time can be reduced. Discuss the advantages and disadvantages of this approach over the more standard Stejskal–Tanner approach.
  - E Calculate the difference in signal intensity obtained for water at an echo-time of 100 ms when acquired with a standard Hahn spin-echo or a CPMG spin-echo pulse train with  $\text{TE}_{\text{CPMG}} = 2 \text{ ms}$ . Assume  $D = 0.7 \mu\text{m}^2 \text{ ms}^{-1}$ ,  $T_2 = 70 \text{ ms}$  and a back ground magnetic field gradient  $G_0 = 2500 \text{ Hz mm}^{-1}$ .
- 3.10** Consider a stimulated echo sequence with two  $5 \text{ ms } 250 \text{ mT m}^{-1}$  TE crusher gradients along the x direction, separated by 50 ms. Dephasing during the TM period (TM = 20 ms) is achieved with a  $2 \text{ ms } 40 \text{ mT m}^{-1}$  TM crusher gradient along the y direction.
- A Calculate the diffusion weighting (i.e. 'b-value') for the sequence.
  - B The creatine resonance at 3.03 ppm is detected with an intensity of 387 during the diffusion weighted experiment. In an earlier experiment with a lower diffusion weighting ( $b = 120 \text{ s mm}^{-2}$ ), the creatine intensity was 1045. Calculate the ADC of creatine.

- C During a measurement with  $TM = 500$  ms the creatine ADC is significantly larger than that calculated under B. Give the most likely explanation for this observation and a possible solution.
- D Calculate the diffusion weighting when the TE crusher gradients are applied simultaneously along all three spatial directions.
- E Calculate the diffusion weighting when the TM crusher gradient is changed to a  $5 \text{ ms } 50 \text{ mT m}^{-1}$  gradient applied along the x and z directions.
- 3.11 A Derive Equation (3.82) from Equation (3.81).
- B For a  $^{13}\text{C}$  fractional enrichment (FE) of compound S of 0.7, calculate the FE of compound P for  $t \rightarrow \infty$ .
- C Given a FE for compound S of 0.7 and  $[P] = 10 \text{ mM}$ , calculate the flux when  $[P^*] = 3 \text{ mM}$  at  $t = 12 \text{ min}$ .
- D Given a FE for compound S of 0.7 and  $[P] = 20 \text{ mM}$ , calculate the flux when  $[P^*] = 6 \text{ mM}$  at  $t = 12 \text{ min}$ .
- 3.12 Show, for 2D diffusion, that the trace of the diffusion tensor is rotationally invariant.

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# 4

## Magnetic Resonance Imaging

### 4.1 Introduction

Magnetic resonance spectroscopy (MRS) has proven to be a powerful technique to supply information about the chemical structure of molecules. *In vitro* MRS on biological or synthetic molecules is usually performed on a uniform sample, i.e. the concentration and composition are constant throughout the sample, such that the sample dimension and location need not be considered as an experimental parameter. However, this condition is usually not met with *in vivo* MRS: the concentration, composition, relaxation and other properties of compounds can vary throughout the object under investigation. Such variations can be considerable in cases where normal and pathological tissues (i.e. tumors, infarctions) are studied. Therefore, *in vivo* MRS is often combined with techniques that allow the detection of spatially localized volumes, in which variations in composition and relaxation are minimal. These localization techniques will be described in detail in Chapters 6 and 7.

Before any localization technique for *in vivo* MRS can be used, one has to know the spatial boundaries and the distribution of different tissues within the object under investigation. Magnetic resonance imaging (MRI) is an ideal technique to provide this and a wealth of additional information about the object under investigation. In 1973, Lauterbur [1] reported the first reconstruction of a proton spin density map (i.e. an image of the water distribution) using NMR. In the same year, Mansfield and Grannell [2] independently demonstrated the Fourier relationship between the spin density and the NMR signal acquired in the presence of a magnetic field gradient. Following these initial reports, MRI rapidly developed to the important imaging modality it is today (see [3–5] for reviews), allowing the noninvasive and nondestructive generation of images of intact living objects. By proper choice of experimental parameters excellent soft tissue contrast can be achieved.

The principles underlying MRI also apply for many *in vivo* MRS techniques. Therefore, the necessity to use MRI for an accurate determination of the origin of the MRS signals

and the similarity between the principles of MRI and MRS provide the justification for this chapter. First, the essential ingredient of any MRI experiment, namely magnetic field gradients, will be discussed in detail, after which the standard spin-warp MRI sequence will be described. Following an introduction to the k-space formalism, which provides a quantitative description of any MRI sequence, the principles of fast MRI methods are discussed. The chapter is concluded with the origin and utilization of several soft tissue contrasts, including  $T_1$ ,  $T_2$  and BOLD. For more extensive treatments of MRI, the reader is referred to a wealth of textbooks (e.g. [3–5]).

## 4.2 Magnetic Field Gradients

As with all NMR techniques, MRI also makes use of the general resonance condition  $\omega_0 = \gamma B_0$  [i.e. Equation (1.11)]. However, instead of providing spectral information (as with MRS), the resonance condition in MRI applications is used to obtain spatial information. The essential concept of MRI is that the resonance frequency  $\omega_0$  is made position-dependent, such that after Fourier transformation the different frequencies correspond to spatial position rather than chemical shift. This can be accomplished by making the external magnetic field position-dependent with magnetic field gradients. The most commonly used magnetic field gradient is a magnetic field of which the amplitude varies linearly with position. Mathematically, a linear magnetic field gradient  $G_z$  in the  $z$  direction is therefore described by:

$$G_z = \left( \frac{dB_z}{dz} \right) = \text{constant} \quad (4.1)$$

where  $B_z$  is the magnetic field strength and  $z$  the position. Magnetic field gradients in the  $x$  and  $y$  directions can be described by analogous formulas. Magnetic field gradients are generated by electrical currents in specially shaped coils within the bore of the magnet. Normally there are three sets of gradients in a (MRI) magnet, the so-called X, Y, and Z gradients, corresponding to the direction along which the magnetic field strength changes. Chapter 10 will deal with the characteristics of gradient coils.

It is important to recognize the difference between the direction of a gradient and the direction of the magnetic field. The magnetic field is *always* directed along the  $z$  axis (parallel to the main magnetic field  $B_0$ ), independent of the gradient orientation. The direction of a gradient refers to the direction in which the strength of the magnetic field varies (Figure 4.1). The magnetic field gradient is positioned around the center of the magnet, i.e. a gradient adds to the main magnetic field on one side of the middle and subtracts from the static field on the other side. Therefore the magnetic field strength of all gradients is zero in the magnet's isocenter.

The addition of a magnetic field gradient  $\mathbf{G}$  to the static magnetic field  $B_0$  generates a total magnetic field at position  $\mathbf{r}$  given by:

$$\mathbf{B}(\mathbf{r}) = B_0 + \mathbf{r}\mathbf{G} \quad (4.2)$$

As a consequence the resonance condition [Equation (1.11)] should be rewritten as:

$$\omega(\mathbf{r}) = \gamma \mathbf{B}(\mathbf{r}) = \gamma B_0 + \gamma \mathbf{r}\mathbf{G} \quad (4.3)$$

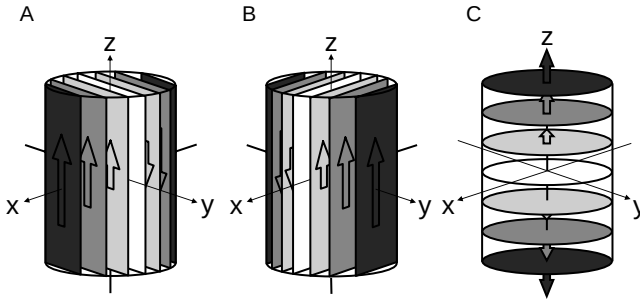


**Figure 4.1** Magnetic field distribution generated by magnetic field gradients in the (A)  $x$ , (B)  $y$  and (C)  $z$  direction. Within each plane the magnetic field strength is identical. The magnetic field strength generated by each gradient increases linearly as a function of position, as indicated by the relative amplitudes of the arrows and the color of the planes. In the middle of the gradient isocenter for each gradient direction (white plane) the magnetic field gradient strength is zero.

i.e. the resonance frequency becomes dependent on position  $\mathbf{r}$ . Equation (4.3) is the basis for all MRI sequences, as well as most localized MRS methods. Using Equation (4.3) all three dimensions of a 3D object can be encoded. The specific encoding can be achieved in a number of ways, all of which use either the frequency or the phase of the signal to obtain spatial information.

### 4.3 Slice Selection

To obtain an image of a 3D object, all three dimensions need to be encoded independently which could be a time-consuming procedure. Therefore, one often reduces the problem to two dimensions by selecting a spatial slice out of a 3D object [6, 7]. A spatial slice can be selected by the combination of a RF pulse and a magnetic field gradient. A magnetic field gradient in the  $z$  direction creates a linear magnetic field distribution as function of the  $z$  position. As a consequence each  $z$  position is characterized by a specific magnetic field and hence a specific resonance frequency. When a RF pulse, specially designed to excite only a selective frequency range (see Chapter 5), is applied simultaneously with the magnetic field gradient, only a selective range of frequencies, and hence positions, is excited (Figure 4.2A). For a 3D object, the action which is schematically drawn in Figure 4.2A, corresponds to the selection of a 2D spatial slice (Figure 4.2B).

The slice thickness is determined by two factors, the magnetic field gradient strength and the bandwidth of the RF pulse. A stronger magnetic field gradient creates a larger range of resonance frequencies across the sample (i.e. the slope of the diagonal line in Figure 4.2A increases), resulting in the selection of a thinner spatial slice when the RF pulse is kept constant (in terms of frequencies the slice thickness has not changed, since the bandwidth of the RF pulse remained constant). Vice versa, the same can be achieved by decreasing the RF pulse bandwidth (e.g. by increasing the pulselength), while keeping the magnetic gradient strength constant. On-resonance, both approaches lead to the same spatial slice.



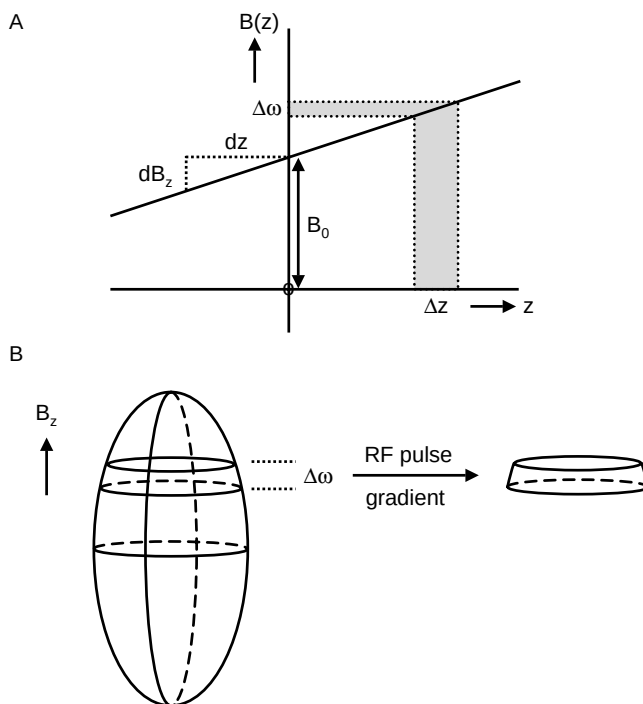


**Figure 4.2** Principle of slice selection with magnetic field gradients. (A) A magnetic field gradient in the  $z$  direction generates a distribution of magnetic field strengths (superimposed on the main magnetic field  $B_0$ ) and consequently of resonance frequencies, which is linearly dependent on the spatial position  $z$ . Therefore, a selective range of frequencies as excited by a frequency-selective RF pulse (see Chapter 5) corresponds directly to a selective range of spatial positions (i.e. a slice). (B) The selection of a range of frequencies by a selective RF pulse in the presence of a magnetic field gradient results in the selection of a spatial slice from a hypothetical 3D object. The required gradient strength and RF offset to select a slice of specific thickness and position are given by Equations (4.4) and (4.5), respectively.

However, off-resonance, the former approach gives smaller chemical shift artifacts and is therefore the preferred method (see Chapter 6).

The spatial position of the slice is also determined by two factors, the magnetic field gradient strength and the transmitter frequency of the RF pulse. The magnetic field gradient creates the spatial distribution of frequencies, but the transmitter frequency (and the bandwidth) of the RF pulse determines which frequencies are being excited. When the transmitter frequency equals the Larmor frequency, spins in the middle of the sample ( $\pm 0.5 \times \text{bandwidth}$ ) are excited. To excite spins at a certain distance from the isocenter, the transmitter frequency should be adjusted according to Equation (4.3).

Since the magnetic field gradient strength affects both the slice thickness and the slice position, it is common practice to first determine the required gradient strength (for a given RF pulselength), after which the position is selected with the transmitter frequency of the

RF pulse. For example, selection of a 2 mm slice, 5 mm out of the isocenter using a RF pulse with a bandwidth of 1000 Hz requires a gradient strength of:

$$G_z = \frac{\Delta\omega}{\gamma \Delta z} = \frac{1000 \text{ Hz}}{4257.6 (\text{Hz G}^{-1}) 0.2 \text{ cm}} = 1.17 \text{ G cm}^{-1} = 11.7 \text{ mT m}^{-1} \quad (4.4)$$

With a fixed gradient strength and a constant RF pulse, the slice position can only be adjusted by changing the transmitter frequency according to:

$$\nu(z) = \nu_0 + \gamma z G_z = \nu_0 + 4257.6 (\text{Hz G}^{-1}) 0.5 \text{ cm} 1.17 (\text{G cm}^{-1}) = \nu_0 + 2500 \text{ Hz} \quad (4.5)$$

where  $\nu_0$  is the Larmor frequency in the absence of magnetic field gradients and  $\gamma = \gamma/2\pi$ . Chapter 5 will give a more detailed discussion of (selective) RF pulses and their characteristic bandwidths.

Slice selection can be performed for only a single spatial slice, but can also be used to select multiple spatial slices. The selection of multiple slices can be performed in a time-efficient manner in which signal from several slices is acquired during the recovery period of a single slice. This interleaved approach gives rise to multi-slice imaging [8]. Suppose the repetition time of a slice-selective imaging sequence is 2500 ms and the total acquisition time (from excitation to the end of acquisition) is 250 ms. The remaining 2250 ms can then be used to excite and acquire nine other spatial slices. Note that, in order to minimize cross-slice interference, both the  $90^\circ$  and  $180^\circ$  RF pulses in the MRI sequence need to be selective and need to select the same spatial slice. Even though all RF pulses are spatially selective, the acquisition of subsequent slices will lead to interference when the selected slices are close to each other (i.e. near-continuous coverage of the object under investigation). This is because the slice profile is not perfectly rectangular, leading to partial excitation and hence overlap of adjacent slices. For this reason, the slices are normally acquired in an interleaved manner (i.e. for 10 slices the acquisition order is 1, 3, 5, 7, 9, 2, 4, 6, 8, 10). In this manner, the effect of neighboring slices is significantly reduced (see also Exercise 4.5). An important point that should be noted is that the frequency profiles of identical, frequency selective  $90^\circ$  and  $180^\circ$  RF pulses seldom have the same bandwidth, such that the selected slices are of different thickness (and/or shape). For instance, the bandwidths of identical  $90^\circ$  and  $180^\circ$  sinc pulses differ by almost a factor of two (see Chapter 5). This has to be taken into account when quantitative, volumetric MRI measurements need to be performed.

## 4.4 Frequency Encoding

After selecting one or more spatial slices, the origin of the MR signal needs to be encoded in two dimensions. Again, Equation (4.3) functions as the basis of spatial encoding. In the slice selection process, the distribution of spatially dependent frequencies was maintained during the RF pulse in order to enable the RF pulse to select (e.g. excite) the desired frequencies. When, after excitation, the distribution of spatially dependent frequencies is maintained during signal acquisition, the FID would hold information about the spatial origin of the signal. Upon Fourier transformation the NMR ‘spectrum’ would resemble a 1D projection of the spin density distribution along the direction of the applied gradient. The simplest pulse sequence to obtain a 1D projection would be a pulse-acquire method with a magnetic field gradient applied during acquisition. However, this experimental set-up is

not optimal in several respects. Due to finite gradient rise times the first few data points are sampled during a time-varying gradient, thereby leading to artifacts when a regular Fourier transformation is applied. Eliminating the first few data points would greatly diminish the obtainable signal-to-noise ratio (SNR). Alternatively, the magnetic field gradient can start before the excitation pulse to ensure a constant gradient during signal acquisition. This would indeed eliminate the distortion problem at the expense of a lower SNR, since the RF pulse in combination with a magnetic field gradient would select a spatial slice (see Section 4.3), thereby only covering part of the sample. An alternative and more convenient way to obtain spatial information is to acquire an echo instead of a FID, as shown in Figure 4.3. This type of echo formation belongs to the class of gradient-echo techniques. Together with spin-echo techniques (see also Section 1.12) they form the basis for the majority of MRI methods.

A gradient-echo sequence consists, besides the excitation pulse, of two gradient pulses. One is applied prior to signal acquisition while the other, being of opposite sign and

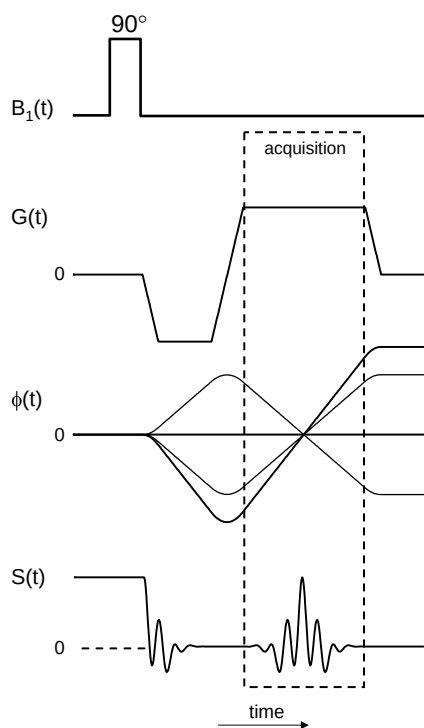


**Figure 4.3** Principle of gradient-echo formation. Following excitation the spins acquire a position-dependent phase  $\phi(t)$  during the negative preparation gradient according to Equation (4.6). Phase cancellation across the entire sample leads to a quick dephasing of the total signal  $S(t)$ . Upon gradient sign reversal the phase of the spins reduces according to Equation (4.8) leading to full echo formation when the area of the positive gradient exactly cancels that of the negative gradient. During the acquisition period the phase changes linearly with time, such that a position-dependent frequency is obtained according to Equation (4.8).

typically having twice the area, is applied during signal acquisition. Formation of the echo containing the spatially dependent frequencies can be explained in a number of ways. In Section 4.6 a concise k-space formalism applicable to any imaging sequence will be presented. For now a more visual approach will be followed.

The function of the first magnetic field gradient in Figure 4.3 is to prepare the transverse magnetization for encoding the spatial information during signal acquisition. At position  $\mathbf{r}$  the effect of the first gradient pulse is the generation of a phase shift given by:

$$\phi_1(\mathbf{r}, t) = \gamma \mathbf{r} \int_0^t \mathbf{G}_1(t') dt' \quad (4.6)$$

which, for a constant amplitude gradient [i.e.  $\mathbf{G}_1(t) = \mathbf{G}_1$ ] simplifies to:

$$\phi_1(\mathbf{r}, t) = \gamma \mathbf{r} \mathbf{G}_1 t \quad (4.7)$$

At the end of the first gradient pulse the transverse magnetization at each position  $\mathbf{r}$  is encoded (i.e. prepared) with a specific phase shift  $\phi_1(\mathbf{r})$ . The second gradient is of opposite sign and the total area is twice that of the first gradient. Therefore, the total applied gradient in the middle of the second gradient is zero and consequently the acquired phase shift also equals zero. Since the transverse magnetization prior to signal acquisition had acquired a linear position-dependent phase shift during the first gradient, spins at different spatial positions have to rotate at different frequencies to fulfill the condition  $\phi(\mathbf{r}) = 0$  in the middle of the second gradient. The frequencies  $\omega(\mathbf{r})$  are related to the initial acquired phase shifts according to:

$$\omega(\mathbf{r}) = \frac{d\phi(\mathbf{r}, t)}{dt} \quad \text{where} \quad \phi(\mathbf{r}, t) = \phi_1(\mathbf{r}, T_1) + \gamma \mathbf{r} \int_0^t \mathbf{G}_2(t') dt' \quad (4.8)$$

which for a constant amplitude gradient [i.e.  $\mathbf{G}_2(t) = \mathbf{G}_2$ ] simplifies to:

$$\phi(\mathbf{r}, t) = \phi_1(\mathbf{r}, T_1) + \gamma \mathbf{r} \mathbf{G}_2 t \quad (4.9)$$

$T_1$  is the length of the first gradient and  $\mathbf{G}_2$  the amplitude of the second gradient, which is opposite to  $\mathbf{G}_1$  such that  $\phi(\mathbf{r}, t)$  decreases over time. Maximum echo formation [i.e.  $\phi(\mathbf{r}) = 0$  for all positions  $\mathbf{r}$ ] occurs when  $\phi_1(\mathbf{r}, T_1) = -\phi_2(\mathbf{r}, T_2)$  or, in other words  $\mathbf{G}_1 T_1 = -\mathbf{G}_2 T_2$  (i.e. when the total, net gradient is zero.  $T_2$  is equal to half the acquisition time). Since signal acquisition was performed during the second ('readout') gradient, the spatially dependent frequencies given by Equation (4.8) are recorded and will give the spatial spin distribution upon Fourier transformation. From the signal evolution  $S(t)$  shown in Figure 4.3 it follows that while the FID signal is distorted by time-varying gradients, the echo is acquired during a period of constant gradient amplitude, thereby leading to an undistorted spatial profile following Fourier transformation. The outlined procedure is generally referred to as frequency encoding and is the most often employed method to obtain 1D spatial information. From Figure 4.3 it can also be deduced that the presence of magnetic field inhomogeneity will lead to an additional phase evolution that is independent of the applied magnetic field gradients. As such the phase-time slopes during signal acquisition are modified by an additional linear phase-time slope, thereby leading to the encoding of a different, incorrect spatial position (see also Exercise 4.7). In other

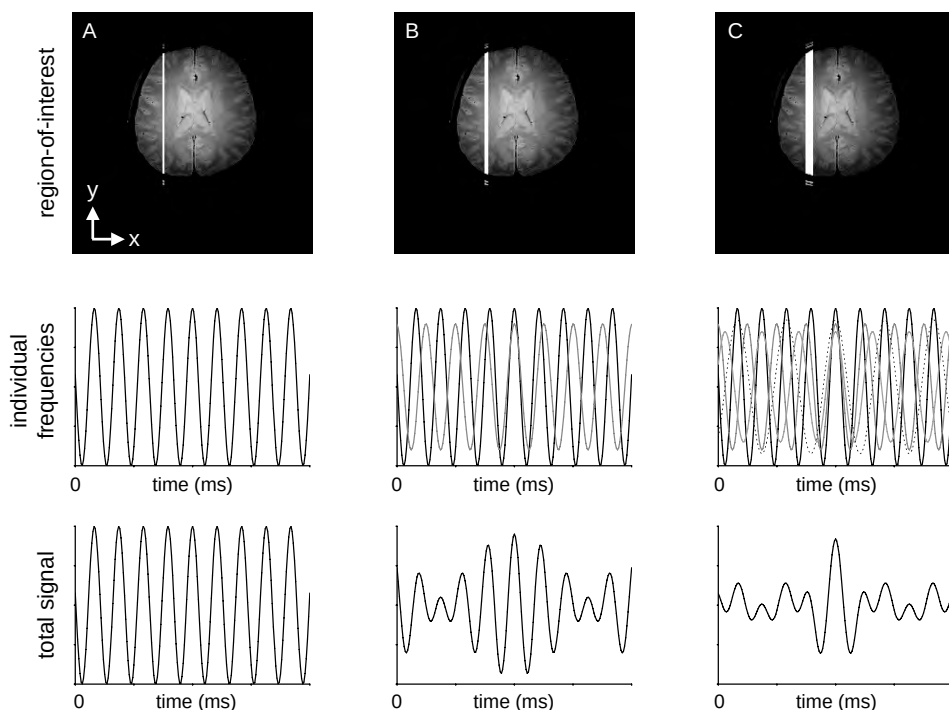


**Figure 4.4** Relationship between spatially encoded frequencies and total sample echo formation. (A) When the frequency encoding gradient is applied along the x direction, a single spatial position in that direction corresponds to a single frequency. (B) Adding another position, with a slightly different frequency and intensity, leads to partial phase cancellation in the total, summed signal. (C) Extending this principle by adding more positions, each with a different frequency and intensity, leads to more phase cancellation in the total, summed signal. Note that no phase cancellation occurs in the middle of the acquisition window, since at that point the phase of all spatial positions is zero (see also Figure 4.3). The exact nature of the phase cancellation is indicative of the sample position and shape and can be revealed through a Fourier transformation as shown in Figure 4.5.

words, magnetic field inhomogeneity leads to geometric image distortion in the frequency encoding direction. For fast MRI methods, like EPI, the distortions in the phase-encoding direction typically far outweigh the distortions in the frequency encoding direction (see Section 4.7.1).

Figure 4.4 schematically shows how signals originating from local positions give rise to the total acquired gradient-echo which will yield the spatial spin density distribution after Fourier transformation. Consider a frequency encoding (‘readout’) gradient in the x direction. A single spatial position in the x direction (Figure 4.4A) corresponds to a single resonance frequency, according to Equation (4.3), and the signal acquired from that position represents the integrated sum of all signal in the y direction (assuming 1D slice selection has already been performed). Another spatial position in the x direction corresponds, according to Equation (4.3), to another resonance frequency (Figure 4.4B)

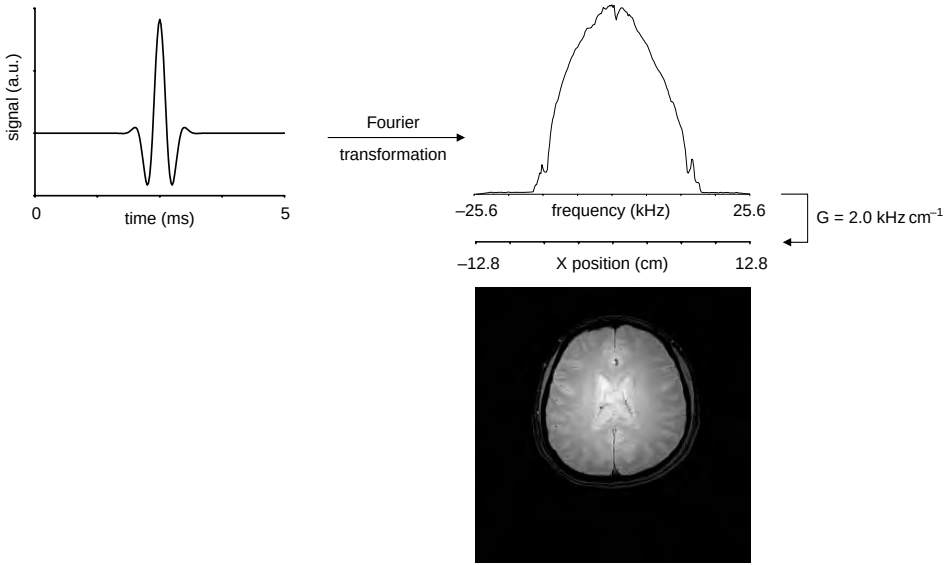


**Figure 4.5** Fourier transformation of a frequency-encoded echo reveals the spin distribution along the frequency-encoding direction. The spin distribution clearly shows the boundaries of the skull, as well as the separation between the two hemispheres. The frequency axis is readily converted to a spatial axis using Equation (4.3).

such that the total acquired signal from both positions leads to partial phase cancellation (Figure 4.4B, bottom). Extending this principle to include more and more positions in the x direction leads to more and more phase cancellation in the total acquired signal (Figure 4.4C, bottom). However, it should of course be realized that the exact nature of phase cancellation is a function of the object size, position and signal distribution. When the principles of Figure 4.4 are extrapolated to the acquisition of signal from the entire human head, a signal shown in Figure 4.5 results. While the time-domain signal may not appear to contain many frequencies, a Fourier transformation reveals that it provides an accurate representation of the signal distribution in the x direction. Note that while the Fourier transformation results in a signal distribution ‘spectrum’ with the axis in frequency units (kHz), knowledge of the gradient strength in combination with Equation (4.3) allows a recalculation of the profile in spatial units (cm).

Quantitatively the formation of an echo as shown in Figure 4.5 can be understood by considering an ensemble of spins. Using a complex notation for the transverse magnetization, i.e.:

$$M_{xy} = M_x + iM_y \quad (4.10)$$

the precession of transverse magnetization can be described as:

$$M_{xy}(\mathbf{r}, t) = M_{xy}(\mathbf{r}, 0)e^{+i\Delta\omega(\mathbf{r})t} \quad (4.11)$$

where

$$\Delta\omega(\mathbf{r}) = (\omega_0 - \omega) + \gamma\mathbf{r}\mathbf{G} = \Delta\omega + \gamma\mathbf{r}\mathbf{G} \quad (4.12)$$

$\omega_0$  represents the Larmor precessional frequency and  $\omega$  the carrier frequency, operative during signal acquisition. The constant frequency term and the position dependent gradient term can easily be separated, giving:

$$M_{xy}(\mathbf{r}, t) = M_{xy}(\mathbf{r}, 0)e^{+i\Delta\omega t}e^{+i\gamma\mathbf{r}\mathbf{G}t} \quad (4.13)$$

Equation (4.13) gives the time-domain signal (i.e. FID or echo) at one position  $\mathbf{r}$ . To obtain the magnetization over the entire sample (and the complete echo) the contributions of all positions in the sample must be integrated. For a sample of length  $L$ , the average magnetization is then given by:

$$M_{xy}(t) = \frac{e^{+i\Delta\omega t}}{L} \int_{-L/2}^{+L/2} M_{xy}(\mathbf{r}, 0)e^{+i\gamma\mathbf{r}\mathbf{G}t} d\mathbf{r} \quad (4.14)$$

Normally  $M_{xy}(\mathbf{r}, 0)$ , which represents the spin density of the sample at position  $\mathbf{r}$ , is not a known analytical function and Equation (4.14) needs to be evaluated numerically. However, for a uniform sample  $M_{xy}(\mathbf{r}, 0) = M_{xy}(0) = \text{constant}$ , the integral can be solved analytically resulting in:

$$M_{xy}(t) = M_{xy}(0) \frac{\sin(\gamma LGt/2)}{\gamma LGt/2} e^{+i\Delta\omega t} \quad (4.15)$$

From Equation (4.15) it follows that the time-domain signal (i.e. FID or echo) from the entire sample rapidly declines in the presence of a magnetic field gradient. Note that the magnetization is initially dephased completely, but upon increasing the gradient (either by the amplitude or duration) the magnetization is partially rephased according to the lobes of a sinc (i.e.  $\sin x/x$ ) function. This is an important consideration which should be taken into account when magnetic field gradients are used as ‘crushers’ (i.e. to remove unwanted transverse magnetization, see Chapters 5–8). The envelope function of Equation (4.15) is given by  $M_{xy}(t) = 2/\gamma LGt$ . Therefore, in order to suppress the signal by a factor  $f$ , the gradient area needs to be at least  $2f/L$ . In the presence of magnetic field inhomogeneity, the required gradient area may be significantly larger, as local magnetic field gradients may counteract the applied crusher gradient. It should be realized that the dephasing of transverse magnetization by magnetic field gradients is a macroscopic process. On a local (microscopic) level the magnetic field gradient merely changes the frequency of the spins. It is therefore always possible to recover dephased signal by applying a magnetic field gradient of opposite sign, provided that the signal has not been irreversibly lost by  $T_2$  relaxation.

For a more realistic (nonrectangular) gradient shape, the loss of phase coherence between transverse magnetization in a macroscopic sample is no longer given by Equation (4.15), but can still be calculated by integrating the exponent of Equation (4.14) with respect to time.

Spatial information in one dimension can be obtained accurately from 1D projections using frequency encoding with magnetic field gradients. However, a 1D projection does not provide information on the internal structure of 2D or 3D objects. Therefore a suitable

method needs to be employed to identify the volume elements that span a multidimensional (e.g. 2D) space. The described frequency-encoding procedure provides a good method for imaging along one of the required spatial coordinates. The spatial distribution along the second dimension can be obtained in several ways. The first method employed [1] was the usage of a second frequency-encoding magnetic field gradient along an orthogonal direction executed in concert with the first frequency-encoding gradient. Since magnetic field gradients are vectors, the effect of two simultaneous gradients can be added in a vectorial manner. Therefore, two simultaneous magnetic field gradients of equal strength execute frequency encoding along an oblique orientation which makes an angle of  $45^\circ$  with either gradient direction. The orientation of the oblique projection (given by angle  $\theta$  relative to gradient  $G_1$ ) can easily be varied by changing the relative amplitudes of the two magnetic field gradients, i.e.  $\theta = \arctan(G_2/G_1)$ . By making a large number of projections between  $\theta = 0^\circ$  and  $180^\circ$  the 2D object can be reconstructed by the method of filtered back-projection, a technique originating from X-ray tomography. Although the technique of projection reconstruction started off the field of MRI [1], its popularity has decreased considerably and its applications are now limited to imaging of very short  $T_2$  nuclei. For routine MRI experiments, the spin-warp imaging sequence [9] is most commonly used. In spin-warp imaging, the second spatial coordinate is obtained by a procedure referred to as phase encoding. With frequency encoding, the frequency of the NMR signal was made position-dependent with a magnetic field gradient prior to and during signal acquisition, resulting in a 1D projection in a single experiment. With phase encoding, the phase of the NMR signal is encoded as a function of position prior to signal acquisition. After multiple experiments in which the phase is appropriately manipulated, spatial information about the second dimension is obtained. Spin-warp imaging integrates these two independent methods of spatial encoding.

## 4.5 Phase Encoding

Figure 4.6 shows a spin-warp MRI sequence employing frequency encoding with a magnetic field gradient  $G_{\text{freq}}$  and phase encoding with a magnetic field gradient  $G_{\text{phase}}$ . To obtain spatial information along the second (i.e. phase encoding) dimension, a number of experiments need to be performed in which the amplitude of gradient  $G_{\text{phase}}$  is changed from  $+G_{\text{phase}}(\text{max})$  to  $-G_{\text{phase}}(\text{max})$ . The principle of phase encoding is not much different from frequency encoding, i.e. the magnetic field gradient  $G_{\text{phase}}$  encodes the phase (and indirectly the frequency) of the transverse magnetization as a function of position. Figure 4.7B shows the 1D projections of the object shown in Figure 4.7A for five different phase-encoding increments. When the phase-encoding gradient is zero, the signal is identical to that of Figure 4.5 and gives the spatial distribution of the object in the x direction. However, when the phase-encoding gradient is not zero, the signal in the y direction is spatially encoded leading to phase cancellation of the directly acquired frequency-encoded direction. As may be recalled from Figure 4.4, a single spatial point in the readout profile in the x direction represents the integrated signal from all positions in the y direction. When one point in the x direction is studied in more detail (Figure 4.7C) it can be seen that the phase-encoding gradient does not lead to phase cancellation in the y direction. Instead the signal is linearly phase-encoded, whereby the amount of acquired phase increases linearly with the applied phase-encoding gradient. Plotting one point in the y direction ('gray ball')



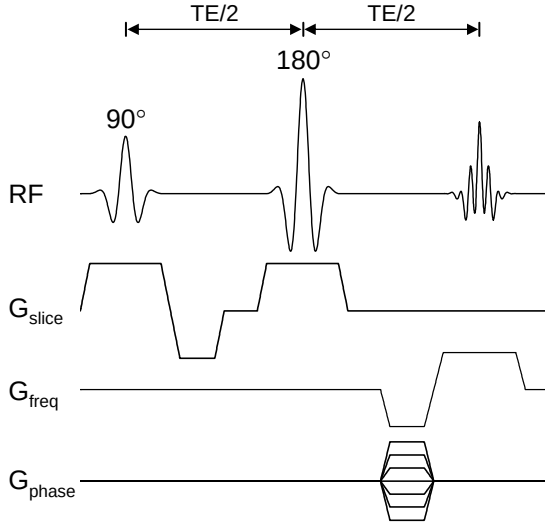


**Figure 4.6** Standard spin-echo or spin-warp MRI sequence. The echo time  $TE$  is defined from the middle of the excitation pulse to the top of the gradient echo, which coincides with the position of the spin-echo. As a rule of thumb, the slice selection gradients during the  $90^\circ$  and  $180^\circ$  pulses are not equal, as the bandwidths of the pulses are different. The negative gradient following the excitation pulse is necessary to refocus signal that was dephased during excitation (for more details see Section 5.3 and Figure 5.7).

as a function of the applied phase-encoding gradient (Figure 4.7D) readily shows that the phase modulation indirectly generates a frequency that is encoded independently of the frequency-encoded direction. In practice, the phase-encoded projections obtained after a 1D Fourier transformation are not shown, but the 2D data matrix is obtained with a 2D Fourier transformation (Figure 4.8).

## 4.6 Spatial Frequency Space

In order to understand the origin of artifacts in imaging or the performance of more complicated imaging sequences, a more concise mathematical representation of imaging is needed. This is achieved by a generalization of Equation (4.11), i.e. a description of the observed signal  $M_{xy}(t)$  in the presence of a time-dependent magnetic field gradient  $G(t)$ :

$$M_{xy}(\mathbf{G}, t) = \int_{-\infty}^{+\infty} M_0(\mathbf{r}) e^{+i\gamma \mathbf{r} \cdot \int_0^t \mathbf{G}(t') dt'} d\mathbf{r} \quad (4.16)$$

where  $M_0(\mathbf{r})$  is the spin density at position  $\mathbf{r}$ , and  $t$  the time after the  $90^\circ$  pulse. Depending on the particular MRI sequence used, Equation (4.16) should be multiplied with factor describing effects of relaxation or diffusion (e.g.  $e^{-t/T_2}$ ). By defining a spatial frequency

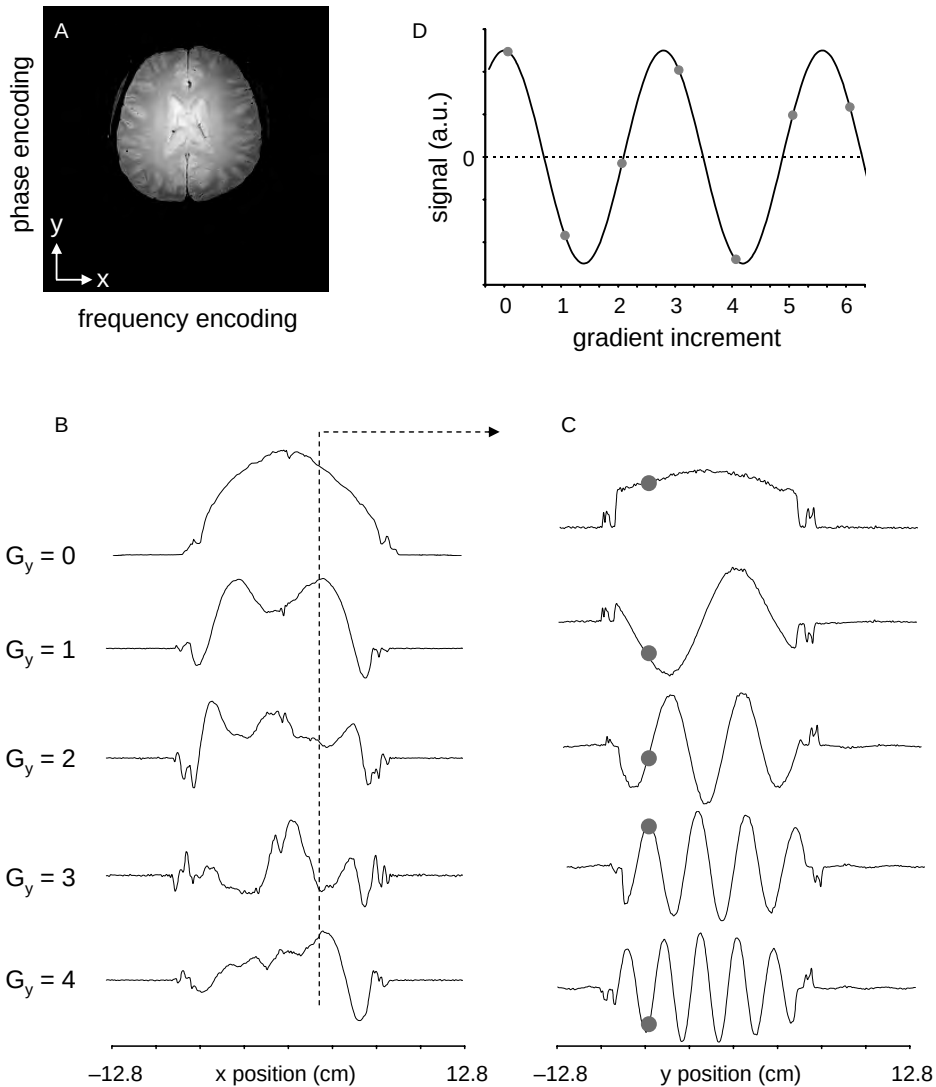


**Figure 4.7** Principle of phase encoding. (A) Object under investigation. (B) 1D phase-encoded spatial profiles acquired in the x direction. Without a phase-encoding gradient ( $G_y = 0$ ) the spatial profile represents the spin density distribution in the x direction. In the presence of a phase-encoding gradient, signal in the y direction experiences a linearly dependent phase shift, leading to phase cancellation and signal loss in the acquired signal along the x direction. (C) When, hypothetically, signal in the y direction could be studied, it is immediately obvious that the phase-encoding gradient leads to an increasing amount of phase modulation, which for a single point ('gray ball') corresponds to (D) a single, independently encoded frequency. However, in a realistic MRI scan no prior information about the y direction is available. Nevertheless, the independent encoding of frequencies in the second dimension is recorded in the specific phase cancellation observed in the acquired signal (B).

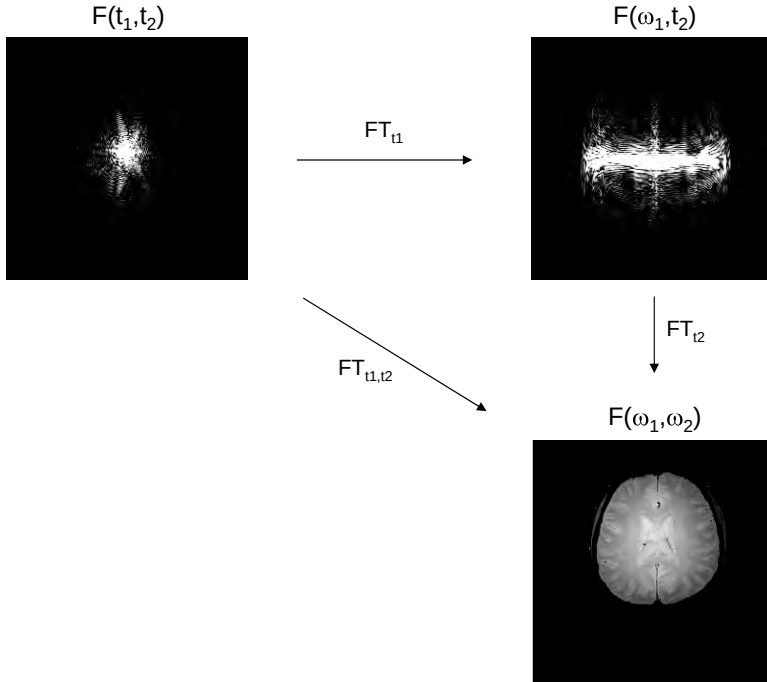


**Figure 4.8** Time- and frequency-domain signals are directly related through sequential 1D or a single 2D Fourier transformation. Rather than showing the individually encoded spatial profiles (Figure 4.7B), the phase-encoded echoes can be directly converted to an image by a 2D Fourier transformation. Again, knowledge of the gradient strengths allows, in conjunction with Equation (4.3), the conversion of the frequency axes into spatial axes.

variable  $\mathbf{k}(t)$  as:

$$\mathbf{k}(t) = \gamma \int_0^t \mathbf{G}(t') dt' \quad (4.17)$$

Equation (4.16) reduces to:

$$M_{xy}(\mathbf{k}(t)) = \int_{-\infty}^{+\infty} M_0(\mathbf{r}) e^{+i\mathbf{k}(t)\mathbf{r}} d\mathbf{r} \quad (4.18)$$

i.e. the observed time-domain signal spanning a 2D  $\mathbf{k}$ -space of spatial frequencies [10–12] equals the inverse Fourier transformation of the spin density. Furthermore,  $M_{xy}(t)$  tends to zero when  $\mathbf{k}(t)$  goes to infinity. Under normal circumstances the decay of  $M_{xy}(t)$  will be very rapid with increasing  $\mathbf{k}(t)$ . However, as long as the magnetization has not been irreversibly dephased by  $T_2$  relaxation,  $M_{xy}(t)$  can always be recalled by letting the  $\mathbf{k}$ -space trajectory return to low  $\mathbf{k}(t)$  values, i.e. a spin echo can be generated when the total integrated

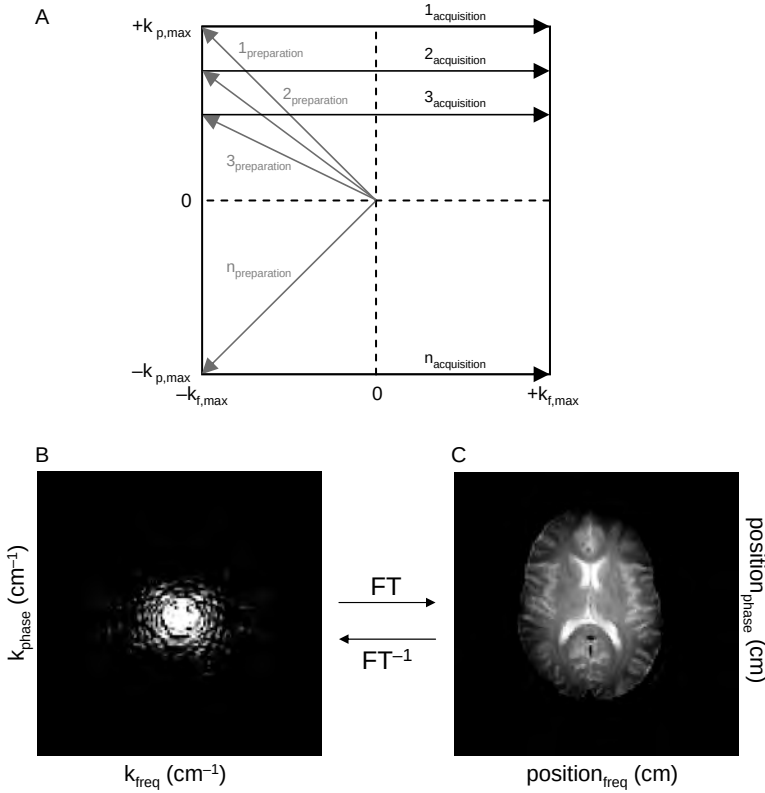


**Figure 4.9** The structure of  $k$ -space with respect to the frequency and phase-encoding gradients during a standard spin-warp MRI sequence. (A) The preparation gradient during the first phase-encoding step moves the start of data acquisition to  $(+k_{p,max}, -k_{f,max})$ . Signal is acquired during the positive frequency-encoding gradient, which corresponds to the  $k$ -space line  $+k_{p,max}$  between  $-k_{f,max}$  and  $+k_{f,max}$ . As the phase-encoding gradient amplitude is decreased in subsequent experiments,  $k$ -space is filled from top to bottom (B) after which Fourier transformation gives the final MR image (C).

gradient area [i.e.  $\mathbf{k}(t)$ ] tends to zero. As an example of the structure of  $k$ -space, consider the spin-warp imaging sequence in Figure 4.6. Figure 4.9A shows the corresponding  $k$ -space representation, in which the horizontal axes indicate  $k_f$  (the  $k$ -parameter of the frequency-encoding direction) and the vertical axes indicate  $k_p$  (the  $k$ -parameter of the phase-encoding direction). Immediately after excitation  $k_f = k_p = 0$ , since no gradient has been applied in either direction. In the first experiment, a maximum positive phase-encoding gradient will be applied, making  $k_p = +k_{p,max}$ . The dephasing gradient in the frequency-encoding direction assures that  $k_f = -k_{f,max}$ . However, no signal acquisition has been performed yet; the spatial frequencies in  $k$ -space are just being prepared. During the acquisition period a rephasing gradient is applied, thereby linearly increasing  $k_{freq}$  over time. At the top of the spin echo,  $k_f = 0$  and maximal signal will be observed (for that particular  $k_p$ , which is

nonzero in the first experiment). At the end of acquisition  $k_f = +k_{f,\max}$ . In the following experiments  $k_p$  will be reduced in discrete steps, such that  $k$ -space is ‘filled’ with spatially encoded spin echoes according to parallel lines. In the middle experiment  $k_p = 0$  and maximum signal will be observed at the top of the echo ( $k_f = 0$ ). Because of the low  $k_f$  and  $k_p$  values, coordinates near the middle of  $k$ -space are called low spatial frequencies, while coordinates at the edges of  $k$ -space are referred to as high spatial frequencies. In general it can be said that low spatial frequencies (which have the highest intensity) correspond to the overall shape of images, while high spatial frequencies represent more detailed features in the final image. Figure 4.10 shows an example of the relative importance of the different  $k$ -space coordinates on the final appearance of the image. An image reconstructed from low spatial frequencies only (Figure 4.10B) has approximately the same signal intensity as the image originating from the entire  $k$ -space data (Figure 4.10A). However, the image appears blurry (i.e. devoid of detailed spatial structures). The high spatial frequencies are required to represent sharp edges accurately. In other words, a data matrix (i.e.  $k$ -space) containing high spatial frequencies results in an image with a higher spatial resolution (Figure 4.10C).

The relation between gradient strength, gradient duration and field of view (FOV) can easily be derived, since it is governed by the Nyquist sampling theory. A gradient- or spin-echo is, just as a regular FID, sampled at discrete time intervals  $\Delta t$ . The Nyquist sampling theory states that a frequency can only be accurately described if it is sampled at least twice per period, i.e. the phase difference between two sample points should be maximally  $2\pi$  over the entire FOV. The phase over the entire FOV can also be expressed as  $\phi = 2\pi \gamma \text{FOVG} \Delta t$ , where  $\gamma = \gamma/2\pi$  is expressed in  $\text{Hz G}^{-1}$  or  $\text{Hz mT}^{-1}$ , resulting in:

$$\text{FOV} = \frac{1}{\gamma G \Delta t} = \frac{1}{\Delta k} \quad (4.19)$$

where  $\Delta k$  is expressed in  $\text{cm}^{-1}$ . Equation (4.19) is valid for the frequency-encoding direction. For phase encoding it must be adjusted according to  $\Delta k = \gamma \Delta G T$ , where  $T$  is the length of the (rectangular) phase-encoding gradient. The bandwidth (in Hz) of the ‘NMR spectrum’ in the frequency-encoding direction equals  $1/\Delta t$  as with spectroscopy. In a typical MRI experiment of human brain,  $\text{FOV} = 24 \text{ cm}$ ,  $G = 5.0 \text{ mT m}^{-1}$  and  $\gamma(^1\text{H}) = 4257.6 \text{ Hz G}^{-1}$ , resulting in  $\Delta t = 19.57 \mu\text{s}$  and a bandwidth of  $51\,091 \text{ Hz}$ . The effective bandwidth in the phase-encoding direction is infinite for spin-warp MRI methods, since the phase-encoding direction does not have a time dependence. This is contrary to the situation in echo-planar imaging (EPI), where the effective bandwidth in the phase-encoding direction can be as low as a few hundred Hz, leading to significant image distortion in the presence of magnetic field inhomogeneity (see Section 4.7.1).

## 4.7 Fast MRI Sequences

The  $k$ -space formalism can be used to explain the principles of any gradient-based MRI method and is particularly useful for the understanding and design of ultra-fast MRI pulse sequences. Fast imaging sequences are crucial for dynamic MRI studies, like functional MRI and for parametric MRI studies, like diffusion tensor imaging (DTI) or  $T_1$  mapping.

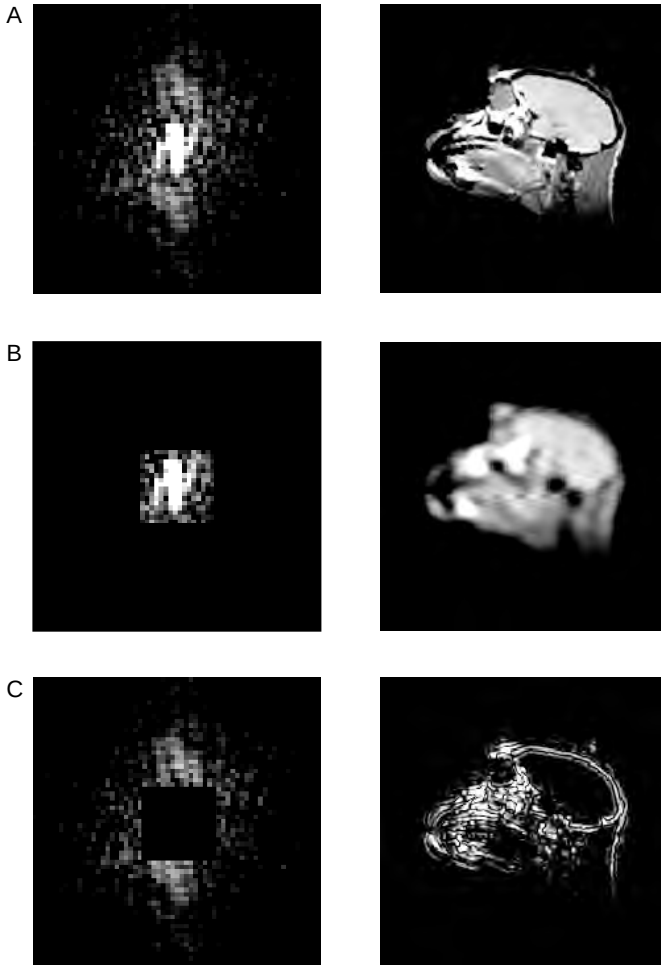


**Figure 4.10** Relationship between spatial frequency or  $k$ -space (left) and image space (right). (A) Fourier transformation of the entire  $k$ -space gives rise to a clear image of the head of a monkey featuring good SNR and many details. (B) Fourier transformation of only the low frequencies in  $k$ -space gives rise to a blurry image with almost the same SNR as in (A) but lacking significant details. (C) The higher spatial frequencies in  $k$ -space give rise to a very detailed image with a highly compromised SNR. Note that the vertical scale in (C) is twice that of (A) or (B).

Rather than providing a complete overview of fast MRI methods, the discussion is limited to EPI, initially proposed by Mansfield [13]. Since EPI is arguably the fastest method and forms the basis for a wide range of other fast MRI sequences it is well-suited to illustrate some of the considerations involved with fast MRI methodology. For more extensive discussions of fast MRI methodology the reader is referred to the literature [3–5,14].

### 4.7.1 Echo-planar Imaging

During regular spin-warp MR imaging, a single line of  $k$ -space consisting of  $N_f$  data points is sampled following excitation.  $N_p$  separate excitations are required to sample  $N_p$   $k$ -space lines to complete a 2D  $N_f \times N_p$   $k$ -space data grid. The total duration of a spin-warp MRI scan is then given by  $N_p \times TR$ , where  $TR$  is the repetition time. The key feature of EPI is that more than one  $k_p$  line of  $k$ -space is sampled following a single excitation. When all  $N_f \times N_p$  data points of  $k$ -space are sampled following a single excitation the technique is referred to as ‘single-shot’ EPI, for which the experimental duration is reduced to  $1 \times TR$ .

The basic pulse sequence for gradient-echo EPI is shown in Figure 4.11A, with the corresponding  $k$ -space trajectory shown in Figure 4.11B. For the sequence shown in Figure 4.11A, the first two gradients bring the start of  $k$ -space sampling to the edge of  $k$ -space at  $(-k_{f,\max}, -k_{p,\max})$ . Sign-reversal of the frequency-encoding gradient leads to the generation and acquisition of the first gradient-echo along the  $k$ -space line  $k_p = -k_{p,\max}$  with  $-k_{f,\max} \leq k_f \leq +k_{f,\max}$ . A so-called phase-encoding blip brings the  $k$ -space position to  $(+k_{f,\max}, -k_{p,\max} + \Delta k_p)$ , after which sign-reversal of the frequency-encoding gradient leads to

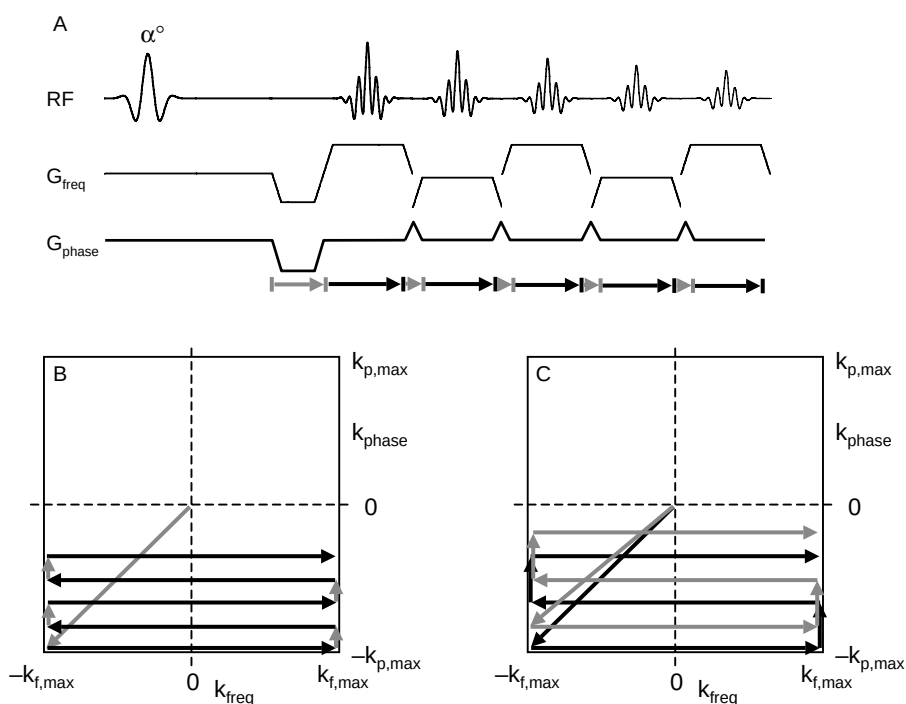


**Figure 4.11** (A) Gradient-echo EPI sequence and (B) corresponding (single-shot)  $k$ -space trajectory. The black  $k$ -space lines correspond to the frequency-encoding gradients during which signal acquisition commences, while gray lines correspond to the phase-encoding blips. (C)  $k$ -space trajectory for a two-shot, interleaved EPI sequence. The black and gray lines now correspond to the  $k$ -space trajectories during the first and second excitation, respectively.

the generation and acquisition of the second gradient-echo along the  $k$ -space line  $k_p = -k_{p,\max} + \Delta k_p$  with  $+k_{f,\max} \leq k_f \leq -k_{f,\max}$ . It is then straightforward to see that all of  $k$ -space can be sampled with a ‘back-and-forth’ frequency-encoding trajectory between  $-k_{f,\max}$  and  $+k_{f,\max}$  in which the second dimension evolves from  $-k_{p,\max}$  to  $+k_{p,\max}$  by constant-amplitude phase-encoding blips of  $\Delta k_p$ . Maximum echo formation is achieved when  $k_p = 0$ , which corresponds to the middle echo in  $k$ -space. The  $k$ -space sampling during spin-echo EPI is similar to that of gradient-echo EPI with the most noticeable difference that the application of a  $180^\circ$  pulse leads to refocusing of phase evolution due to magnetic field inhomogeneity for the echo in the center of  $k$ -space.

Direct Fourier transformation of the acquired EPI data does typically not result in a recognizable image due to differences between the odd and even echoes. Firstly, the odd and even echoes are acquired in opposite directions in  $k$ -space, such that the odd (or even) echoes need to be time-reversed. However, eddy currents, gradient asymmetry and magnetic field inhomogeneity can all cause residual differences between even and odd echoes (Figure 4.12A), leading to so-called N/2 ghosting in the image domain (Figure 4.12B). The use of a reference dataset (Figure 4.12C), acquired without phase-encoding blips, is a robust method to significantly reduce the N/2 ghosts.

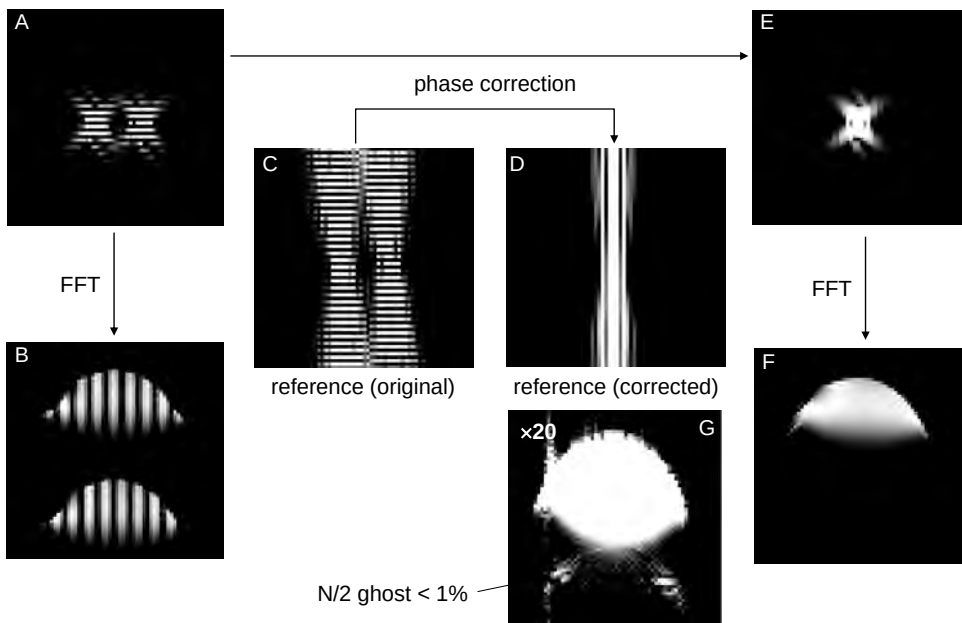


**Figure 4.12** Basic EPI signal processing. (A) Upon phase reversal of every other  $k$ -space line, the odd and even echoes may be misaligned due to experimental imperfections and timing errors, leading to (B) significant N/2 ghosting. Under the assumption that all echoes in a nonphase-encoded reference dataset (C) should be identical, a correction term for each echo can be calculated and applied to (D) the reference dataset or (E) the phase-encoded EPI dataset. (F and G) Fourier transformation of the corrected dataset shows greatly reduced N/2 ghosting.



Without phase-encoding gradients all echoes in the reference data set should be identical (aside from  $T_2^*$  relaxation, Figure 4.12D). Therefore any differences between echoes must be attributed to experimental imperfections and can be used to correct the phase-encoded EPI dataset (Figure 4.12E) resulting in greatly reduced N/2 ghosting (Figure 4.12F and G).

As was realized by Mansfield at a very early stage, the successful implementation of EPI is to a large degree synonymous with the development of fast, strong and shielded magnetic field gradients (see Chapter 10). As a result of those developments, EPI is now routinely used for functional MRI, diffusion tensor imaging and other studies. However, it should always be realized that EPI is exceptionally sensitive to experimental imperfections, like eddy currents, gradient asymmetry and especially magnetic field inhomogeneity. Consider a pixel with a magnetic field inhomogeneity  $\Delta B_0$  imposed on the main magnetic field  $B_0$ . The general imaging equation relating the detected signal  $M_{xy}$  to the k-space sampling [i.e. Equation (4.18)] must then be extended to:

$$M_{xy}(k_f, k_p) = \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} M_0(r_f, r_p) e^{+i(k_f r_f + k_p r_p + \gamma \Delta B_0(r_f, r_p)t)} dr_f dr_p \quad (4.20)$$

for a 2D imaging method in the spatial  $(r_f, r_p)$  plane. Under the assumption of infinitesimal gradient rise times it can be shown (see Exercise 4.13) that the magnetic field inhomogeneity  $\Delta B_0 = (2\pi \Delta \nu / \gamma)$  leads to a pixel shift in the frequency- and phase-encoding directions given by:

$$\Delta r_f = N_f \left( \frac{\Delta \nu}{SW} \right) \quad \Delta r_p = N_p N_f \left( \frac{\Delta \nu}{SW} \right) \quad (4.21)$$

It follows that the pixel shift in the frequency encoding is  $N_p$  times smaller than in the phase-encoding direction and can, for typical EPI spectral bandwidths SW (50–200 kHz), be ignored. The pixel shift in the phase-encoding direction can, however, become significant for typical magnetic field inhomogeneity encountered at higher magnetic fields. Besides improving the magnetic field homogeneity (see Chapter 10), the pixel shift can only be reduced by increasing the bandwidth (at the expense of a reduced SNR) or decreasing the number of phase-encoding increments  $N_p$  per excitation.

In the case when not all  $N_p$  phase-encoding increments are acquired following a single excitation, the technique is often referred to as multi-shot EPI. When all  $N_p$  phase-encoding increments are acquired in  $N_i$  separate excitations or interleaves, the geometric distortion in the phase-encoding direction reduces to:

$$\Delta r_p = \left( \frac{N_p}{N_i} \right) N_f \left( \frac{\Delta \nu}{SW} \right) \quad (4.22)$$

A two-shot or four-shot EPI often gives remarkable improvements in image quality, as can be seen from Figure 4.13. Furthermore, acquiring k-space over multiple excitations also allows a reduction of the minimum attainable echo-time, which can be an effective method to improve sensitivity or image contrast. The only downside of multi-shot EPI is that the experimental duration becomes  $N_i \times TR$ , which can present an obstacle when very fast dynamic processes are studied (cardiac MRI, fast functional responses). While there are several ways of acquiring  $N_i$  interleaves, the most commonly used method is shown in

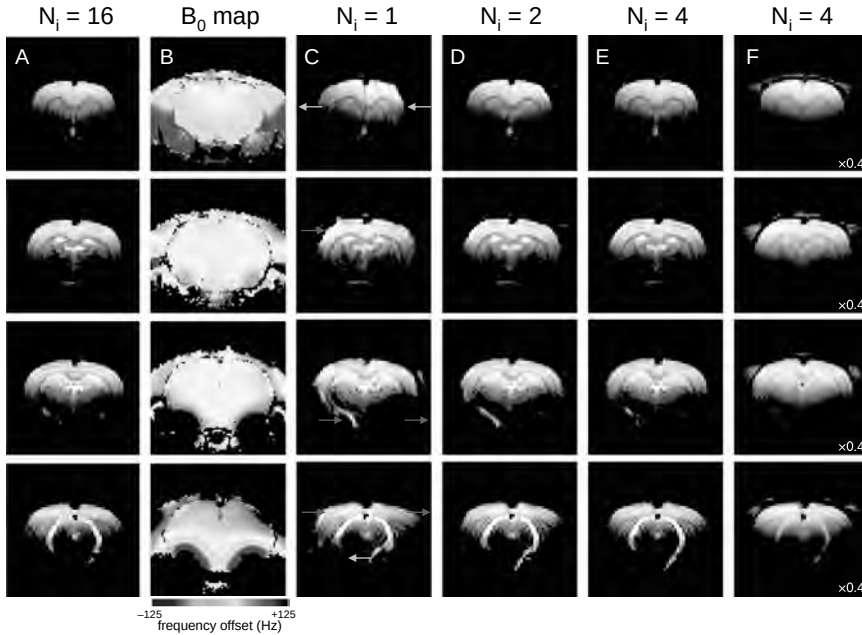


**Figure 4.13** Spin-echo echo-planar images acquired from rat brain at 9.4 T with surface coil transmission and reception (coil diameter = 14 mm). All images (16 slices of 1 mm slice thickness) were acquired as a  $64 \times 64$  data matrix over a  $25.6 \times 25.6$  mm FOV, with the phase-encoding gradient blips in the x direction (horizontal) and an imaging bandwidth of 200 kHz. A single, globally optimized shim setting was used for all experiments. (A) Images acquired with 16 interleaves ( $TE = 60$  ms) closely resemble a conventional spin-echo MRI. (B) Magnetic field  $B_0$  map acquired over the same slices, see Section 4.8.3 for more details. (C–E) Echo-planar images acquired with (C) one, (D) two and (E) four interleaves ( $N_i$ ) at  $TE = 60$  ms. The single-shot EPI images in (C) show severe geometric distortion that is directly related to the magnetic field inhomogeneity shown in (B). Red and blue arrows indicate pixel shifts towards the right and left, respectively, and correspond to positive and negative magnetic field offsets. With (D) two and (E) four interleaves, the pixel shifts reduce two- and fourfold, respectively. However, even with four interleaves noticeable image distortion remains. (F) The shorter acquisition time per excitation with four interleaves allows a reduction of the echo-time to 35 ms, thereby providing 2.5 times more signal. However, the geometric distortion between (E) and (F) is identical. (See color plate 2)

Figure 4.11C. During the first excitation, k-space lines  $(1, N_i + 1, 2N_i + 1, \dots, N_p - N_i + 1)$  are sampled, followed by k-space lines  $(2, N_i + 2, 2N_i + 2, N_p - N_i + 2)$  during the second excitation and so on until the entire  $N_f \times N_p$  k-space data grid has been sampled. This type of interleaved k-space sampling ensures a relatively smooth  $T_2^*$ -weighted k-space filtering, thereby minimizing artifacts and optimizing sensitivity.

Note that in addition to geometric distortions as shown in Figure 4.13, EPI, as well as all other gradient-echo-based MRI methods, also suffer from through-plane signal loss in areas of low magnetic field homogeneity. While the geometric distortions of gradient-echo

EPI and spin-echo EPI are identical, the through-slice signal loss in spin-echo EPI is greatly reduced as the  $180^\circ$  pulse refocuses the through-slice phase evolution at the position of the middle echo, corresponding to the middle of k-space.

#### 4.8 Contrast in MRI

One of the most challenging tasks of MRI is to create appropriate contrast for the discrimination of areas of interest. In principle, any difference in the properties of spins can be used to create contrast. The most obvious parameters are spin density, longitudinal relaxation time  $T_1$  and transverse relaxation time  $T_2$ . A conventional spin-warp imaging sequence can exploit all these differences by proper adjustment of the experimental parameters TR (repetition time) and TE (echo time). For a spin-warp imaging sequence, the signal at the top of the echo is given by:

$$M_{xy}(TR, TE) = M_0(1 - e^{-TR/T_1})e^{-TE/T_2} \quad (4.23)$$

Herein a  $90^\circ$  excitation pulse has been assumed and effects of the refocusing pulse (i.e. inversion of recovered longitudinal magnetization during  $TE/2$ ) have been neglected. Figure 4.14A shows the signal dependence on TR ( $TE = 0$ ) for two pixels with  $T_1 = 900$  ms

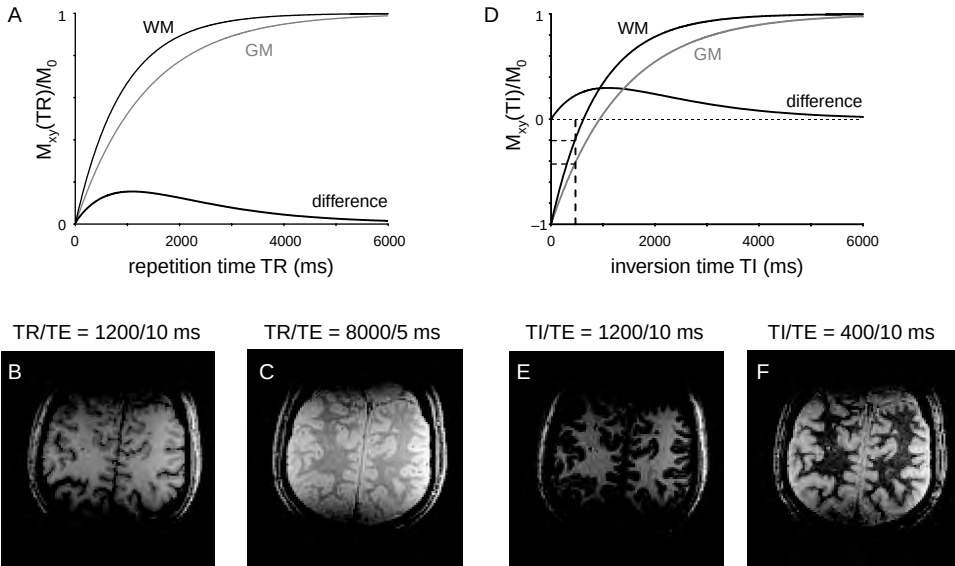


**Figure 4.14** (A) Saturation recovery signal curves for white matter (WM) and gray matter (GM) at 4.0 T with  $T_1$  values of 900 and 1350 ms, respectively. The difference between the curves indicates a maximum at  $TR \sim 1200$  ms. Saturation recovery images from human brain at 4.0 T acquired with (B)  $TR/TE = 1200/10$  ms and (C)  $TR = 8000/5$  ms. The image in (C) closely resembles a 'proton-density' image. (D) Inversion recovery signal curves for  $T_1 = 900$  ms (WM) and  $T_1 = 1350$  ms (GM). Inversion recovery images from human brain at 4.0 T acquired with (E)  $TI/TE = 1200/10$  ms and (F)  $TI/TE = 400/10$  ms.

and 1350 ms, roughly corresponding to gray and white matter at 4.0 T, respectively [15]. Maximum ' $T_1$  contrast' is achieved when  $TR \sim 1200$  ms. However, the image acquired with  $TR = 1200$  ms (Figure 4.14B) is largely devoid of contrast, largely because the differences in proton density between gray and white matter (Figure 4.14C) cancel out the  $T_1$  contrast. The amount of  $T_1$  contrast can be significantly enhanced by extending the sequence with an inversion pulse and a recovery delay TI, such that the signal at the top of the echo is given by:

$$M_{xy}(TR, TE) = M_0(1 - 2e^{-TI/T_1})e^{-TE/T_2} \quad (4.24)$$

where a long repetition time ( $TR > 5T_1$ ) and a perfect inversion pulse have been assumed. Figure 4.14D shows the signal dependence on TI for  $T_1 = 900$  and 1350 ms. Again maximum  $T_1$  contrast is generated at  $TI \sim 1200$  ms and while the corresponding image (Figure 4.14E) has improved gray/white matter contrast, the cancellation of  $T_1$  contrast by differences in proton density largely prevents a clear discrimination between cerebral tissue types. However, for  $TI < 800$  ms the gray/white matter  $T_1$  contrast is inverted relative to the situation when  $TI > 1000$  ms. Acquiring an image with  $TI = 400$  ms therefore results in excellent image contrast (Figure 4.14F) since the  $T_1$  and proton density differences between gray and white matter enhance each other. From Figure 4.14 it follows that the best image contrast is determined by an interplay between several parameters, like proton density, and  $T_1$  and  $T_2$  relaxation. As a result, a single 'parameter-weighted' (e.g. ' $T_1$ -weighted') image can not provide unambiguous information about that parameter and should be used with care (e.g. all ' $T_1$ -weighted' images in Figure 4.14 are also heavily weighted by proton density differences). A more quantitative approach in which one of the parameters (e.g.  $T_1$ ) is uniquely determined *independent* of the other parameters offers a more objective strategy for tissue characterization and will be discussed next.

#### 4.8.1 $T_1$ and $T_2$ Relaxation Mapping

Because MRI images are always affected by a combination of  $T_1$ ,  $T_2$ , spin density and other effects, the generation of qualitative contrast on an unknown sample may result in ambiguous results. Furthermore, it is impossible to determine which effects are involved when temporal changes in the images are observed. Therefore, it is often desirable to generate calculated images ('maps'), representing one of the parameters, from MRI data that are increasingly weighted for the parameter of choice. For instance a  $T_1$  map can be calculated from a series of images with increasing repetition (or inversion) time. Figure 4.15 shows  $T_1$  and  $T_2$  maps that were calculated from a series of  $T_1$ - and  $T_2$ -weighted images. Besides the quantitative nature of the maps, they also generate new contrast, since the effects from other parameters (e.g. spin density) are entirely excluded.

Time constraints are critical for most clinical applications. As such, for MRS studies most experimental time should be used on the acquisition of spectroscopic data, whereas supporting MRI data should be acquired in the shortest amount of time.  $T_1$  and  $T_2$  measurements of complete relaxation curves can be prohibitively long and one typically has to resort to faster, more efficient methods. Dozens of methods and hundreds of papers have appeared on the fast measurement of  $T_1$  relaxation time constants, as reviewed by Kingsley [16]. Here one of the more popular methods, based on the early papers of Look and Locker [17–19], will be described that can measure  $T_1$  relaxation time constants in a

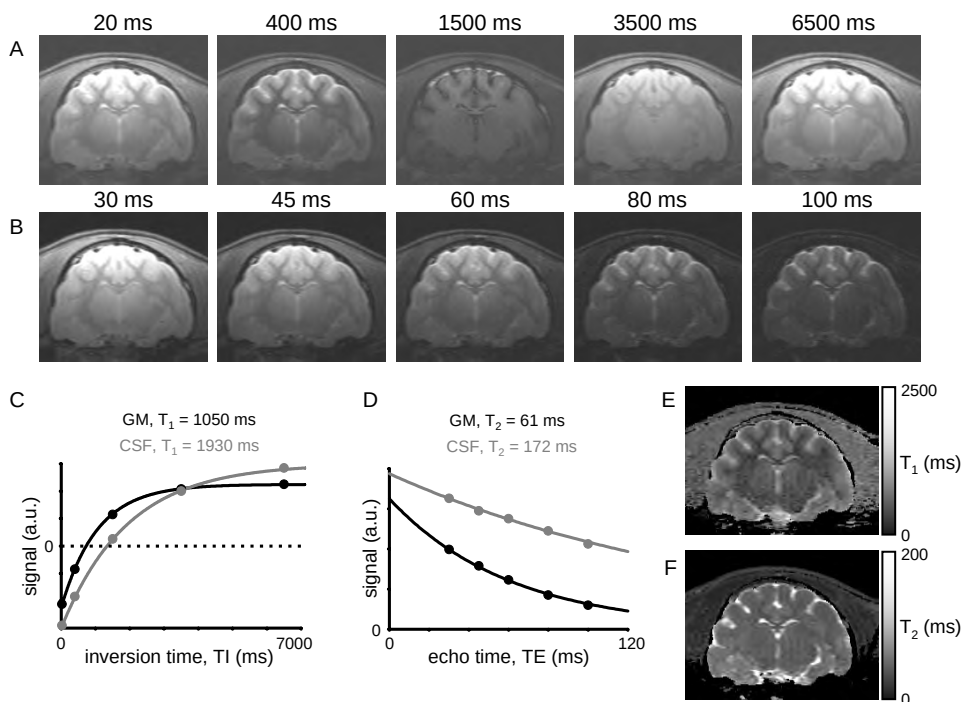


**Figure 4.15**  $T_1$  and  $T_2$  relaxation mapping. (A) Five ' $T_1$ -weighted' images acquired from cat brain at 4.7 T with an inversion recovery sequence ( $TR/TE = 7000/30$  ms) and inversion delays of 20, 400, 1500, 3500 and 6500 ms. (B) Five ' $T_2$ -weighted' images acquired with a spin-echo sequence ( $TR = 3000$  ms) and echo delays of 30, 45, 60, 80 and 100 ms. (C and D) Representative (C)  $T_1$  signal recovery and (D)  $T_2$  signal decay curves for single pixels in cerebral gray matter and CSF, respectively, together with the best fits according to Equation (4.23) or (4.24). Quantitative (E)  $T_1$  and (F)  $T_2$  relaxation maps obtained by fitting the 'weighted' data in (A) and (B) on a pixel-by-pixel basis as shown in (C) and (D).

single experiment. For other methods, the reader is referred to the literature [16], as well as Exercise 4.12. The measurement of  $T_2$  relaxation is typically less time-consuming, as the complete  $T_2$  relaxation curve can be sampled through a multi-echo acquisition [20,21].

## 4.8.2 Fast $T_1$ and $T_2$ Relaxation Mapping

During a conventional inversion recovery  $T_1$  measurement, the thermal equilibrium magnetization is initially inverted. A single point in the recovery of longitudinal magnetization due to  $T_1$  relaxation is then detected by exciting the magnetization at a specific time following inversion. The complete  $T_1$  relaxation curve is then obtained by repeating the experiment for different inversion times. Inversion recovery is the most robust method for  $T_1$  measurements. However, because the longitudinal magnetization must be completely

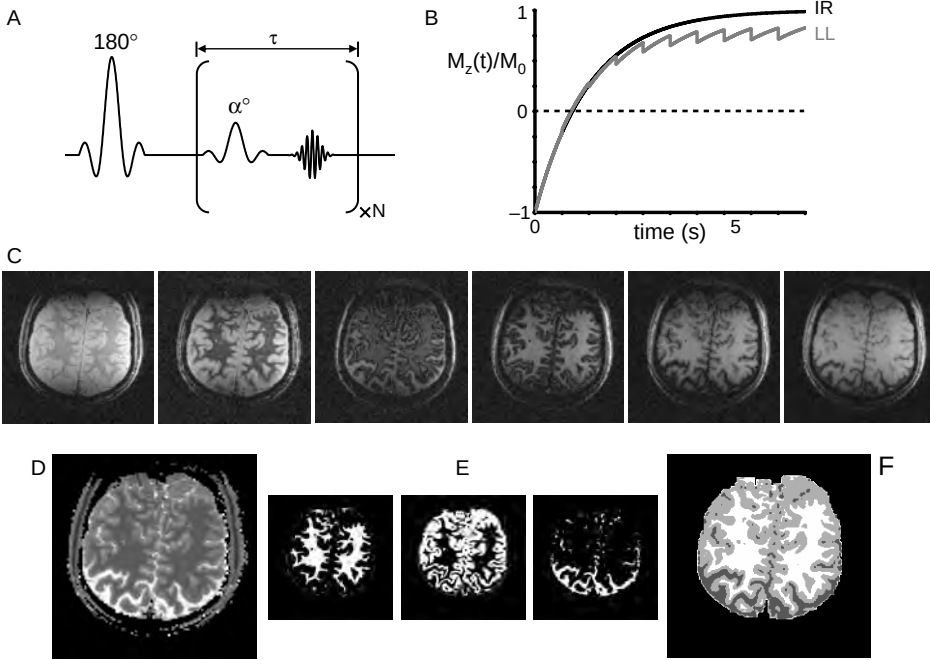


**Figure 4.16** Fast  $T_1$  relaxation mapping based on the Look–Locker method. (A) Basic pulse sequence without MR imaging gradients. (B) Signal recovery curves for inversion recovery (IR) and Look–Locker (LL) experiments. The signal recovery during a LL experiment can be described by an apparent  $T_1$  relaxation time constant,  $T_1^*$ , according to Equation (4.26). (C) Look–locker  $T_1$ -weighted images acquired from human brain at 4.0 T with  $\alpha = 30^\circ$  and  $\tau = 250$  ms. (D) Calculated  $T_1$  relaxation map obtained through pixel-by-pixel fitting of the data in (C) with Equation (4.25). (E and F) Prior knowledge on the  $T_1$  ranges of different tissue types can be used to segment the  $T_1$  relaxation map into white matter (left in E, white in F), gray matter (middle in E, light gray in F) and CSF (right in E, dark gray in F).

recovered before the following inversion is applied ( $TR = 5T_1$ ), it is also the slowest method for  $T_1$  measurements.

During the Look–Locker  $T_1$  measurement, the thermal equilibrium magnetization is also initially inverted (Figure 4.16A). However, rather than applying  $90^\circ$  excitations, the recovering magnetization is sampled by small nutation angle excitations ( $\alpha < 10\text{--}15^\circ$ ) which perturb the longitudinal magnetization only slightly. As a result, the longitudinal magnetization can be sampled repeatedly as the magnetization returns to its equilibrium state (Figure 4.16B). The time dependence of the longitudinal magnetization in the presence of repeated excitation can be described as:

$$M_z(\alpha, T_1, n\tau) = C_1(\alpha, T_1)[1 - C_2(\alpha, T_1)e^{-n\tau/T_1^*}] \quad (4.25)$$

where  $C_1$  and  $C_2$  are complicated expressions of the nutation angle  $\alpha$  and relaxation time constant  $T_1$  [15,17–19] and  $n$  is an integer between 1 and the total number of excitations

N. The repeated excitation essentially reduces  $T_1$  to an apparent relaxation time constant,  $T_1^*$ , which is given by:

$$\frac{1}{T_1^*} = \frac{1}{T_1} - \frac{\ln(\cos \alpha)}{\tau} \quad (4.26)$$

Therefore, the signal intensity versus time curve can be fitted with a three-parameter fit ( $C_1, C_2, T_1^*$ ), similar to a regular inversion recovery method. With a known nutation angle  $\alpha$ , the relaxation time  $T_1$  can be deduced from the fitted  $T_1^*$  according to Equation (4.26). When the nutation angle is not known a separate experiment is required to spatially map the nutation angle, as will be discussed in Section 4.8.4.

Figure 4.16C shows a typically Look–Locker dataset acquired on human brain with  $\alpha = 30^\circ$  and  $\tau = 250$  ms. For a  $128 \times 128$  image resolution over eight spatial slices and eight time points, the  $T_1$  measurement required circa 8 min. A conventional inversion recovery method would require 68 min. Fitting each image pixel to Equation (4.25) allows the generation of a quantitative  $T_1$  map, as shown in Figure 4.16D. Using predetermined  $T_1$  ranges for different tissue types, the  $T_1$  map can be objectively segmented into white matter, gray matter and CSF (Figure 4.16E and F). Tissue segmentation is a crucial requirement when performing metabolite quantification, as it allows an accurate estimation of partial volume effects between different tissue types and it shows the exact spatial origin of the spectroscopic water and metabolite signals.

### 4.8.3 Magnetic Field $B_0$ Mapping

Knowledge of the magnetic field distribution within the object under investigation is important for a wide range of applications, including post-acquisition frequency correction in MRSI (see Chapter 7), EPI image distortion correction and optimization of the magnetic field homogeneity through shimming (see Chapter 10).

The magnetic field distribution is typically obtained from the additional phase evolution during a period of free precession. While the method can essentially be based on any MRI sequence, the principle will be demonstrated for a fast gradient-echo sequence as shown in Figure 4.17A. In the first (reference) experiment ( $\tau = 0$ ), the signal at the top of the gradient echo at position  $\mathbf{r}$  can be described as:

$$M_{xy,1} = M_{xy}(\mathbf{r}, t) = M_{xy}(\mathbf{r}, 0)e^{-TE/T_2(\mathbf{r})}e^{-i\Delta\omega(\mathbf{r})TE} \quad (4.27)$$

where  $\Delta\omega = \gamma \Delta B_0$  is representative of macroscopic magnetic field inhomogeneity  $\Delta B_0$ . In the second experiment a small delay  $\tau$  is inserted prior to acquisition to allow free precession under the influence of the local magnetic field  $B_0$ . The signal at the top of the gradient echo at position  $\mathbf{r}$  is then given by:

$$M_{xy,2} = M_{xy}(\mathbf{r}, t + \tau) = M_{xy}(\mathbf{r}, 0)e^{-(TE+\tau)/T_2(\mathbf{r})}e^{-i\Delta\omega(\mathbf{r})(TE+\tau)} \quad (4.28)$$

The additional phase evolution of scan 2 relative to scan 1,  $\Delta\varphi(\mathbf{r}) = \Delta\omega(\mathbf{r})\tau$  is directly proportional to the magnetic field inhomogeneity. The phase difference between the two scans can be calculated using complex division according to:

$$\Delta\varphi = \arctan\left(\frac{R_1 I_2 - I_1 R_2}{R_1 R_2 + I_1 I_2}\right) \quad (4.29)$$

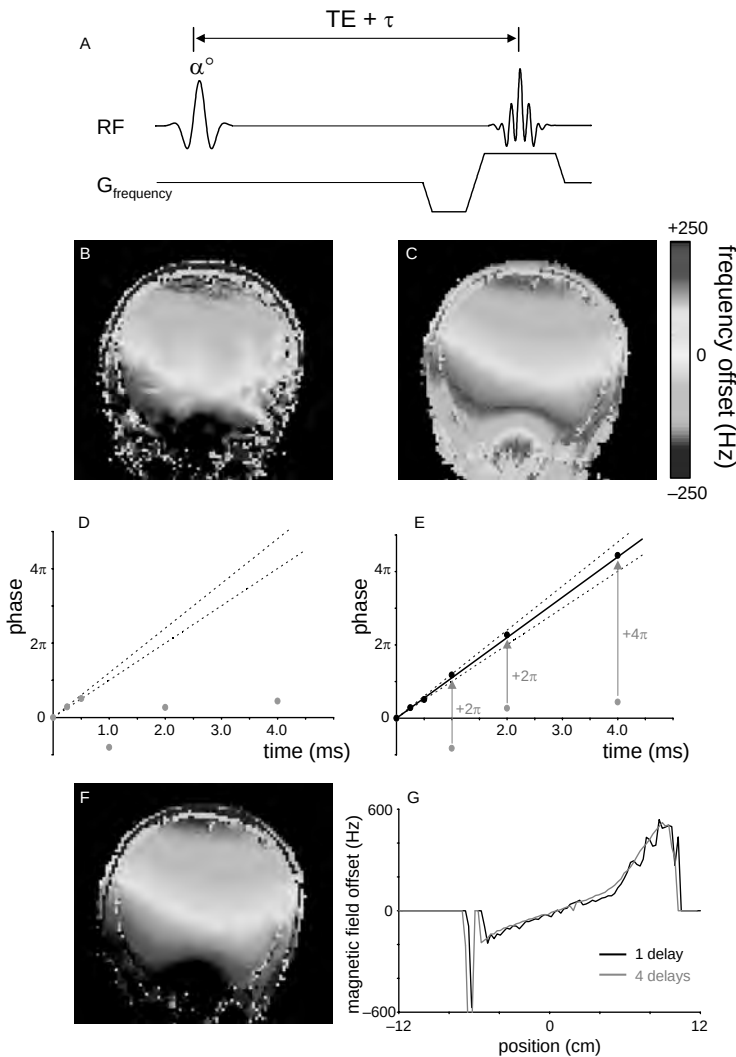


**Figure 4.17** Principle of magnetic field  $B_0$  mapping. (A) Basic pulse sequence in which  $\tau$  is a variable delay in addition to the constant echo time  $TE$ . (B) For a short delay  $\tau = 0.33$  ms, no phase wraps occur at the expense of a relatively low SNR in the calculated frequency offset or  $B_0$  map. (C) For a long delay  $\tau = 3.0$  ms, the SNR has greatly improved at the expense of phase wrapping artifacts. The advantages of short and long delays can be combined as shown in (D and E) through the procedure of 1D temporal unwrapping. Acquiring several images with increasing delays  $\tau$  allows the elimination of phase wrapping artifacts even when the majority of images is initially phase wrapped [MR pixels 4, 5 and 6 in (D) are wrapped]. Under the assumption that the first data point ( $\tau > 0$ ) is not wrapped, a linear trend can be established (D). All other data points can then simply be unwrapped by multiples of  $2\pi$  until they fall close to the line established by the previous points (E). Unwrapping followed by linear regression of a four-delay dataset ( $\tau = 0.33, 1.0$  and  $3.0$  ms) gives a  $B_0$  map with excellent SNR and no phase wraps (F). (G) Comparison of a vertical trace through the field maps in (B) and (F) demonstrating the SNR advantage of additional longer delays. (See color plate 3)



where R and I refer to the real and imaginary components of the observed signals, respectively, and 1 and 2 refer to the signals acquired with  $\tau = 0$  and  $\tau > 0$ , respectively. The additional delay  $\tau$  (typically between 0 and 5 ms) slightly decreases the signal intensity due to  $T_2$  relaxation. However, since this only affects the signal intensity it has no consequence for the phase calculation according to Equation (4.29).

The most commonly encountered problem in  $B_0$  mapping is phase wrapping (Figure 4.17C). The arctan function in Equation (4.29) only calculates the phase between  $-\pi$  and  $+\pi$ . When the actual phase evolution is very large (i.e. for  $\Delta\omega/2\pi = 550$  Hz and  $\tau = 4.0$  ms,  $\Delta\phi = 4.4\pi$ ), the calculated phase will wrap, through an integer number of  $2\pi$  cycles, back into this range (e.g.  $4.4\pi - 2 \times 2\pi = 0.4\pi$ ). In general phase wrapping leads to an incorrect estimation of the local magnetic field (e.g.  $0.4\pi$  would be equivalent to a 50 Hz offset). However, in the presence of a wide range of magnetic field inhomogeneity, phase wrapping will also lead to discrete phase jumps (e.g. two adjacent points with real phases of  $0.95\pi$  and  $1.05\pi$  will appear at  $0.95\pi$  and  $-0.95\pi$ ), making it impossible to accurately estimate global magnetic field homogeneity (Figure 4.17C). The problem of discrete phase jumps can be solved through multidimensional spatial phase unwrapping algorithms (e.g. [22] and references therein). However, these algorithms are typically not straightforward and are computationally intensive. A simpler solution is to choose the evolution time  $\tau$  small enough so that phase wrapping does not occur (e.g. the actual phases are between  $-\pi$  and  $+\pi$ , Figure 4.17B). Unfortunately, in the presence of noise the accuracy of the estimated offsets is not optimal when using a small  $\tau$  delay. Accurate estimates of magnetic field offsets are obtained when the number of evolution delays as well as the delay durations are extended (see Figure 4.17F and G). While the acquired phase during the longer delays will likely be wrapped, the initial delay is chosen such that no phase wrap occurs (Figure 4.17D). Then based on the linear phase–time trend established by the first delay, the subsequent delays can be unwrapped by adding or subtracting an integer number of  $2\pi$  to the calculated phase (Figure 4.17E). Once all phases have been temporally unwrapped, a linear least-squares fit of the phase–time curve will provide a best estimate of the magnetic field offset (Figure 4.17F and G). Typical computation times for phase calculation, phase unwrapping and linear least-squares fitting for a  $128 \times 128 \times 64$  dataset is several seconds.

While the calculated  $B_0$  map is in principle independent of the employed pulse sequence type (gradient-echo, spin-echo, EPI), the pulse sequence can nevertheless have a significant effect on the appearance of the  $B_0$  map. Gradient-echo-based  $B_0$  mapping can be fast and reliable, but the images may show areas of signal loss when strong magnetic field inhomogeneity is encountered due to phase cancellation during the initial echo-time TE. In these cases it is better to utilize spin-echo-based  $B_0$  mapping, since phase evolution due to magnetic field inhomogeneity is refocused at the top of the echo.  $B_0$  maps based on EPI can be acquired very rapidly. However, EPI image quality (signal loss and image distortion) is heavily influenced by magnetic field inhomogeneity and as such is less ideal to provide reliable  $B_0$  maps.

#### 4.8.4 Magnetic Field $B_1$ Mapping

Spatial homogeneity of the field generated by RF coils is important for the unambiguous interpretation of images, optimizing sensitivity and minimizing RF-related artifacts. However, inhomogeneous RF fields are frequently encountered in *in vivo* NMR applications

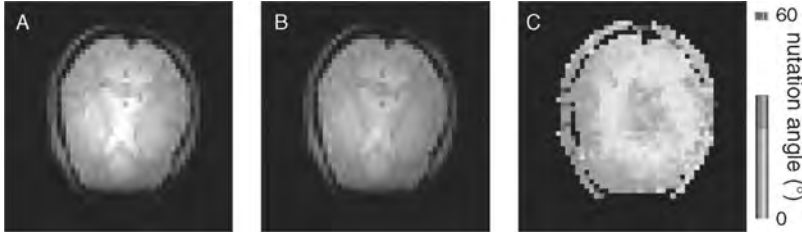


**Figure 4.18** Principle of magnetic field  $B_1$  mapping. From the two images acquired with nutation angles  $\theta_1$  (A) and  $\theta_2$  (B), a nutation angle map (C) can be generated which is directly proportional to the transmit  $B_1$  magnetic field (see Exercise 4.9 for more details). (See color plate 4)

at high magnetic fields (see Chapter 10) or with RF coils that are inherently inhomogeneous (i.e. surface coils). It becomes therefore important to measure the RF field quantitatively and a number of MRI techniques are available to achieve this goal. Most techniques rely on the relation between pulselength  $T$ , RF field amplitude  $B_1$ , nutation angle  $\theta$  and observed signal  $M_{xy}$ , which for a simple (fully relaxed) pulse-acquire experiment is given by:

$$M_{xy} = M_0 \sin \theta, \text{ with } \theta = \gamma B_1 T \quad (4.30)$$

where  $M_0$  is the thermal equilibrium magnetization. Acquiring two fully relaxed images with two different nutation angles  $\theta_1$  and  $\theta_2 = 2\theta_1$  allows the calculation of the nutation angle according to:

$$\theta_1 = \arccos \left( \frac{M_{xy}(\theta_2)}{2M_{xy}(\theta_1)} \right) \quad (4.31)$$

where  $M_{xy}(\theta_1)$  and  $M_{xy}(\theta_2)$  are the detected signals following nutation angles  $\theta_1$  and  $\theta_2$ , respectively. The  $B_1$  field can subsequently be calculated through Equation (4.30). Since the method is based on only two measurements, the accuracy of the method is highest in the 80–100° range of nutation angles. Figure 4.18 shows an example of  $B_1$  magnetic field mapping using a fast modification of the principle outlined by Equations (4.30) and (4.31) (see also Exercise 4.9).

#### 4.8.5 Alternative Image Contrast Mechanisms

Throughout some of the other chapters a number of different image contrast mechanisms have been discussed. Diffusion-weighted and diffusion-tensor imaging were introduced in Section 3.4, while MT contrast imaging was discussed in Section 3.3.6. Over the last three decades a wide variety of other contrast mechanisms have been discovered and implemented. Among others these include MR angiography [23–25], susceptibility-weighted MRI [26], diffusion kurtosis imaging [27], manganese-enhanced MRI (MEMRI) [28, 29], cardiac tagging [30], bolus tracking [31] and functional MRI based on BOLD [32–34], cerebral blood flow [35] or cerebral blood volume [36–38]. Although many of these techniques can be used in combination with MRS studies and may help in the understanding of certain principles, they are outside the scope of this book. The interested reader is referred to some excellent books on MRI techniques [3–5]. Since fMRI based on

the BOLD mechanism is such an important imaging modality with direct applications to fMRS [39], it will be briefly discussed here.

#### 4.8.6 Functional Imaging

Functional imaging techniques, NMR-based or otherwise, are aimed at the detection of the spatial distribution of neuronal activity in response to a stimulus (Figure 4.19). Although neuronal activity can be defined in several ways, it typically entails a number of events that

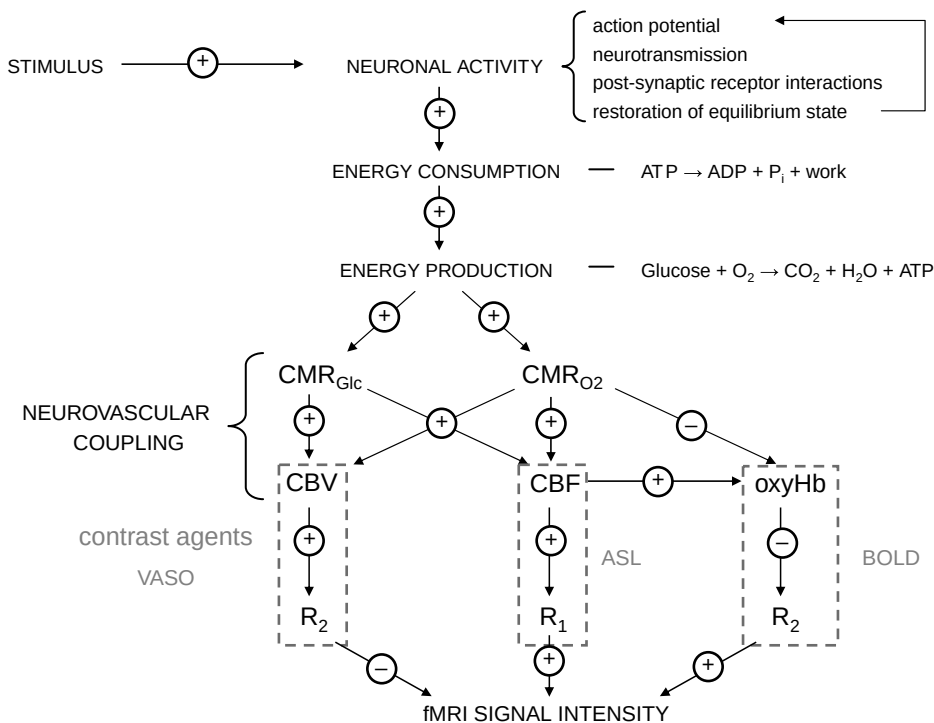


**Figure 4.19** Mechanisms underlying fMRI signal intensity changes. The neuronal activity following a stimulus is associated with an increase in energy consumption, followed by an increase in energy production. Glucose oxidation is the primary mechanism for cerebral energy production, such that  $CMR_{Glc}$  and  $CMR_{O_2}$  must also increase in response to a stimulus. The glucose and oxygen must ultimately be supplied from the blood stream, leading to an increase in CBF and CBV. Since the CBF increases more than  $CMR_{O_2}$ , there will be a net increase in the amount of diamagnetic oxyhemoglobin. This will in turn lead to a decrease in the apparent transverse relaxation rate  $R_2$  (or  $R_2^*$ ), which will lead to a positive BOLD signal. NMR can also be sensitized for CBF and CBV after which stimulus-induced signal changes are observed through changes in the apparent longitudinal and transverse relaxation rates  $R_1$  and  $R_2$ , respectively. It should be obvious that fMRI techniques measure hemodynamic responses that, through a largely unknown neurovascular coupling, are related to the underlying electrical and metabolic processes.  $^{13}C$  NMR can directly measure glucose oxidation and glutamatergic neurotransmission, thereby providing more direct information about the metabolic processes (see Chapter 3).

include an action potential which leads to neurotransmission and post-synaptic receptor interactions. Following these events the equilibrium state must be re-established, which includes the restoration of the intra-extracellular ion balance and recycling of neurotransmitters, such that the system is ready for another action potential. For most functional imaging methods the essential feature is that the restoration of an equilibrium state leads to an increase in energy consumption, which must ultimately also lead to an increase in energy production. Since the oxidation of glucose is the primary pathway for energy production in the brain, an increase in energy production must lead to an increase in the cerebral metabolic rates of glucose ( $CMR_{Glc}$ ) as well as oxygen ( $CMR_{O_2}$ ). The glucose and especially oxygen reserves inside the brain are very limited, such that increased demands for glucose and oxygen must be supplied by the blood, thus leading to an increase in cerebral blood flow (CBF) and cerebral blood volume (CBV). The coupling between the metabolic parameters  $CMR_{Glc}$  and  $CMR_{O_2}$  and the hemodynamic parameters CBF and CBV is often referred to as the neurovascular coupling. The neurovascular coupling, however, is still poorly understood. As a result, a significant fraction of the fMRI literature is dedicated to the study of the exact functional dependence of the measured parameters (CBF and CBV) on the parameters of interest ( $CMR_{Glc}$  and  $CMR_{O_2}$ ). CBF and CBV can both be measured with NMR. CBF is most commonly measured through the use of arterial spin labeling (ASL) methods [35] in which the water spins in the carotid arteries are perturbed (saturated or inverted). During a delay period the perturbed water spins flow to the cerebral region of interest where they reduce the longitudinal magnetization. A difference image with and without arterial spin labeling will show signal that is directly proportional to CBF, provided that the longitudinal  $T_1$  relaxation is known. CBV can be measured through the use of intravascular contrast agents [36]. The intravascular (or ‘blood pool’) contrast agent effectively eliminates the signal from the blood compartment. When the CBV increases, the amount of blood compartment within a MRI pixel increases which leads to a decrease in signal intensity as the blood does not contribute any signal intensity. Recently, a completely noninvasive alternative for CBV measurements has been described that does not require a blood pool agent [37, 38].

Even though NMR is able to measure CBF and CBV, these methods only represent a small fraction of the fMRI studies. An effect that is specific for NMR-based functional imaging can be found in the magnetic characteristics of hemoglobin. It has long been known that deoxyhemoglobin (i.e. hemoglobin without oxygen bound to it) is paramagnetic, whereas oxyhemoglobin is diamagnetic. As will be discussed in more detail in Chapter 10, the magnetic susceptibility difference between diamagnetic water and paramagnetic hemoglobin will lead to a disturbance of the magnetic field surrounding the red blood cells and blood vessels carrying the deoxyhemoglobin (Figure 4.20A). When CBF and CBV increase in response to a stimulus, the amount of deoxyhemoglobin inside red blood cells decreases, thereby leading to a reduction of the magnetic field disturbance (Figure 4.20B). NMR sequences can be sensitized for magnetic field distributions, for example through the use of a gradient-echo MRI sequence (Figure 4.17A). During a resting condition in which no experimental stimuli are applied, the deoxyhemoglobin leads to a certain field disturbance which can be separated into intravascular and extravascular, static and dynamic effects. Figure 4.20A shows a dynamic, extravascular effect in which a water molecule diffuses through the magnetic field disturbance caused by the deoxyhemoglobin. As the water diffuses it experiences a range of magnetic fields and hence resonance frequencies, which will lead to phase dispersal and thus signal loss in a gradient-echo

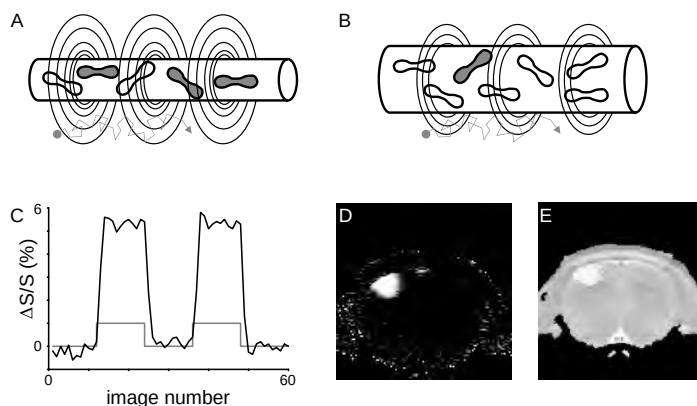


**Figure 4.20** Principle of BOLD fMRI. (A) In a ‘resting’ state the paramagnetic deoxyhemoglobin in red blood cells generates a significant magnetic field disturbance surrounding the cells and blood vessels. Through a number of intra- and extravascular effects this magnetic field inhomogeneity leads to signal loss in  $T_2$ - and  $T_2^*$ -weighted MRI sequences. (B) As a result of the interplay between CBF, CBV and  $\text{CMR}_{\text{O}_2}$  changes following a stimulus there is a net decrease in the amount of deoxyhemoglobin, and thus less signal loss during a  $T_2^*$ -weighted sequence, giving rise to a positive BOLD effect. (C) Temporal BOLD response during single forepaw stimulation in the rat at 9.4 T as extracted from a single image pixel in the somatosensory cortex. Each image was acquired in circa 5 s with  $\text{TE} = 15$  ms. A significant signal increase of 5–6% can be observed during the two stimulation blocks (gray line) relative to the baseline state. (D) Difference image, calculated as  $(S_{\text{stimulated}} - S_{\text{baseline}})/S_{\text{baseline}}$ , showing good localization of the forepaw region in the somatosensory cortex. (E) Cross-correlation map overlaid on an anatomical  $T_1$  map. Only pixels with a cross-correlation coefficient of  $>0.8$  are shown. (See color plate 5)

MRI sequence. When during stimulation (Figure 4.20B) CBF and CBV increase and thus the amount of deoxyhemoglobin decreases, the phase dispersal of the diffusing water in the extravascular compartment will be less, such that more signal will be obtained. Note that the amount of deoxyhemoglobin in the blood only decreases because the increase in CBF exceeds the increase in  $\text{CMR}_{\text{O}_2}$ . Therefore, the so-called BOLD (blood oxygen level dependent) contrast [32–34] is typically positive when comparing an ‘activated’ state with a ‘baseline’ state (Figure 4.20C–E). In reality, the signal is a complicated function of the various intra- and extravascular contributions, the magnetic field strength, echo-time, pulse sequence, blood vessel size and orientation and other parameters. The interested reader is referred to the rich literature on fMRI [40–42]. For intracellular metabolites the situation is considerably simplified, as any effect must be attributed to a dynamic, extravascular contribution [39].

## 4.9 Parallel MRI

Scan time reduction in MRI is typically achieved by (1) decreasing the repetition time or (2) increasing k-space sampling per excitation. A completely different approach to scan time reduction is provided by parallel MRI methods which take advantage of the local spatial

sensitivity information of an array of multiple receive coils to partially replace spatial encoding by magnetic field gradients. Since signal can be acquired simultaneously (i.e. in parallel) by the multiple receive coils, a reduction in k-space sampling directly results in a reduced image acquisition time while maintaining full spatial resolution.

The most well known and commonly used parallel MRI techniques are SMASH (simultaneous acquisition of spatial harmonics) [43], SENSE (sensitivity encoding) [44] and GRAPPA (generalized autocalibrating partially parallel acquisitions) [45]. All parallel MRI methods share the same basic idea of complementing incomplete k-space sampling with additional spatial information obtained from local RF coils. The methods mainly differ (1) in the required amount of prior knowledge on coil sensitivity profiles and (2) in the manner by which the spatial information is combined. SMASH and SENSE both require accurate knowledge of the sensitivity profiles of each coil in a multi-coil receiver array. Sensitivity maps can be obtained as described in Section 4.8.4.

In the following, the specifics of SENSE parallel MRI will be discussed in more detail. For details on the implementation and reconstruction involved with other parallel MRI methods the reader is referred to the original literature [43–45], as well as reviews [46,47].

Consider the object shown in Figure 4.21A. The image scan time can be reduced fourfold by only acquiring every fourth line in k-space, which is equivalent to a fourfold reduction of the FOV in one direction. Signal outside of the reduced FOV will be sampled at an apparent lower frequency in order to satisfy the Nyquist sampling condition (see also Section 1.9.3). As a result, signal from outside the reduced FOV is aliased to within the FOV leading to the image shown in Figure 4.21B. The folded image can only be unambiguously unfolded with the aid of additional information about the spatial origins of the signal. Parallel MRI methods provide the additional information through the spatially dependent sensitivity of multiple RF coils. Suppose that signal from the object is acquired with four independent receiver coils as shown in Figure 4.21C. The signal originating from coil 2 is shown in Figure 4.21D, which will lead to the image shown in Figure 4.21E when the FOV is reduced fourfold. Signals originating from spatial positions outside of the FOV will still fold back into the reduced FOV, but in the case of Figure 4.21E it is known that all observed signal originates from within the sensitive volume of receiver coil 2. Being a parallel MRI method, signal from all four coils is acquired simultaneously as shown in Figure 4.21F. The signal  $I_{\text{coil},k}(x, y)$  acquired by coil  $k$  at position  $(x, y)$  is the sum of the signal in the original object  $I_{\text{object}}(x, y_m)$  at the four  $y$  positions that fold back to position  $(x, y)$  in the reduced FOV image multiplied by the coil sensitivity  $C_k(x, y_m)$  at those positions, i.e.:

$$I_{\text{coil},k}(x, y) = \sum_{m=1}^4 C_k(x, y_m) I_{\text{object}}(x, y_m) \quad (4.32)$$

For the more general case of  $K$  coils and  $M$  spatial locations,  $K$  linear equations with  $M$  unknowns can be constructed and written in matrix form as:

$$\mathbf{I}_{\text{coil}} = \mathbf{C} \cdot \mathbf{I}_{\text{object}} \quad (4.33)$$

where  $\mathbf{I}_{\text{coil}}$  is a  $(K \times 1)$  vector and represents the measured complex signal intensity at a chosen pixel for each of the  $K$  coils.  $\mathbf{I}_{\text{object}}$  is a  $(M \times 1)$  vector and represents the signal in the full FOV image.  $\mathbf{C}$  is a  $(K \times M)$  sensitivity matrix describing the sensitivity of all  $K$  coils at all  $M$  spatial positions. Once the sensitivity matrix  $\mathbf{C}$  is known, the unfolded image

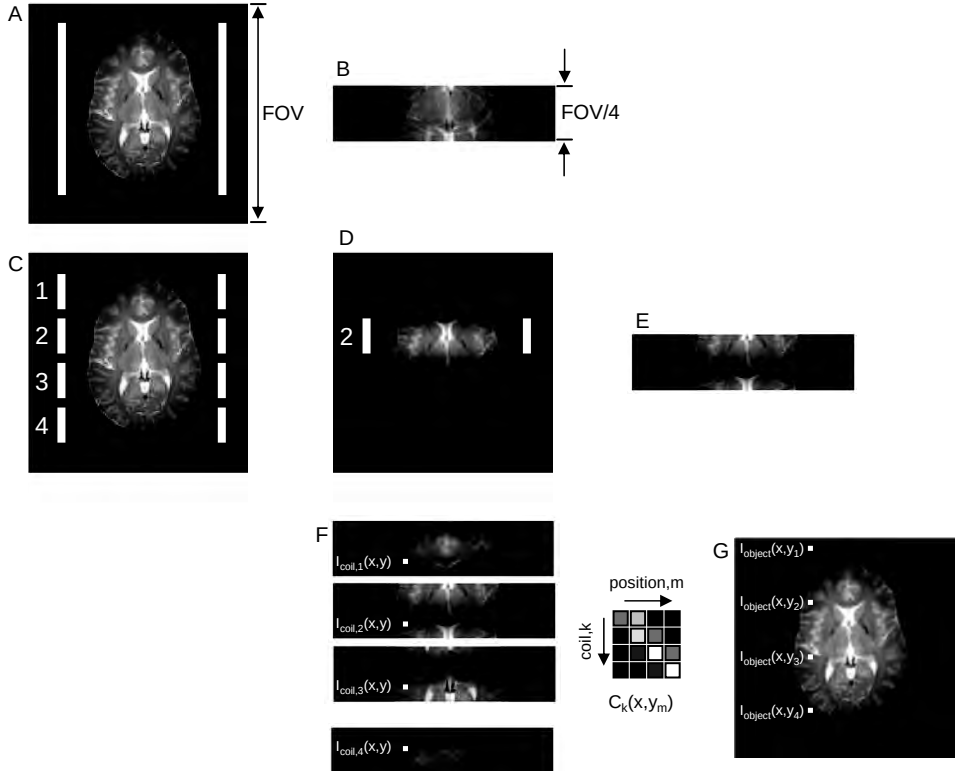


**Figure 4.21** Principle of parallel imaging with the SENSE algorithm. (A) Full FOV image of a human head. (B) A fourfold reduction of the FOV results in severe aliasing from which the original image can not be recovered without additional information. (C) In parallel imaging the additional information is supplied by the sensitivity profiles of different RF coils, such as (D) the sensitivity profile shown for coil 2. (E) A fourfold reduction of the FOV will still lead to aliasing, but with the prior knowledge that all aliased and nonaliased signal is coming from coil 2. (F) Parallel imaging allows the simultaneous detection of signal from all four coils, after which (G) the full FOV, unfolded image can be obtained through some matrix algebra given by Equation (4.34).

over the full FOV,  $I_{\text{object}}$ , can be calculated according to:

$$I_{\text{object}} = (\mathbf{C}^H \mathbf{C})^{-1} \mathbf{C}^H \cdot \mathbf{I}_{\text{coil}} \quad (4.34)$$

Equation (4.34) assumes that there is no noise correlation between the coils. In reality, coil sensitivity profiles overlap and are not completely independent from one another, necessitating the extension of Equation (4.34) with receiver noise correlation matrices [44].

The matrix inversion in Equation (4.34) can only be performed uniquely when the number of unknown spatial locations  $M$  is equal to or smaller than the number of coils  $K$ , thereby limiting the maximum scan time reduction to the number of coils. To reconstruct

the full unfolded image, Equation (4.34) is repeated for each pixel position in the folded images. The SNR of SENSE compared with a conventional experiment is given by:

$$\text{SNR}_{\text{SENSE}} = \frac{\text{SNR}_{\text{conv}}}{g M^{1/2}} \quad (4.35)$$

where the factor  $M^{1/2}$  originates from the fact that less time is spent on data acquisition and  $g$  represents a geometry factor and is dependent on the RF coil geometry and interactions. For a perfectly constructed coil array,  $g = 1$  and the sensitivity of SENSE and conventional acquisitions is identical for the same experimental duration.

## 4.10 Exercises

**4.1** Consider a spin-warp MRI sequence ( $\text{TR} - 90^\circ - \text{TE}/2 - 180^\circ - \text{TE}/2 - \text{acquisition}$ ) and two tissue types with the following characteristics:

Tissue 1:  $M_0 = 0.8$ ,  $T_1 = 2.0$  s,  $T_2 = 0.06$  s.

Tissue 2:  $M_0 = 0.7$ ,  $T_1 = 1.0$  s,  $T_2 = 0.03$  s.

**A** At  $\text{TR} = 5.0$  s calculate the echo time  $\text{TE}$  which gives the largest absolute signal difference between the two tissues. Ignore  $T_1$  relaxation during  $\text{TE}$ .

**B** At  $\text{TE} = 10$  ms calculate the repetition time  $\text{TR}$  which gives the largest absolute signal difference between the two tissues.

**C** Calculate the echo-time  $\text{TE}$  at which the  $T_2$ -weighting cancels the combined proton-density/ $T_1$ -weighting at a repetition time  $\text{TR}$  of 2.0 s.

**4.2** MRI signal is acquired with a gradient-echo sequence as 256 complex points over 1.28 ms during a readout gradient of  $2.4466 \text{ G cm}^{-1}$ . Signal excitation is achieved with a 1.5 ms sinc pulse (bandwidth = 3750 Hz). All gradients are ramped up and down between zero and their final amplitude in 500  $\mu\text{s}$ .

**A** Calculate the FOV in the frequency-encoding ('readout') direction.

**B** For the same resolution and FOV as calculated under (A), calculate the phase-encoding gradient increments (in  $\text{G cm}^{-1}$ ) for a total phase-encoding gradient duration of 1.2 ms (including ramps).

**C** Calculate the slice selection gradient amplitude for 4.0 mm slices.

**D** For a maximum system gradient amplitude of  $2.5 \text{ G cm}^{-1}$  calculate the minimum achievable echo-time  $\text{TE}$  (from the middle of the excitation pulse to the top of the echo) for the parameters calculated under (A)–(C). Hint: Magnetic field gradients in orthogonal directions can temporally overlap during certain periods within a sequence.

**4.3** Consider the following sequences:

1. A multi-slice spin-echo sequence with  $\text{TR}/\text{TE} = 3000/12.5$  ms.

2. A fast multi-slice gradient-echo method (FLASH) with  $\text{TR} = 10$  ms,  $\text{TE} = 4$  ms and nutation angle  $\alpha = 15^\circ$ .

3. A multi-slice, spin-echo EPI sequence with  $\text{TR}/\text{TE} = 3000/20$  ms and 8 echoes per excitation.

**A** For an image resolution of  $32 \times 32 \times 32$  calculate the total experimental duration and determine the fastest sequence.

**B** Repeat the calculation for an image resolution of  $128 \times 128 \times 128$ .



- 4.4** A fast gradient-echo FLASH image is acquired as a  $96 \times 96$  data matrix over a  $19.2 \times 19.2$  cm FOV with a 2.0 mm thick slice, TR/TE = 25/5 ms, 1 average and nutation angle  $\alpha = 20^\circ$ . Complete dephasing of transverse magnetization prior to each excitation can be assumed.
- A** For the corpus callosum with  $T_1/T_2 = 1500/75$  ms an absolute SNR of 40 was obtained. Which of the following modifications provides the largest and smallest improvement in absolute SNR?
- Increasing the FOV  $21.6 \times 21.6$  cm.
  - Increasing the number of averages to 2.
  - Decreasing the nutation angle to  $15^\circ$ .
  - Increasing the repetition time to 100 ms.
- B** Which modification provides the largest improvement in SNR per unit of time?
- C** Calculate the Ernst angle and determine the increase in SNR relative to  $\alpha = 20^\circ$ .
- 4.5** **A** Derive an expression for the longitudinal steady-state magnetization for a multi-slice gradient-echo sequence with *sequential* slice selection. Assume an in-slice nutation angle  $\alpha_1$ , an effective nutation angle  $\alpha_2$  on either side of the slice due to an imperfect slice profile and further assume that only adjacent slices affect each other.
- B** Derive an expression for the longitudinal steady-state magnetization for a multi-slice gradient-echo sequence with *interleaved* slice selection.
- C** Calculate the signal gain of interleaved over sequential slice selection for a multi-slice gradient-echo sequence selecting 20 slices in 2000 ms for  $T_1 = 1000$  ms and near-perfect slice profiles ( $\alpha_1 = 85^\circ$ ,  $\alpha_2 = 10^\circ$ ).
- D** Repeat the calculation in (C) for more realistic slices ( $\alpha_1 = 75^\circ$ ,  $\alpha_2 = 35^\circ$ ).
- 4.6** Two multi-slice, gradient-echo MR image datasets are acquired with TR/TE = 4000/4 ms and a 1 ms Gaussian excitation pulse with RF power settings of +6 dB and +12 dB. The absolute signal intensity of a specific pixel is 1025 and 768 in the two images, respectively.
- A** Calculate the nutation angle for the mentioned pixel position.
- B** Given the poorly defined slice profile of a Gaussian RF pulse (see also Chapter 5), there exists a range of nutation angles across the slice which will lead to an incorrect estimation. Based on simulations, a Gaussian pulse calibrated to give a  $110^\circ$  rotation on-resonance, gives the maximum amount of the signal from the entire slice. Pulses calibrated as  $36.7^\circ$  and  $183.4^\circ$  provide exactly 50 % of the maximum signal. Recalculate the on-resonance nutation angle for the mentioned pixel position.
- 4.7** The magnetic field homogeneity is measured with a multi-slice gradient-echo sequence in which the echo time is incremented from 4.0 ms to 5.0, 6.0 and 8.0 ms. For a given pixel, the acquired phases are calculated as  $-86^\circ$ ,  $+76^\circ$ ,  $-128^\circ$  and  $-171^\circ$ , respectively.
- A** Under the assumption that the maximum magnetic field inhomogeneity is less than 500 Hz, calculate the frequency offset for the mentioned pixel position using (1) the first two data points and (2) all available data. Hint: Use a calculator.
- B** When the signal is acquired as a  $64 \times 64$  matrix over a 50 kHz bandwidth with a conventional spin-warp MRI sequence, calculate the spatial displacement

in the frequency- and phase-encoding directions for a magnetic field offset of 400 Hz.

- 4.8 A** The Lissajou k-space trajectory is generated by the following gradient waveform combinations:

$$G_x(t) = \frac{G_{\max} T}{\nu_x} \cos\left(\frac{2\pi\nu_x t}{T}\right) \quad \text{and} \quad G_y(t) = \frac{G_{\max} T}{\nu_y} \cos\left(\frac{2\pi\nu_y t}{T}\right)$$

Draw the Lissajou k-space trajectory for  $\nu_x = 9$ ,  $\nu_y = 10$  and  $0 \leq t \leq T$ .

- B** The Rosette k-space trajectory is generated by the following gradient waveform combinations:

$$G_x(t) = \frac{G_{\max} T}{\nu_1 + \nu_2} \cos\left(\frac{2\pi(\nu_1 + \nu_2)t}{T}\right) + \frac{G_{\max} T}{\nu_1 - \nu_2} \cos\left(\frac{2\pi(\nu_1 - \nu_2)t}{T}\right)$$

$$G_y(t) = \frac{G_{\max} T}{\nu_1 + \nu_2} \sin\left(\frac{2\pi(\nu_1 + \nu_2)t}{T}\right) - \frac{G_{\max} T}{\nu_1 - \nu_2} \sin\left(\frac{2\pi(\nu_1 - \nu_2)t}{T}\right)$$

Draw the Rosette k-space trajectory for  $\nu_1 = 10$ ,  $\nu_2 = 1$  and  $0 \leq t \leq T$ .

- C** Discuss differences, advantages and disadvantages of the alternative trajectories over standard rectangular k-space sampling.
- 4.9** As detailed in Section 4.8.4 spatial mapping of the transmit  $B_1$  field can be achieved with two fully relaxed measurements of two different excitation angles  $\theta_1$  and  $\theta_2 = 2\theta_1$ . A time-efficient alternative is given by the sequence: TR –  $\theta$  – acquisition 1 –  $\theta$  – acquisition 2.
- A** Under the assumption that (1)  $TR \gg T_1$  and (2) acquisition time  $1 \ll T_1$ , derive the expression for the nutation angle as function of the signals detected during acquisition times 1 and 2.
- B** Determine the optimum nutation angle (i.e. the nutation angle that can be determined with the highest sensitivity). Note: Use standard error propagation theory and assume uncorrelated noise between the two measurements. Also:  $\frac{d(\arccos x)}{dx} = \frac{-1}{\sqrt{1-x^2}}$ .
- C** Determine the optimum nutation angle for the standard method described in Section 4.8.4.
- 4.10 A** For a magnetic field ‘crusher’ gradient of 2.0 ms duration, calculate the required amplitude (in  $\text{mT m}^{-1}$ ) to suppress the transverse magnetization by a factor of 100. Assume a sample of length  $L = 5$  cm.
- B** A 5.0 ms optimized excitation pulse ( $BW = 5.0$  kHz) selects a 4.0 mm thick slice from a homogeneous, water-filled cube with 4.0 cm long sides. Calculate the suppression factor of the transverse magnetization at the end of the pulse. Assume that the magnetization is effectively excited at the midpoint of the RF pulse.

- 4.11 A** For two relaxation times  $T_{1A}$  and  $T_{1B}$  calculate the recovery delay TR that leads to the largest contrast in a standard gradient-echo sequence. Assume a  $90^\circ$  excitation pulse and complete removal of transverse magnetization prior to excitation.
- B** Show that a similar expression is valid for maximum  $T_2$  contrast.
- 4.12** In Section 4.8.2 the Look–Locker method for fast  $T_1$  measurements was described. An alternative is provided by  $T_1$  measurements based on two experiments with different repetition times. The signal intensity  $S_1$  and  $S_2$  of two spin-echo MRI experiments with repetition times TR1 and TR2 can be described by:

$$S_1 = S_0(1 - e^{-TR_1/T_1})e^{-TE/T_2}$$

$$S_2 = S_0(1 - e^{-TR_2/T_1})e^{-TE/T_2}$$

- A** Derive an expression for the relaxation time  $T_1$  when  $TR_2 = 2TR_1$  and when  $TR_2 = 3TR_1$ .
- B** For the experiment in which  $TR_2 = 2TR_1$  determine the optimal repetition time TR1 to determine a relaxation time constant  $T_1$  of 1000 ms.
- 4.13** Consider a single-shot EPI sequence with the following parameters:  $64 \times 64$  data matrix over a  $20 \times 20$  cm FOV, imaging bandwidth = 100 kHz and 100  $\mu$ s gradient ramp duration.
- A** Calculate the effective spectral width across the FOV in the phase-encoding direction.
- B** Derive Equation (4.21) for the spatial displacements during EPI.
- C** For a pixel with a frequency offset of +150 Hz, for example due to residual magnetic field inhomogeneity, calculate the pixel shifts in the frequency and phase-encoding directions.
- 4.14** Consider a 2D image with a  $128 \times 128$  resolution over a 24 cm FOV.
- A** Calculate the linear phase that must be applied in k-space to shift the image by 1/3 of the FOV.
- B** Calculate the required first-order image phase correction required to display the image as an in-phase, real image.
- C** Calculate the relative SNRs per unit time for a  $64 \times 64$  image obtained by (1) downsampling the  $128 \times 128$  image (i.e. adding 4 pixels) or (2) acquiring a new image at a  $64 \times 64$  resolution.
- 4.15** A 2 cm diameter cylinder filled with water (4 cm length) is placed in a 4 T magnet with its longest axis parallel to the main magnetic field.
- A** Draw the relative 1D spatial profiles when signal is acquired during a frequency-encoding gradient applied in the x, y and z directions, respectively.
- B** Draw the spatial profile when signal is acquired in the presence of simultaneous x and y gradients of equal amplitude.
- C** Consider two 3 cm diameter spheres filled with water ( $H_2O$ , 4.7 ppm) and methanol ( $CH_3OH$ , 3.3 ppm), respectively, placed 5 cm apart (center-to-center) in the x direction of a 4 T magnet and being aligned in the y and z directions. FOV = 10 cm, dwell time of 1  $\mu$ s, 256 complex acquisition points. Assume equal  $T_1$  and  $T_2$  relaxation parameters for water and methanol. Further assume that the density of methanol is 80% of water.

Draw the spatial profile when signal is acquired with a spin-echo sequence ( $TE = 6.3$  ms) in the presence of frequency-encoding gradients in the x and y directions, respectively.

**D** Draw the spatial profile when signal is acquired with a gradient-echo sequence ( $TE = 6.3$  ms) in the presence of frequency-encoding gradients in the x and y directions, respectively.

**E** Draw the spatial profiles in the x direction when the data is acquired over a spectral width of 20 kHz at 11.75 T (spin-echo sequence, water/methanol phantom).

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# 5

## Radiofrequency Pulses

### 5.1 Introduction

Radiofrequency (RF) pulses are required in all Fourier transform magnetic resonance imaging (MRI) and spectroscopy (MRS) experiments. They are the primary tools used by the experimentalist to perform the desired spin manipulations like excitation, inversion and refocusing. Short, intense (i.e. ‘hard’), constant-amplitude RF pulses (‘square’ RF pulses) are most often used to achieve uniform excitation of the spins across a given frequency range. However, many *in vivo* NMR experiments require more sophisticated RF pulses. Often a certain degree of selectivity is required, i.e. instead of uniform excitation, a well-defined and limited range of frequencies has to be excited, for instance in water suppression and spatial localization. Selective RF pulses can be designed using Fourier transform theory, the Bloch equations or elaborate optimization procedures. In other circumstances, the  $B_1$ -dependence of the nutation angle needs to be minimized in order to reduce artifacts and/or increase sensitivity. This can be achieved with composite RF pulses or with RF amplitude- and frequency-modulated adiabatic RF pulses.

*In vivo* NMR spectroscopy makes use of all features encountered in RF pulse design. In fact, successful *in vivo* NMR studies depend heavily on the thorough understanding and correct implementation of RF pulses. This chapter will deal with the description of RF pulses, emphasizing theoretical principles, practical utility and experimental pitfalls.

### 5.2 Square RF Pulses

When the amplitude of the RF pulse  $\gamma B_1$  is strong enough such that off-resonance effects  $\Delta\Omega$  (i.e. the difference between the Larmor frequency  $\omega_0$  and the frequency  $\omega$  of the irradiating field  $B_1$ ) can be neglected, i.e.  $|\gamma B_1| \gg |\Delta\Omega|$ , the nutation angle  $\theta$  is linearly

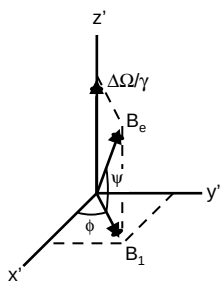


**Figure 5.1** Magnetic field vectors in the rotating frame of reference  $x'y'z'$ . The  $\mathbf{B}_1$  magnetic field, applied over a phase angle  $\phi$  away from the  $x'$  axis, in combination with an off-resonance vector  $\Delta\Omega/\gamma$  make up an effective magnetic field  $\mathbf{B}_e$ , according to Equation (5.2).

proportional to  $\gamma B_1$  and is given by:

$$\theta = \gamma B_1 T \quad (5.1)$$

where  $T$  is the pulse length. However, in many experimental situations the width of the spectra is comparable to the applied RF field strength and off-resonance effects cannot be ignored. In this situation the magnetization will not rotate about  $\mathbf{B}_1$ , but about a tilted effective field  $\mathbf{B}_e$  (Figure 5.1) the amplitude of which is given by:

$$B_e = |\mathbf{B}_e| = \sqrt{B_1^2 + \left(\frac{\Delta\Omega}{\gamma}\right)^2} \quad (5.2)$$

The effective field is tilted towards the  $z'$ -axis over an angle  $\psi$  given by:

$$\psi = \arctan\left(\frac{\Delta\Omega}{\gamma B_1}\right) \quad (5.3)$$

Equation (5.1) should therefore, in the case of nonvanishing off-resonance effects, be modified to:

$$\theta = \gamma B_e T \quad (5.4)$$

Equations (5.2) and (5.4) show the offset compensation of square RF pulses, i.e. for larger frequency offsets  $\Delta\Omega$ , the effective nutation angle increases such that the magnetization will still finish close to the transverse plane. Although Equation (5.4) gives a quantitative description of the effective nutation angle, the actual rotation about the effective field is more complex. Rotations around the effective field are most easily described by rotations about the principal axis  $x'$ ,  $y'$  and  $z'$  in matrix notation. For a rotation around the  $x'$  axis over an angle  $\theta$ , the rotation matrix  $\mathbf{R}_x(\theta)$  is given by:

$$\mathbf{R}_x(\theta) = \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos \theta & \sin \theta \\ 0 & -\sin \theta & \cos \theta \end{pmatrix} \quad (5.5)$$

resulting, for instance, for an initial thermal equilibrium magnetization  $\mathbf{M}_z$  in excitation of magnetization  $\mathbf{M}_z \rightarrow \mathbf{M}_z \cos \theta - \mathbf{M}_y \sin \theta$ . The matrices for rotations about the  $y'$  and  $z'$



axes are given by:

$$\mathbf{R}_y(\theta) = \begin{pmatrix} \cos \theta & 0 & -\sin \theta \\ 0 & 1 & 0 \\ \sin \theta & 0 & \cos \theta \end{pmatrix} \quad (5.6)$$

$$\mathbf{R}_z(\theta) = \begin{pmatrix} \cos \theta & \sin \theta & 0 \\ -\sin \theta & \cos \theta & 0 \\ 0 & 0 & 1 \end{pmatrix} \quad (5.7)$$

These rotation matrices are derived for an anticlockwise rotation about the relevant axis (i.e. when viewed along this axis towards the origin). Using Equations (5.5)–(5.7) allows the description of the rotations of magnetization  $\mathbf{M}$  around the effective field  $\mathbf{B}_e$  in consecutive steps by repeated application of the appropriate rotation matrix for nontilted axes. For example, the rotations induced by a RF pulse along the  $x'$  axis over an angle  $\theta$  with an effective field  $\mathbf{B}_e$  tilted over  $\psi$  to the  $z'$  axis is equivalent to a rotation through  $-\psi$  about  $y'$  followed by a rotation through  $\theta$  about  $x'$  and then followed by a rotation of  $+\psi$  about  $y'$ . The corresponding rotation matrix for the rotation is the product of the three corresponding matrices given by Equations (5.5)–(5.7) and is given by:

$$\mathbf{R}_x(\theta, \psi) = \begin{pmatrix} \cos^2 \psi + \cos \theta \sin^2 \psi & \sin \psi \sin \theta & \sin \psi \cos \psi (1 - \cos \theta) \\ -\sin \psi \sin \theta & \cos \theta & \cos \psi \sin \theta \\ \sin \psi \cos \psi (1 - \cos \theta) & -\cos \psi \sin \theta & \sin^2 \psi + \cos \theta \cos^2 \psi \end{pmatrix} \quad (5.8)$$

This rotation matrix is valid for a  $B_1$  field along the  $x'$  axis of the rotating frame and it can be shown that Equation (5.8) is valid for any RF pulse. The rotation matrix for a RF pulse with phase  $\phi$  relative to the  $x'$  axis is obtained with an additional rotation about  $z'$ , i.e.:

$$\mathbf{R}_x(\phi, \theta, \psi) = \mathbf{R}_z(\phi) \mathbf{R}_x(\theta, \psi) \mathbf{R}_z(-\phi) \quad (5.9)$$

It is straightforward to confirm the validity of Equation (5.8) by considering the case of a strong RF pulse, such that off-resonance effects can be neglected. In that case,  $\psi = 0^\circ$  such that  $\cos \psi = 1$ ,  $\sin \psi = 0$ ,  $\mathbf{B}_e = \mathbf{B}_1$  and Equation (5.8) then reduces to the rotation matrix given by Equation (5.5). For the situation in which no  $\mathbf{B}_1$  field is present (i.e.  $\cos \psi = 0$ ,  $\sin \psi = 1$  and  $\mathbf{B}_e = \Delta\Omega/\gamma$ ) Equation (5.8) reduces to the description of the precession of transverse magnetization about  $z'$  given by Equation (5.7).

Consider the thermal equilibrium magnetization along the  $z'$  axis such that  $\mathbf{M}(0) = (0, 0, M_0)$ . Application of a RF pulse along the  $x'$  axis of duration  $T$  will create transverse magnetization according to Equations (5.5)–(5.9) which is given by:

$$M_x = M_0 \frac{\gamma B_1 \Delta\Omega}{\gamma^2 B_1^2 + \Delta\Omega^2} \{1 - \cos[(\gamma^2 B_1^2 + \Delta\Omega^2)^{1/2} T]\} \quad (5.10)$$

$$M_y = M_0 \frac{\gamma B_1}{(\gamma^2 B_1^2 + \Delta\Omega^2)^{1/2}} \sin[(\gamma^2 B_1^2 + \Delta\Omega^2)^{1/2} T] \quad (5.11)$$

$$M_z = \frac{M_0}{\gamma^2 B_1^2 + \Delta\Omega^2} \{\Delta\Omega^2 + \gamma^2 B_1^2 \cos[(\gamma^2 B_1^2 + \Delta\Omega^2)^{1/2} T]\} \quad (5.12)$$

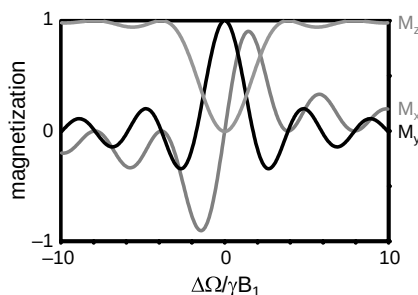


**Figure 5.2** Frequency dependence of the response to a  $90^\circ$  square RF pulse for  $M_x$ ,  $M_y$  and  $M_z$  as calculated with the rotation matrices given by Equations (5.5)–(5.7). Note that the frequency offset  $\Delta\Omega$  is normalized for the RF amplitude  $\gamma B_1$ .

Figure 5.2 shows the profiles of  $M_x$ ,  $M_y$  and  $M_z$  for a  $90^\circ$  nutation angle (applied along the  $-x'$  axis), as calculated using Equations (5.10)–(5.12) respectively. On-resonance, the pulse only generates  $M_y$ , but off-resonance  $M_x$  is created as well, leading to a frequency-dependent phase shift.

Fourier transformation stands at the heart of modern NMR and as a result the frequency profiles of many of the commonly encountered RF pulses have been calculated using Fourier transformation. For a square pulse of length  $T$  the transverse components  $M_x$  and  $M_y$  of the frequency profile, as calculated by Fourier transformation, are given by:

$$M_x = M_0 \frac{1 - \cos(\Delta\Omega T)}{\Delta\Omega T} \quad (5.13)$$

$$M_y = M_0 \frac{\sin(\Delta\Omega T)}{\Delta\Omega T} \quad (5.14)$$

It is clear when comparing Equations (5.10)–(5.12) and Equations (5.13) and (5.14) that the Fourier transform of a RF pulse does not accurately predict the frequency profile. This is because the Fourier transformation is a linear operation [e.g.  $\text{FT}(\alpha) + \text{FT}(\beta) = \text{FT}(\alpha + \beta)$ ], whereas signal excitation in NMR is nonlinear [e.g.  $M_{xy}(\theta = 40^\circ) + M_{xy}(\theta = 60^\circ) \neq M_{xy}(\theta = 100^\circ)$ ]. Only in the range of very small nutation angles (where the NMR response increases almost linearly with the nutation angle, i.e.  $\sin\theta \approx \theta$  for  $\theta < 30^\circ$ ) does the frequency profile calculated by Fourier transformation approximate the actual profile. Figure 5.3 compares the frequency profile of  $M_{xy} [= (M_x^2 + M_y^2)^{1/2}]$  and the phase of  $M_{xy} [= \arctan(M_y/M_x)]$  as calculated using Equations (5.10)–(5.12) or Fourier analysis. The deficiencies of the Fourier analysis are clearly visible; the profile close to on-resonance is too narrow, the zero-crossings are at incorrect frequencies and the phase is predicted to be linear, while the actual phase deviates slightly from exact linearity.

Figure 5.4 shows the frequency profiles as calculated using Equations (5.10)–(5.12) for on-resonance nutation angles of  $30^\circ$ ,  $90^\circ$  and  $180^\circ$ . Note that even though the frequency profile of a  $30^\circ$  pulse is uniform over a relatively large bandwidth, the profile of a  $180^\circ$  pulse achieves perfect inversion on-resonance only. The offset dependence of the transverse magnetization following a square excitation pulse necessitates a first-order phase correction of the spectrum as discussed in Section 1.7. This deviation from perfect behavior can also

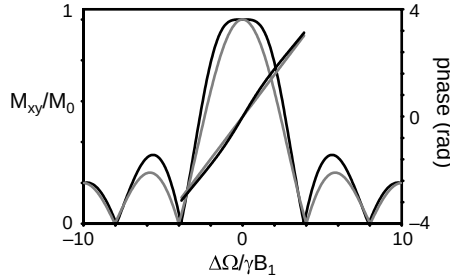


**Figure 5.3** Transverse magnetization  $M_{xy}$  and corresponding phase following a  $90^\circ$  square RF pulse calculated with rotation matrices (black lines) or Fourier transformation (gray lines).

be compensated within the experiment (for sufficiently strong RF pulses). For a strong  $90^\circ$  pulse, the approximation  $\mathbf{B}_e \approx \mathbf{B}_1$  holds and Equations (5.10)–(5.12) yield  $M_x/M_0 \approx -\Delta\Omega/\gamma B_1$ . Following an ideal  $90^\circ$  pulse and delay  $t$ , the transverse magnetization will precess in the transverse plane such that  $M_x/M_0 = -\sin(\Delta\Omega t) \approx -\Delta\Omega t$ . Equating these two expressions for  $M_x$  yields:

$$t \approx \frac{1}{\gamma B_1} = \frac{2T}{\pi} \quad (5.15)$$

Therefore, a real (finite) pulse can be approximated by an ideal (infinitely short) RF pulse followed by a free precession delay  $t$  given by Equation (5.15). Especially in multipulse sequences where phase accumulation of individual RF pulses can lead to significant artifacts, it is important to correct for phase evolution during RF pulses. Practical examples will be given in Chapter 6 for binomial RF pulses used for water suppression.

To obtain the desired nutation angle for a particular application, the pulse length of square RF pulses is normally incremented in subsequent experiments with a constant (high) transmitter power. This is because square pulses are normally used to achieve nonselective

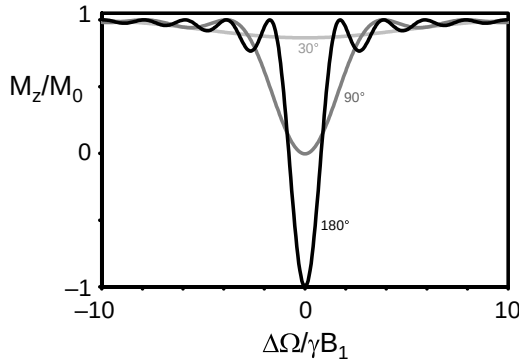


**Figure 5.4** Longitudinal magnetization  $M_z$  following a  $30^\circ$ ,  $90^\circ$  and  $180^\circ$  square RF pulse. Note that the desired rotations are only executed on-resonance.

excitation (or other manipulations). Setting the transmitter power to a relatively high level, allows short pulse lengths for a given nutation angle and consequently a wide bandwidth and uniform excitation. When the calibration experiment is properly executed (in a homogenous RF field), a curve as shown in Figure 5.5A will be obtained, which is described by  $\sin(\gamma B_1 T)$ . To avoid saturation by incomplete  $T_1$  relaxation, the repetition time TR of the experiment should be long compared to the longest  $T_1$  relaxation time,  $T_{1\max}$  (i.e.  $TR > 4-5T_{1\max}$ ). The highest signal corresponds to a  $90^\circ$  nutation angle, after which the signal reduces to zero and becomes maximum negative for  $180^\circ$  and  $270^\circ$  nutation angles, respectively. Obviously, the experiment needs to be performed on-resonance, to avoid undesirable off-resonance phase shifts (Figure 5.2). Furthermore, when the calibration experiment is performed off-resonance, the pulse length corresponding to the highest signal represents overestimation of the actual on-resonance  $90^\circ$  pulse length. Figure 5.5B shows the situation when the repetition time of the calibration experiment is too short ( $TR \sim T_1$ ). The curve can no longer be described by a simple sinusoidal function, but needs to be described by Equation (1.72). When the highest signal is taken as the  $90^\circ$

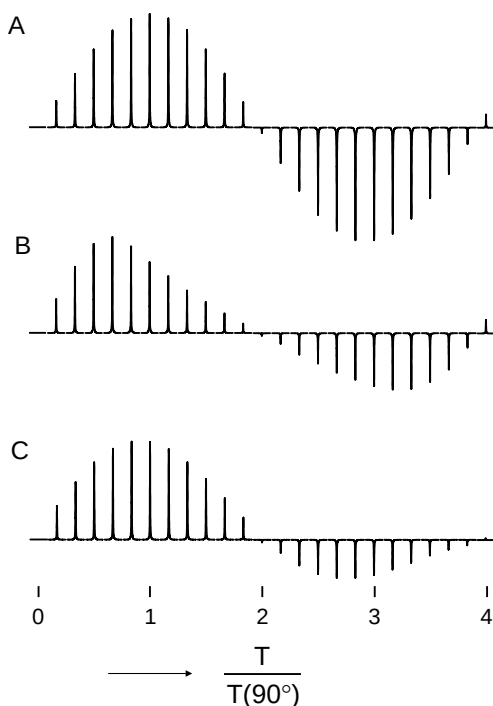


**Figure 5.5** Pulse length calibration of a square RF pulse. (A) On-resonance, under conditions of complete longitudinal relaxation, the transverse magnetization follows a sinusoidal modulation as a function of pulse length according to Equation (5.11). (B) Under conditions of rapid pulse repetition ( $TR \sim T_1$ ), the observed signal intensity becomes dependent upon  $T_1$ , TR and the nutation angle [according to Equation (1.72)]. (C) In the presence of  $B_1$  magnetic field inhomogeneity, the observed signal intensity results from a variety of different nutation angles making accurate pulse length calibration difficult.

nututation angle, a significant underestimation of the actual  $90^\circ$  pulse length results. In principle, the obtained calibration curve could still be used to retrieve the actual  $90^\circ$  nututation angle by fitting the curve with Equation (1.72). However, this requires an accurate knowledge of the  $T_1$  relaxation time, which is seldom true prior to a calibration experiment. In Chapter 4, it is described that knowledge of the nututation angles allows a fast and accurate determination of the  $T_1$  relaxation time. Note, that the pulse length corresponding to a  $180^\circ$  pulse is relatively insensitive to the repetition time making it, in principle, suitable for pulse calibration. However, in situations of significant  $B_1$  magnetic field inhomogeneity (as, for example, encountered with surface coils), the zero-crossing in the calibration curve corresponding to a  $180^\circ$  nututation angle may no longer be observed. Figure 5.5C shows the calibration curve in case the  $B_1$  magnetic field inhomogeneity is  $\pm 50\%$  of the nominal  $B_1$  field strength. It can be seen that especially for longer pulse lengths (corresponding to  $\geq 180^\circ$  nututation angles in a homogeneous  $B_1$  field),  $B_1$  magnetic field inhomogeneity results in severe signal loss. Even though the  $180^\circ$  nututation angle can still be determined from Figure 5.5C, it will be impossible in the presence of stronger  $B_1$  inhomogeneity. A solution to this problem is given by composite and adiabatic RF pulses as described in Sections 5.6–5.8.

### 5.3 Selective RF Pulses

For many applications selective excitation is required. In principle this can be achieved by prolonging the duration of a square RF pulse. However, even though most of the excited spins are within a selective frequency band, there is also significant excitation of spins off-resonance, due to the sinc-like excitation profile (see Figures 5.2–5.4). With the development of MRI and localized MRS methods came the demand for RF pulses with well-defined, selective frequency profiles. Here, the principles of frequency-selective RF pulses are described, as well as their design and optimization.

#### 5.3.1 Sinc Pulses

Even though Fourier transform theory does not accurately predict the off-resonance performance of RF pulses for nututation angles larger than  $30^\circ$ , it has nevertheless been used to design selective RF pulses. The ultimate frequency-selective excitation profile is a step function which, according to Fourier transform theory, can be achieved by a sinc-shaped RF pulse of duration  $T$  given by:

$$B_1 = B_{1\max} \frac{\sin(2n\pi t/T)}{(2n\pi t/T)} \quad \text{for } -T/2 \leq t \leq +T/2 \quad (5.16)$$

where  $B_1 = B_{1\max}$  for  $t = 0$ . Since this pulse would need infinite duration (due to the asymptotic decay of the sinc function), it is normally truncated at a zero-crossing making so-called ‘five-lobe’ ( $n = 3$ ) or ‘seven-lobe’ ( $n = 4$ ) sinc pulses. Figure 5.6A–C shows the frequency profiles of the transverse and longitudinal magnetization of a five-lobe sinc pulse with nututation angles of  $30^\circ$ ,  $90^\circ$  and  $180^\circ$ , respectively. Besides small oscillations arising from the necessary truncation of the RF pulse, a  $30^\circ$  sinc profile gives an approximately square frequency profile. The violation of the small nututation angle approximation of a  $90^\circ$  sinc pulse primarily results in a deviation from the ideal phase behavior which will be

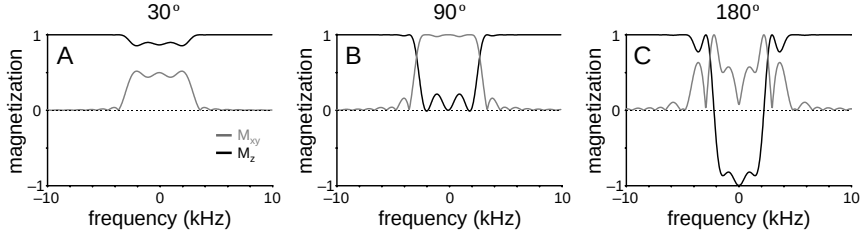


**Figure 5.6** Frequency profiles of a 1 ms 5-lobe sinc pulse for  $M_{xy}$  and  $M_z$ . The on-resonance nutation angle was calibrated as (A)  $30^\circ$ , (B)  $90^\circ$  and (C)  $180^\circ$ . Note that the  $180^\circ$  sinc inversion pulse generates a considerable amount of transverse magnetization due to the imperfect frequency inversion profile.

discussed below. Small amplitude effects can also be observed at the edges of the excitation profile. Because the small nutation angle approximation completely breaks down for the  $180^\circ$  sinc pulse, the profile deviates further from the ideal situation.

In Section 5.2 the frequency profile of a short, hard RF pulse was calculated together with the exact compensation for free precession during the pulse, given by Equation (5.15). For a shaped RF pulse this will be more complicated. In general, the frequency profile of a shaped RF pulse is calculated by segmenting the pulse in parts small enough such that the RF amplitude does not vary (i.e. the parts can be considered as hard pulses). In principle, the frequency profile can then be calculated by repeated multiplication of the rotation matrices given by Equations (5.5)–(5.7). While this is a common and attractive method to numerically evaluate the performance of RF pulses in the absence of relaxation, it leads to tedious calculations prone to errors when performed manually. As an alternative, the Bloch equations can be evaluated. For an arbitrary RF pulse of amplitude  $B_1(t)$  and phase  $\phi(t)$ , the Bloch equations in the rotating frame of reference (ignoring relaxation) are given by:

$$\frac{dM_x(t)}{dt} = -\Delta\Omega M_y(t) - \gamma B_1(t) \sin \phi(t) M_z(t) \quad (5.17)$$

$$\frac{dM_y(t)}{dt} = \Delta\Omega M_x(t) + \gamma B_1(t) \cos \phi(t) M_z(t) \quad (5.18)$$

$$\frac{dM_z(t)}{dt} = \gamma B_1(t) \sin \phi(t) M_x(t) - \gamma B_1(t) \cos \phi(t) M_y(t) \quad (5.19)$$

where  $\Delta\Omega = (\omega_0 - \omega)$ , the difference between the Larmor frequency  $\omega_0$  and the RF pulse frequency  $\omega$ . These Bloch equations can be evaluated exactly by numerical integration, but often the first two differential equations are separated from the third by the so-called small nutation angle approximation, which assumes that the RF pulse has negligible effect on the longitudinal magnetization, such that it can be considered constant and equal to the equilibrium magnetization, i.e.  $M_z(t) = M_0 = \text{constant}$ . In this case the first two Bloch equations can be combined to give:

$$\frac{dM_{xy}(t)}{dt} = i\Delta\Omega M_{xy}(t) + i\gamma B_1(t) e^{+i\phi(t)} M_0 \quad (5.20)$$

where  $M_{xy} = M_x + iM_y$  and  $i^2 = -1$ . The differential equation can be solved for pulse duration  $T$  to give:

$$M_{xy}(t) = i\gamma M_0 \int_{-T/2}^{+T/2} B_1(t) e^{+i\phi(t)} e^{+i\gamma \int_t^{+T/2} \Delta\Omega dt'} dt \quad (5.21)$$

or

$$M_{xy}(t) = i\gamma M_0 e^{+i\gamma \Delta\Omega T/2} \int_{-T/2}^{+T/2} B_1(t) e^{+i\phi(t)} e^{-i\gamma \Delta\Omega t} dt \quad (5.22)$$

From Equation (5.22) it can be seen that, within the small nutation angle approximation (i.e.  $M_z \approx M_0$ ), the frequency profile (or spectral density function) of the magnetization is the Fourier transformation of the applied RF pulse. Furthermore, for time-symmetric RF pulses there will be a linear phase shift of  $\gamma\Delta\Omega T/2$  across the frequency profile. Figure 5.7A shows the trajectories for magnetization during a 1 ms, 5-lobe sinc pulse (calibrated for a  $90^\circ$  nutation angle on-resonance) at frequency offsets  $\Delta\Omega$  of 0, 0.15 and 0.3 kHz. At the end of the RF pulse, spins at different frequency offsets  $\Delta\Omega$  have acquired different phases in accordance with Equation (5.22). Figure 5.7B shows a simulation of the transverse magnetization at the end of the RF pulse for all frequency offsets  $|\Delta\Omega| < 10$  kHz. Figure 5.7C demonstrates that the acquired phase is close to linear and can therefore be corrected by incorporating the second half of the RF pulse in the first half of the echo-time as shown in Figure 5.7D, which will lead to the refocused frequency profile shown in Figure 5.7E. When the RF pulse is executed in the presence of a magnetic field gradient (as for example required during slice selection), the acquired phase will become spatially dependent, leading to severe signal loss. Therefore, similar to the refocusing of a linear phase in the spectral domain (Figure 5.7C–E), the spatial phase need to be refocused by a refocusing gradient with an area that cancels the acquired phase during the RF pulse (Figure 5.7D). When working outside of the small nutation angle approximation, the refocusing gradient requires empirical optimization to ensure maximum signal recovery. The spin-echo sequence of Figure 5.7D therefore compensates for both spectral and spatial frequency evolution during the excitation RF pulse. By adding a frequency encoding magnetic field gradient in the direction of a slice selection, the sequence of Figure 5.7D can be used to obtain the spatial profile of the excitation pulse (Figure 5.7F).

Calibration of the nutation angle of sinc and other selective pulses normally differs from the procedure described for square RF pulses. Because sinc pulses are most often used for their frequency selective characteristics, it is not advisable to increment the pulse length during a calibration experiment since this would change the excitation bandwidth (i.e. the selectivity). Most commonly, the pulse length of a selective RF pulse is kept constant (such that the excitation bandwidth is constant), after which the transmitter power is incremented during the calibration experiment. For linear power amplifiers, the obtained calibration curve would be identical (in appearance) to those shown in Figure 5.5. However, the output of most power amplifiers is nonlinear and often their output is given in units of decibel. Figure 5.8. shows a typical calibration curve in which the transmitter power is incremented with 0.5 dB units. The highest signal represents a  $90^\circ$  excitation. The RF amplitude of an

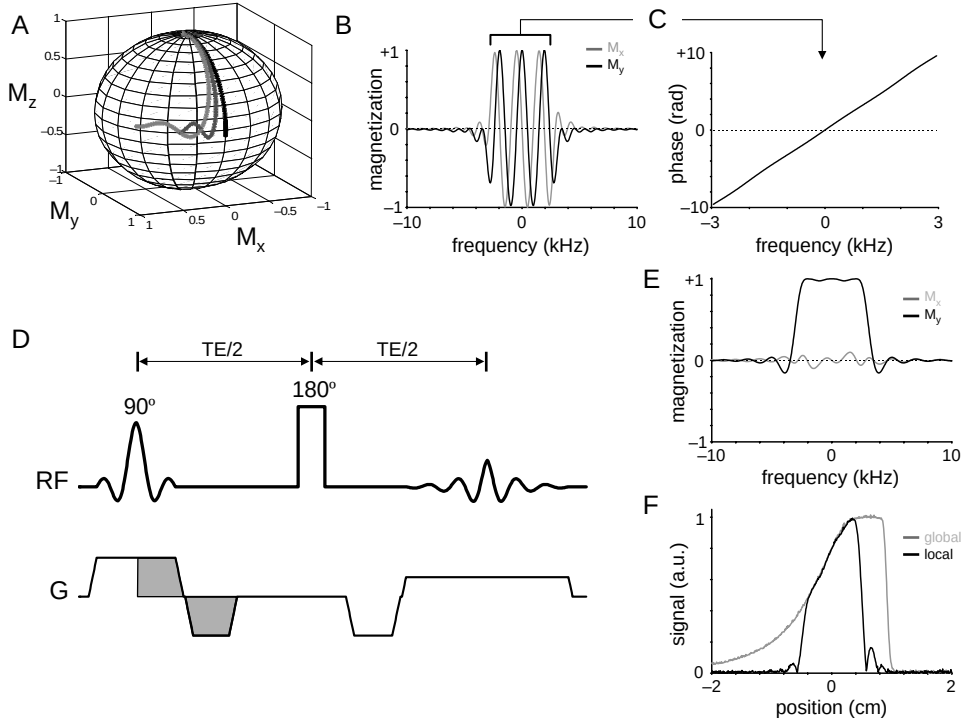


**Figure 5.7** Phase evolution during RF pulses. (A) 3D magnetization trajectories for three frequency offsets (0.0, 0.15 and 0.30 kHz) during a 1 ms 5-lobe sinc excitation pulse. (B) Transverse magnetization at different frequency offsets acquire a different phase at the end of the pulse. However, since (C) the phase is largely linear (phase slope = 3.256 rad kHz<sup>-1</sup>), it can be refocused by (D) including the second half of the sinc pulse in the first TE/2 period of a spin-echo sequence, resulting in (E) a phase-refocused frequency profile. When the sinc excitation pulse is used in conjunction with a magnetic field gradient for slice selection, the phase evolution during the pulse will lead to a spatial phase distribution and consequently signal loss. The application of a negative gradient following the pulse leads to complete phase refocusing, such that the sequence in (D) can be used to (F) visualize the slice selection profile. The global profile is obtained with a non-selective excitation pulse in the absence of slice selection gradients, whereas the local profile is obtained with a selective pulse as depicted in (D).

arbitrary calibration point is related to the RF amplitude required for a 90° nutation angle  $B_1(90^\circ)$  according to:

$$B_1 = B_1(90^\circ)e^{+\Delta\text{dB}/20} \quad (5.23)$$

where  $\Delta\text{dB}$  is the change in transmitter output in units of dB. For a +6 dB change, the nutation angle will be doubled, as can also be deduced from Figure 5.8 [there are 6 dB units between  $B_1(90^\circ)$  and  $B_1(180^\circ)$ ]. In many MRI applications the power for the 90° and 180° RF pulses in a spin echo are calibrated simultaneously by maximizing the echo intensity. In this case the calibration curve will be described by a  $\sin^3\theta$  dependence upon



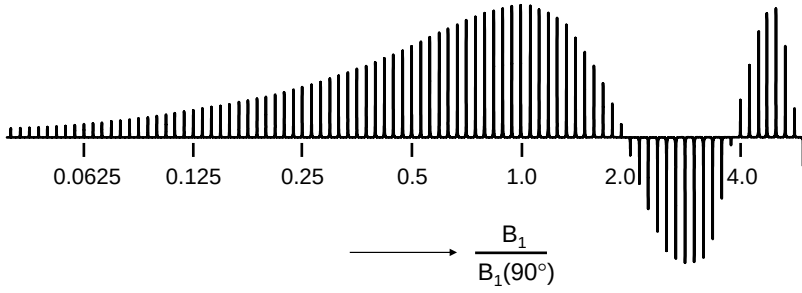


**Figure 5.8** RF  $B_1$  magnetic field calibration of an amplitude-modulated RF pulse (e.g. sinc). The observed signal intensity deviates from a sinusoidal dependence due to the nonlinear output of the RF amplifier.

the  $B_1$  magnetic field strength. When the  $90^\circ$  and  $180^\circ$  RF pulses consist of different pulse shapes, the appropriate power relation between the pulses needs to be taken into account during simultaneous calibration. This power relation can for instance be obtained from simulations based on the Bloch equations. Note that on most RF amplifiers the output is not absolutely zero at the lowest power setting (first calibration point in Figure 5.8), but is merely maximally attenuated. This point should be taken into account for more specialized pulses like DANTE (Section 5.5) and adiabatic pulses (Section 5.7) when zero power output is required.

### 5.3.2 Gaussian and Hermitian Pulses

An even simpler approach towards frequency-selective excitation is provided by a Gaussian RF pulse envelope, since the Fourier transformation of a Gaussian time-domain signal yields a Gaussian-shaped frequency-domain signal [1]. A Gaussian RF pulse is described by:

$$B_1(t) = B_{1\max} e^{-\beta(2t/T)^2} \quad \text{for } -T/2 \leq t \leq +T/2 \quad (5.24)$$

where  $\beta$  determines the truncation of the asymptotic Gaussian function, and is given by  $\beta = -\ln(B_1(T/2)/B_{1\max})$ . Figure 5.9 shows the inversion profile of a Gaussian RF pulse. An important parameter to describe RF pulses is the product of bandwidth  $\Delta\omega$  and pulse length  $T$  (sometimes referred to as the  $R$  value). The bandwidth  $\Delta\omega$  is being measured as the frequency width at half maximum of either  $M_{xy}$  or  $M_z$ , depending on whether an excitation or inversion pulse is considered. This product is constant for a given RF pulse and nutation angle. Table 5.1 shows the  $R$  values for (selective) RF pulses useful for *in vivo* NMR. Most of the reported RF pulses in Table 5.1 will be discussed throughout this chapter. The  $R$  value is a convenient parameter for calculations in many experiments. For example, suppose that the required gradient strength to select a 0.5 cm slice with a 1.0 ms  $90^\circ$  Gaussian pulse needs to be calculated. The combination of Equation (4.3) and the  $R$  value of Table 5.1 yields a direct answer, since the bandwidth of the pulse is:

$$\Delta\omega = \frac{R}{T} = 2710 \text{ Hz} \quad (5.25)$$

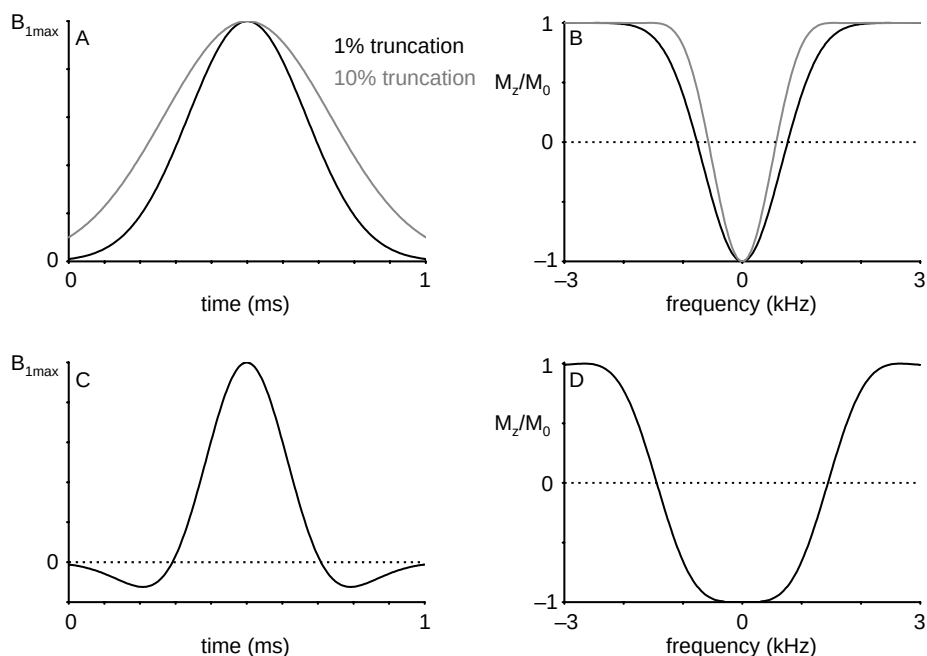


**Figure 5.9** (B, D) Frequency profiles of 1 ms (A) Gaussian and (C) Hermitian inversion RF pulses. Truncation of a Gaussian RF pulse leads to a narrower, Gaussian-shaped frequency inversion profile.

after which the gradient strength can be calculated according to:

$$G = \frac{\Delta\omega}{\gamma x} = 12.7 \text{ mT m}^{-1} \quad (5.26)$$

From this example it should be clear that a low R value is desirable when gradient strength is limited. However, when sufficient gradient strength is available pulses with higher R values are recommended, since they can minimize chemical shift artifacts in spectral localization sequences (i.e. a different spatial localization for resonances with different Larmor frequencies, see Chapter 6). A Gaussian pulse is also very popular for water suppression (see Chapter 6) again due to its relatively low R value in combination with reasonable frequency selectivity. In order to selectively excite a bandwidth of 250 Hz (i.e. the water resonance), 10.8 ms Gaussian pulses are required, whereas a sinc pulse would need a 23.9 ms pulse length. The longer pulse lengths required for sinc pulses would certainly lead to  $T_1$  and  $T_2$  relaxation during the pulse and therefore to incomplete water suppression.

Besides the R values, Table 5.1 also shows  $B_{1max}$  and  $B_{1rms}$  parameters.  $B_{1max}$  is defined as the required RF amplitude to generate a  $90^\circ$  nutation angle (unless specified otherwise) relative to a square RF pulse with the same pulse length. For example, a 125  $\mu\text{s}$  square RF pulse requires  $B_{1max} = 2.0$  kHz for a (on-resonance)  $90^\circ$  excitation. The RF amplitude of

**Table 5.1** Characteristics of amplitude-modulated RF pulses.

| Pulse                       | $\alpha$ | $B_{1\max}$ | $B_{1\text{rms}}$ | $R_{\text{BW}}^a$ | $R_{\text{TW}}^b$ |
|-----------------------------|----------|-------------|-------------------|-------------------|-------------------|
| Square                      | 90°      | 1.00        | 1.00              | 1.37              | 0.61              |
| Square                      | 180°     | 2.00        | 2.00              | 0.80              | 0.60              |
| Sinc                        | 90°      | 5.70        | 1.52              | 5.98              | 0.95              |
| Sinc                        | 180°     | 11.4        | 3.04              | 4.48              | 1.01              |
| Gauss, 1 %                  | 90°      | 2.43        | 1.00              | 2.71              | 1.86              |
| Gauss, 1 %                  | 180°     | 4.86        | 2.00              | 1.53              | 1.34              |
| Gauss, 10 %                 | 90°      | 1.77        | 1.00              | 2.00              | 1.14              |
| Gauss, 10 %                 | 180°     | 3.54        | 2.00              | 1.14              | 0.94              |
| Hermitian                   | 90°      | 5.08        | 1.14              | 5.43              | 2.56              |
| Hermitian                   | 180°     | 12.96       | 2.88              | 3.49              | 1.81              |
| Optimized sinc <sup>c</sup> | 180°     | 21.8        | 3.72              | 5.75              | 1.35              |
| SLR linear <sup>d</sup>     | 90°      | 6.15        | 1.38              | 6.08              | 1.69              |
| SLR linear <sup>d</sup>     | 90°      | 12.6        | 1.66              | 12.02             | 1.74              |
| SLR linear <sup>d</sup>     | 90°      | 19.7        | 1.88              | 17.99             | 1.68              |
| SLR refocused <sup>d</sup>  | 90°      | 6.66        | 1.61              | 6.86              | 1.38              |
| SLR refocused <sup>d</sup>  | 90°      | 12.0        | 2.03              | 12.80             | 1.46              |
| SLR refocused <sup>d</sup>  | 90°      | 17.4        | 2.27              | 18.74             | 1.49              |
| SLR <sup>d</sup>            | 180°     | 30.1        | 3.85              | 6.04              | 1.84              |
| SLR <sup>d</sup>            | 180°     | 74.0        | 5.05              | 12.26             | 1.90              |
| SLR <sup>d</sup>            | 180°     | 117         | 5.71              | 18.24             | 1.89              |

<sup>a</sup> $R_{\text{BW}}$  = bandwidth (Hz)  $\times$  pulse length (ms) measured at  $M_{xy}/M_0 = 0.50$  for 90° and  $M_z/M_0 = 0.0$  for 180° RF pulses.

<sup>b</sup> $R_{\text{TW}}$  = transition width (Hz)  $\times$  pulse length (ms) measured at  $0.05 \leq M_{xy}/M_0 \leq 0.95$  for 90° and  $-0.90 \leq M_z/M_0 \leq 0.90$  for 180° RF pulses.

<sup>c</sup>Data from Mao *et al.* [2].

<sup>d</sup>See Tables 5.2 and 5.3 for Fourier coefficients.

a 2.0 ms 90° sinc pulse can then easily be calculated as:

$$B_1 = \frac{125}{2000} \times \frac{5.98}{1.00} \times 2.0 \text{ kHz} = 0.748 \text{ kHz} \quad (5.27)$$

$B_{1\text{rms}}$  is defined as the root mean square RF amplitude required for a 90° nutation angle (unless specified otherwise). The square of  $B_{1\text{rms}}$  is directly proportional to the average power. From Table 5.1 it follows that a square pulse is optimal in terms of required peak power [ $\sim (B_{1\max})^2$ ] and average power [ $\sim (B_{1\text{rms}})^2$ ]. In other words, the price for improved selectivity is increased power requirements. It should further be noted that for symmetrical RF pulses  $B_{1\max}$  increases linearly with increasing R value (and hence the bandwidth).

Sinc and Gaussian shaped RF pulses were among the first shaped RF pulses simply because their performance could largely be predicted by Fourier transformation. Using arguments based on average Hamiltonian expansion, Warren [3] was able to predict the effects of arbitrarily shaped RF pulses. Based on fairly qualitative arguments following from the first two terms of the perturbation expansion, so-called Hermitian 90° and 180° RF pulses were proposed, which can be described by

$$B_1(t) = B_{1\max} [1 - 0.667(6t/T)^2] e^{-(6t/T)^2} \quad (5.28)$$

and

$$B_1(t) = B_{1\max} [1 - 0.956(6t/T)^2] e^{-(6t/T)^2} \quad (5.29)$$

respectively, with  $-T/2 \leq t \leq +T/2$ . Figure 5.9D shows simulations based on the Bloch equations for a  $180^\circ$  Hermitian RF pulse, given by Equation (5.29). The inversion profile of a  $180^\circ$  Hermitian RF pulse is similar to that of a Gaussian RF pulse. However, the R value is twice as large. The inversion profile of the  $180^\circ$  Hermitian RF pulse is a substantial improvement over the profile generated by a  $180^\circ$  sinc pulse (Figure 5.6C). The refocusing capabilities of  $180^\circ$  RF pulses, as required in spin-echo sequences will be discussed in Section 5.7.3.

### 5.3.3 Multifrequency RF Pulses

For many applications, like spectral editing, multi-volume localization and signal suppression, it may be desirable to utilize RF pulses that achieve a specific rotation at multiple, selective frequency bands. Figure 5.10 shows the principle for generating these so-called multifrequency RF pulses. An optimized SLR  $180^\circ$  pulse (see Section 5.4.1) inverts magnetization over a selective frequency range centered on the Larmor frequency (Figure 5.10A and B). The selective inversion band can be shifted ( $\Delta\Omega/2\pi$ ) Hz off-resonance (Figure 5.10C and D) by imposing a phase ramp  $\phi(t)$  during the RF pulse given by:

$$\phi(t) = \int_0^t \Delta\Omega dt = \Delta\Omega t \quad (5.30)$$

where  $T$  is the pulse length. A RF pulse that inverts simultaneously at both frequencies can be generated by adding the complex RF pulse shapes of Figure 5.10A and C, given the multifrequency pulse shown in Figure 5.10E. Note that the peak amplitude of the combined RF pulse is twice as high as the separate single-banded RF pulses. This can pose problems when more than two bands need to be selected as for instance in Hadamard encoding (see Chapter 7). While several solutions to this problem have been proposed [4], restrictions on available RF peak power have limited the applications of multifrequency RF pulses to low-order Hadamard matrices ( $n \leq 8$ ).

An assumption underlying the simple addition of two RF pulses as outlined in Figure 5.10 is that the rotations required to invert one frequency band do not affect the rotations that lead to the inversion of the other frequency band. This assumption is valid when the frequency bands are well separated as shown in Figure 5.10. However, when the frequency separation between the inversion bands gets smaller, the frequency profile quickly deteriorates. Figure 5.11A shows a double-frequency inversion pulse based on an optimized Shinnar–Le Roux inversion pulse, as will be detailed in Section 5.4. It follows that the inversion profile quickly deteriorates when the frequency separation is reduced. Note that even though the inversion profile for the longitudinal magnetization appears reasonable for frequency separation  $>4$  kHz, the pulse generates significantly more transverse magnetization. This puts higher demands on signal dephasing by magnetic field ‘crusher’ gradients.

Steffen *et al.* [5] have presented an elegant method to reduce the deterioration of multifrequency RF pulses applied at nearby frequencies. Essentially by tracking the phase evolution generated by pulse 1 at the frequency of pulse 2 and applying this as a correction, they were able to greatly improve the frequency profile of multifrequency RF pulses (Figure 5.11B). Similar improvements can be achieved by incorporating the positions of the frequency bands in a pulse optimization algorithm, as will be discussed next.

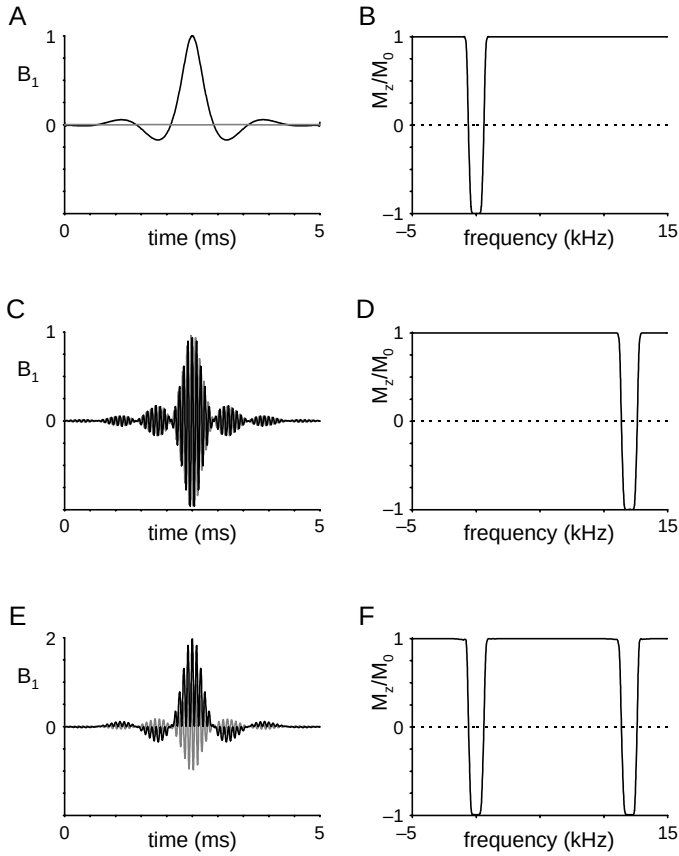


**Figure 5.10** Principle of multifrequency pulse generation. (A) A SLR-optimized inversion pulse produces (B) a frequency-selective inversion profile centered around 0 kHz. (C) In the presence of a linear phase ramp, the SLR-optimized inversion pulse becomes complex [i.e. having  $B_{1x}$  (black line) and  $B_{1y}$  (gray line) modulations] leading to (D) a frequency-shifted inversion profile. Complex addition of the pulses in (A) and (C) gives (E) a multifrequency RF pulse that produces (F) simultaneous selective inversion bands at two different frequencies. Note that the maximum  $B_1$  amplitude of the multifrequency pulse is twice as high as that of each single-frequency pulse.

## 5.4 Pulse Optimization

For a given RF pulse the Bloch equations can be solved to reveal the excitation or inversion profile [e.g. see Equations (5.17)–(5.19)]. While the calculation is often performed numerically, the ‘forward’ solution can be obtained in a straightforward manner. The ‘inverse’ problem, however, in which the RF pulse is calculated for a given frequency profile is much more difficult. For small nutation angles, the inverse problem was solved by taking the inverse Fourier transformation of the frequency profile, resulting in sinc and Gaussian RF pulses. But, as shown in Figure 5.6, this approach does not work well for larger nutation

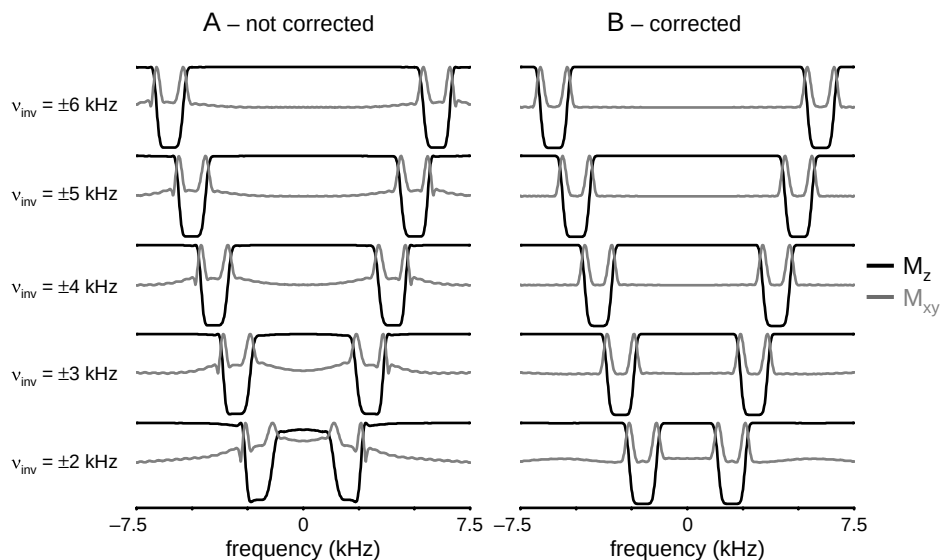


**Figure 5.11** Effect of reducing the inversion band separation of multifrequency RF pulses. (A) As the separation between selective inversion bands is reduced, the rotations required for one inversion band increasingly affect the rotations for the other inversion band, leading to a larger deviation of the ideal, single-frequency inversion profile. (B) By determining and correcting the mutual interactions of the two selective inversion profiles, the ideal inversion profiles can be maintained for much smaller frequency separations. See text for more details.

angles, due to nonlinearities in the Bloch equations that are not accounted for during a linear Fourier transform operation. In the large nutation angle regime, improved RF pulses can be obtained through iterative numerical optimization methods, like gradient descent algorithms, optimal control theory [6], simulated annealing and neural networks [7]. These optimization have resulted in a number of greatly improved RF pulses, like the optimized  $180^\circ$  pulse by Mao *et al.* [8] and self-refocused RF pulses by Geen *et al.* [9, 10]. However, most of these methods are computationally very intensive, have limited flexibility and can not guarantee the most optimal RF pulse (i.e. there is a risk of being ‘trapped’ in local, suboptimal minima).

#### 5.4.1 Shinnar–Le Roux Algorithm

The Shinnar–Le Roux (SLR) algorithm [11–16] allows the inverse problem to be solved directly by providing an invertible relationship between a complex RF pulse (with amplitude  $B_1(t)$  and phase  $\phi(t)$ ) and two complex exponentials  $A_N(z)$  and  $B_N(z)$ , where  $B_N(z)$  is an approximation to the desired magnetization profile and  $A_N(z)$  is calculated from  $B_N(z)$  to generate an unique, minimum power RF pulse, i.e.:

$$\{B_1(t), \phi(t)\} \xleftrightarrow{\text{SLR}} \{A_N(z), B_N(z)\} \quad (5.31)$$

This relationship makes RF pulse design equivalent to the design of two polynomials describing the frequency-domain response. This is a routine problem encountered and solved in finite impulse response (FIR) digital filter design.

An extensive treatment of the SLR algorithm is given by Pauly *et al.* [15] and others [16, 17]. Here a brief, qualitative overview will be given followed by several examples of useful RF pulses designed with the SLR algorithm. Figure 5.12 shows a flowchart of the steps involved in calculating an optimal RF pulse for a given frequency profile. The

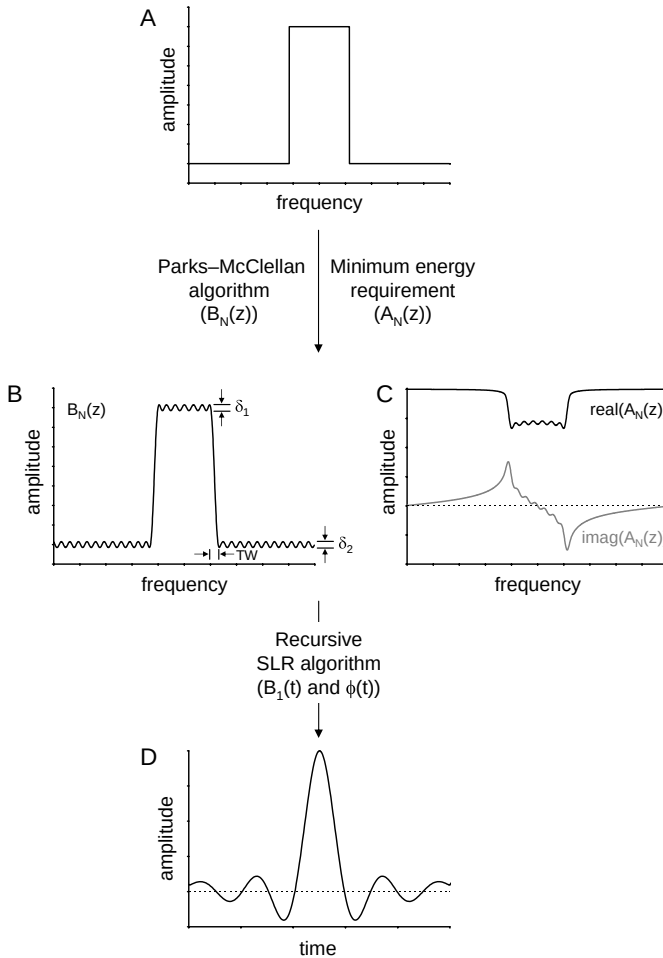


**Figure 5.12** Flowchart for the SLR pulse optimization algorithm. Following the definition of (A) a desired frequency profile, the required (B)  $B_N$  and (C)  $A_N$  polynomials are generated with the Parks-McClellan algorithm with considerations to a minimum energy requirement. The amount of ripples inside and outside the frequency profile ( $\delta_1$  and  $\delta_2$ , respectively) and the transition width ( $TW$ ) are the primary design parameters. (D) Following the construction of the appropriate polynomials, the SLR algorithm recursively calculates the corresponding RF pulse parameters, amplitude  $B_1(t)$  and phase  $\phi(t)$ .

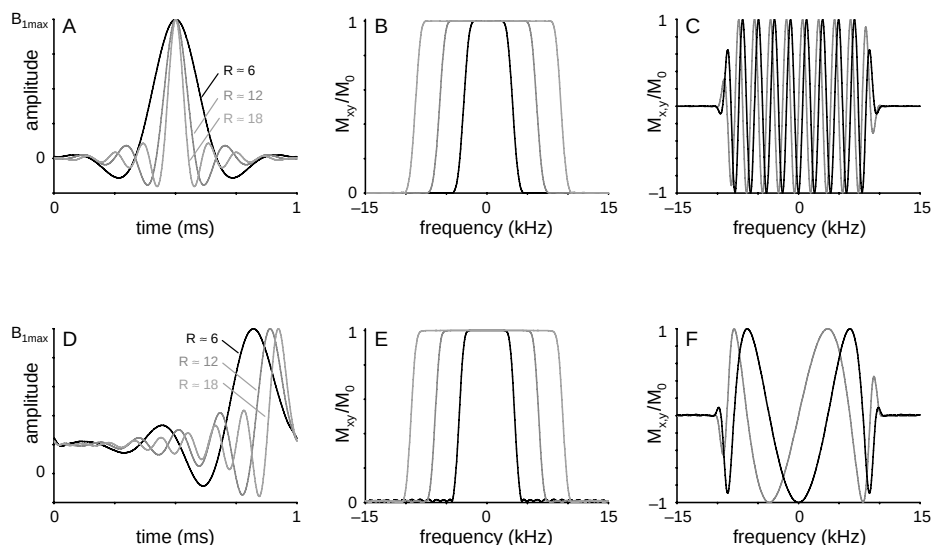


**Figure 5.13** (A) Linear phase and (D) minimum phase excitation pulses optimized with the SLR algorithm. (B, E) Absolute valued  $M_{xy}$  and (C, F) phase-sensitive  $M_x$  and  $M_y$  frequency profiles for (B, C) linear phase and (E, F) minimum phase RF pulses. Only the RF pulses with the largest R value of 18 are shown in (C, F). Table 5.1 summarizes the pulse characteristics, whereas Table 5.2 shows the Fourier coefficients required to reconstruct the RF pulses.

first step is the definition of an ideal frequency profile (Figure 5.12A). However, since this profile can not be achieved with a finite-length RF pulse, it needs to be converted to a more realistic profile with a finite transition width and ripples in the pass and stop bands. Pauly *et al.* [15] have shown that an optimal frequency profile can be obtained, expressed as a polynomial  $B_N$ , through the Parks-McClellan algorithm used in FIR filter design. The algorithm ensures the best filter design (i.e. narrowest transition bandwidth) given the pass- and stop-band ripples. Since the algorithm generates constant ripples, the corresponding pulses are also referred to as being of an equi-ripple design. To proceed with the SLR algorithm a second polynomial  $A_N$  is required. For a given frequency profile and  $B_N$  polynomial there is an infinite number of possibilities for the  $A_N$  polynomial. However, Le Roux [15] showed that the minimum phase polynomial  $A_N$  yields the pulse that has the lowest RF power deposition, and is therefore generally desired. The minimum phase  $A_N$  polynomial can be calculated directly from the  $B_N$  polynomial.

Following some additional inputs, like the phase of the frequency profile (linear, minimum or maximum phase), the SLR algorithm proceeds by recursively calculating the RF pulse amplitude  $B_1$  and phase  $\phi$  from the polynomials  $A_N$  and  $B_N$ .

Figures 5.13 and 5.14 show several examples of SLR pulses calculated using the Matpulse program described by Matson [16] and available on-line at the Metabolic Magnetic Resonance Research and Computing Center of the University of Pennsylvania. Figure 5.13A shows linear phase frequency-selective excitation pulses with R values of circa 6, 12 and 18, together with their excitation profile (Figure 5.13B). Table 5.1 summarizes their performance in terms of maximum and mean RF amplitude, R value and selectivity. The



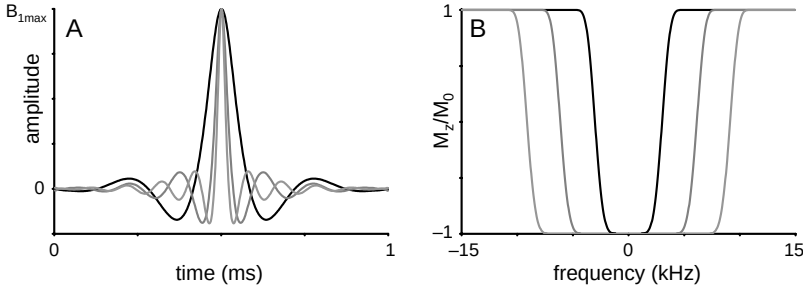


**Figure 5.14** (A) Inversion pulses optimized with the SLR algorithm and (B) the corresponding frequency inversion profiles. Table 5.1 summarizes the pulse characteristics, whereas Table 5.3 shows the Fourier coefficients required to reconstruct the RF pulses.

selectivity is defined as the pass bandwidth ( $M_{xy}/M_0 > 0.50$  for excitation or  $M_z/M_0 < -0.0$  for inversion) divided by the transition width ( $0.05 < M_{xy}/M_0 < 0.95$  for excitation and  $-0.90 < M_z/M_0 < 0.90$  for inversion). A higher selectivity is synonymous with a frequency profile that approximates the ideal step-function frequency profile (as shown in Figure 5.12A). It follows that excitation pulses optimized with the SLR algorithm display greatly improved frequency profiles over conventional shaped RF pulses. Furthermore, the optimization procedure readily allows an increase of the intrinsic excitation bandwidth (accompanied by an increase in peak RF amplitude), thereby greatly improving the selectivity of the frequency profile. When the linear phase across the profile is undesirable (Figure 5.13C), it is possible to design minimum-phase excitation pulses (Figure 5.13D). While their excitation frequency profile is similar to the linear-phase pulses (Figure 5.13E), the phase across the frequency profile is minimal (Figure 5.13F). Through a simulated annealing optimization algorithm Geen *et al.* [9, 10] have designed inherently refocused RF pulses under the acronym BURP (band-selective pulses with uniform response and pure phase) with a residual phase close to zero across the excitation profile, at the expense of a slight increase of signal excitation outside the pass band. Similar minimum-phase RF pulses have been used to achieve ultra-short echo-times ( $TE = 1$  ms) in localized MRS [18]. Note that maximum-phase excitation pulses can be obtained through time-reversal of minimum-phase pulses. Maximum-phase pulses can be useful for applications where signal dephasing is required, as for example encountered in outer volume suppression (see Chapter 7).

Figure 5.14A shows SLR-optimized inversion pulses together with their frequency-selective inversion profile. Since the inversion pulses do not rely on the small nutation approximation, their frequency profile is greatly improved with uniform inversion in the passband and negligible signal perturbation in the stopband. Table 5.1 summarizes the characteristics of the pulses shown in Figure 5.14A. While the pulses shown in Figures 5.13 and 5.14 can be generated through the Matpulse program [16], Tables 5.2 and 5.3 express the pulse shapes as coefficients of a limited Fourier series. The corresponding RF pulse shape (normalized to 1.0 for the maximum amplitude) can be calculated according to:

$$f_B(t) = A_0 + \sum_{n=1}^{n_{\max}} \left[ A_n \cos\left(\frac{2\pi n t}{T}\right) + B_n \sin\left(\frac{2\pi n t}{T}\right) \right] \quad (5.32)$$

**Table 5.2** Fourier coefficients for linear and refocused (minimum) phase excitation pulses obtained with the Shinner-Le Roux (SLR) pulse optimization algorithm.<sup>a,b</sup>

| n  | SLR 90° - linear <sup>c</sup> |                |                |                |                |                | SLR 90° - refocused |                |                |                |                |                |
|----|-------------------------------|----------------|----------------|----------------|----------------|----------------|---------------------|----------------|----------------|----------------|----------------|----------------|
|    | R = 6                         |                | R = 12         |                | R = 18         |                | R = 6               |                | R = 12         |                | R = 18         |                |
|    | A <sub>n</sub>                | B <sub>n</sub> | A <sub>n</sub> | B <sub>n</sub> | A <sub>n</sub> | B <sub>n</sub> | A <sub>n</sub>      | B <sub>n</sub> | A <sub>n</sub> | B <sub>n</sub> | A <sub>n</sub> | B <sub>n</sub> |
| 0  | 0.16248152                    | 0.07926787     | 0.05078526     | 0.15016398     | 0.00000000     | 0.08315260     | 0.00000000          | 0.00000000     | 0.05736874     | 0.00000000     | 0.10733739     | 0.04101031     |
| 1  | -0.33473283                   | -0.15568177    | -0.10383633    | 0.19305354     | -0.23411937    | 0.14400077     | -0.08421706         | 0.14400077     | 0.08541360     | -0.0763002     | 0.04994930     | -0.10503248    |
| 2  | 0.31861783                    | 0.15832894     | 0.10238266     | -0.11611928    | -0.29165608    | -0.02087969    | -0.16994331         | -0.16994331    | 0.00316519     | -0.11754264    | -0.05017866    | -0.06913492    |
| 3  | -0.13415863                   | -0.16016458    | -0.10380703    | -0.20334391    | 0.11054030     | 0.02688607     | 0.0352382           | 0.0352382      | 0.00994613     | -0.09994613    | 0.00610658     | 0.1040452      |
| 4  | 0.02225950                    | 0.16450203     | 0.10432680     | 0.01999193     | 0.02688607     | -0.17436244    | 0.04634288          | -0.17436244    | 0.0023186      | 0.0023186      | 0.007054397    | 0.04444313     |
| 5  | -0.00511679                   | -0.16193649    | -0.10506775    | 0.00407566     | 0.0032382      | 0.03958135     | 0.03958135          | 0.03958135     | -0.00315922    | -0.00315922    | 0.00727440     | -0.00176137    |
| 6  | 0.00156740                    | 0.08371448     | 0.10762871     | -0.0008750     | -0.0024942     | -0.00175806    | -0.00175806         | -0.00175806    | 0.00162391     | 0.00162391     | 0.00242586     | 0.00133322     |
| 7  | -0.00088433                   | -0.01839556    | -0.11074206    | -0.00084618    | -0.00367055    | 0.00103345     | 0.00103345          | 0.00103345     | 0.00161452     | 0.00161452     | 0.00025175     | -0.00044629    |
| 8  | 0.00013945                    | 0.00737875     | 0.11078835     | -0.00056449    | -0.00233207    | 0.00083545     | -0.00083545         | -0.00083545    | 0.00053659     | 0.00053659     | -0.00136098    | -0.00086665    |
| 9  | -0.00008296                   | -0.00405617    | -0.06001204    | 0.00022508     | -0.00187182    | 0.00231749     | 0.00231749          | 0.00231749     | -0.0002341     | -0.0002341     | -0.00058156    | -0.00114407    |
| 10 | 0.00005558                    | 0.00266805     | 0.01607527     | 0.00048078     | -0.00175806    | -0.00083545    | -0.00083545         | -0.00083545    | 0.00065681     | 0.00065681     | -0.00089172    | -0.00086665    |
| 11 | 0.00006721                    | -0.00158901    | -0.00774033    | 0.00056717     | -0.00156218    | -0.00103345    | -0.00103345         | -0.00103345    | 0.000246314    | 0.000246314    | -0.00099439    | -0.00061096    |
| 12 | 0.00010308                    | 0.00105189     | 0.00487509     | 0.00061858     | -0.00136616    | -0.00117903    | -0.00117903         | -0.00117903    | 0.00131397     | 0.00131397     | 0.00025175     | -0.00044629    |
| 13 | 0.00012230                    | -0.00065938    | -0.00348665    | 0.00065605     | -0.00119940    | 0.00103345     | 0.00103345          | 0.00103345     | 0.00053659     | 0.00053659     | -0.00136098    | -0.00086665    |
| 14 | 0.00014492                    | 0.00036897     | 0.00243179     | 0.00066749     | -0.00103345    | -0.00083545    | -0.00083545         | -0.00083545    | 0.00065681     | 0.00065681     | -0.00058156    | -0.00114407    |
| 15 | 0.00015219                    | -0.00026906    | -0.00180295    | 0.00067343     | -0.00093827    | -0.00044511    | -0.00044511         | -0.00044511    | 0.0002341      | 0.0002341      | -0.00089172    | -0.00086665    |
| 16 | 0.00016416                    | 0.00008059     | 0.00133012     | 0.00067005     | -0.00083584    | -0.00026498    | -0.00026498         | -0.00026498    | 0.00058156     | 0.00058156     | -0.00136098    | -0.00086665    |
| 17 | 0.00017416                    | -0.00011846    | -0.00097056    | 0.00065389     | -0.00074915    | -0.00026771    | -0.00026771         | -0.00026771    | 0.0002341      | 0.0002341      | -0.00089172    | -0.00086665    |
| 18 | 0.00018167                    | -0.00003266    | 0.00075516     | 0.00064013     | -0.00067090    | -0.00025640    | -0.00025640         | -0.00025640    | 0.00058156     | 0.00058156     | -0.00136098    | -0.00086665    |
| 19 | 0.00018828                    | -0.00008225    | -0.00050716    | 0.00062404     | -0.00060449    | -0.00021260    | -0.00021260         | -0.00021260    | 0.00058156     | 0.00058156     | -0.00136098    | -0.00086665    |
| 20 | 0.00019865                    | -0.00005674    | 0.00042757     | 0.00060931     | -0.00054598    | -0.00018750    | -0.00018750         | -0.00018750    | 0.00058156     | 0.00058156     | -0.00136098    | -0.00086665    |

<sup>a</sup> The pulses can be generated by substituting the Fourier coefficients in Eq. [5.32].

<sup>b</sup> The R-value is defined as the product of the excitation bandwidth at half maximum and the pulse length.

<sup>c</sup> For linear phase RF pulses all coefficients B<sub>n</sub> are zero.

**Table 5.3** Fourier coefficients for frequency-selective inversion pulses obtained with the SLR pulse optimization algorithm.<sup>a-c</sup>

| SLR 180° |                |    |                |      |                |    |                |      |                |    |                |
|----------|----------------|----|----------------|------|----------------|----|----------------|------|----------------|----|----------------|
| R=6      |                |    |                | R=12 |                |    |                | R=18 |                |    |                |
| n        | A <sub>n</sub> | n  | A <sub>n</sub> | n    | A <sub>n</sub> | n  | A <sub>n</sub> | n    | A <sub>n</sub> | n  | A <sub>n</sub> |
| 0        | 0.06635181     | 0  | 0.02702691     | 0    | 0.01714172     | 0  | 0.01714172     | 0    | 0.01714172     | 0  | 0.01714172     |
| 1        | -0.13458090    | 1  | -0.05390668    | 21   | -0.00855701    | 1  | -0.03421674    | 21   | -0.01895516    | 41 | -0.00247516    |
| 2        | 0.15170590     | 2  | 0.05438521     | 22   | 0.00703153     | 2  | 0.03435725     | 22   | 0.01695494     | 42 | 0.00232921     |
| 3        | -0.19982295    | 3  | -0.05456922    | 23   | -0.00643979    | 3  | -0.03439342    | 23   | -0.01496220    | 43 | -0.00203528    |
| 4        | 0.17146113     | 4  | 0.05492238     | 24   | 0.00518968     | 4  | 0.03459552     | 24   | 0.01349978     | 44 | 0.00191889     |
| 5        | -0.09676891    | 5  | -0.05326782    | 25   | -0.00483605    | 5  | -0.03472722    | 25   | -0.01195154    | 45 | -0.00167024    |
| 6        | 0.05893062     | 6  | 0.07345793     | 26   | 0.00379127     | 6  | 0.03483642     | 26   | 0.01086741     | 46 | 0.00157515     |
| 7        | -0.03770459    | 7  | -0.10515570    | 27   | -0.00362445    | 7  | -0.03479243    | 27   | -0.00964598    | 47 | -0.00136829    |
| 8        | 0.02576376     | 8  | 0.09576095     | 28   | 0.00274458     | 8  | 0.03112749     | 28   | 0.00882870     | 48 | 0.00129420     |
| 9        | -0.01748007    | 9  | -0.07541780    | 29   | -0.00271675    | 9  | -0.03588703    | 29   | -0.00784569    | 49 | -0.00111994    |
| 10       | 0.01243958     | 10 | 0.05969441     | 30   | 0.00195887     | 10 | 0.06241820     | 30   | 0.00722680     | 50 | 0.00105849     |
| 11       | -0.00844882    | 11 | -0.04802775    | 31   | -0.00204281    | 11 | -0.07145163    | 31   | -0.00643001    | 51 | -0.00091635    |
| 12       | 0.00602830     | 12 | 0.03864893     | 32   | 0.00138194     | 12 | 0.06448437     | 32   | 0.00596445     | 52 | 0.00086838     |
| 13       | -0.00396913    | 13 | -0.03195968    | 33   | -0.00155051    | 13 | -0.05645268    | 33   | -0.00531325    | 53 | -0.00075383    |
| 14       | 0.00289058     | 14 | 0.02610504     | 34   | 0.00096498     | 14 | 0.04885679     | 34   | 0.00495710     | 54 | 0.00070878     |
| 15       | -0.00181555    | 15 | -0.02211101    | 35   | -0.00119024    | 15 | -0.04219754    | 35   | -0.00440550    | 55 | -0.00061974    |
| 16       | 0.00141186     | 16 | 0.01823381     | 36   | 0.00065040     | 16 | 0.03665936     | 36   | 0.00411548     | 56 | 0.00057952     |
| 17       | -0.00079600    | 17 | -0.01576166    | 37   | -0.00092624    | 17 | -0.03179856    | 37   | -0.00364448    | 57 | -0.00050937    |
| 18       | 0.00073058     | 18 | 0.01304492     | 38   | 0.00042258     | 18 | 0.02789577     | 38   | 0.00341476     | 58 | 0.00047143     |
| 19       | -0.00030914    | 19 | -0.01149885    | 39   | -0.00072962    | 19 | -0.02435225    | 39   | -0.00300713    | 59 | -0.00042235    |
| 20       | 0.00040858     | 20 | 0.00950882     | 40   | 0.00025909     | 20 | 0.02158768     | 40   | 0.00282545     | 60 | 0.00038187     |

<sup>a</sup>The pulses can be generated by substituting the Fourier coefficients in Equation (5.32).

<sup>b</sup>The R value is defined as the product of the inversion bandwidth at  $M_z = 0$  and the pulse length.

<sup>c</sup>The coefficients  $B_n$  are zero for all RF pulses.

where  $f_B(t)$  is the RF amplitude modulation function,  $A_n$  and  $B_n$  are the Fourier coefficients and  $0 \leq t \leq T$ . The SLR algorithm has been used and expanded to design low-power RF pulses [19–21], multifrequency RF pulses [22] and spectral-spatial RF pulses [23].

## 5.5 DANTE RF Pulses

For a given amplitude-modulated RF pulse, the pulse length, bandwidth and pulse power are inherently related to each other. Therefore, to achieve very selective excitation, as for example is needed in water suppression, long pulse lengths are required and in order to retain a constant nutation angle, the RF amplitude should be decreased. This can cause deviations from the desired excitation profile when the pulse shape is not properly executed as a result of nonlinearity and limited dynamic range in the low power range of the RF amplifier. An alternative for long, low power RF pulses to achieve selective excitation is the so-called DANTE (delays alternating with nutation for tailored excitation) pulse sequence. DANTE consists of a series of short, high power (i.e. ‘hard’) RF pulses interleaved with delays [24–26]. Figure 5.15 shows a DANTE sequence consisting of  $n$  RF pulses of individual duration  $\tau$ , interleaved by delays of individual duration  $\Delta t$ . The total on-resonance nutation angle is simply given by the sum of the nutation angles generated by the  $n$  individual RF pulses. However, off-resonance the magnetization acquires a phase shift  $\Delta\phi = \Delta\Omega(\tau + \Delta t)$ , where  $\Delta\Omega/(2\pi)$  is the frequency offset in Hz during each of the pulse segments  $\tau$  and delays  $\Delta t$ . This is only valid under the assumption of a linear response of the RF pulse (i.e. small nutation angle).

Figure 5.15C–F shows the trajectory of magnetization during a DANTE pulse sequence (Figure 5.15A) at frequency offsets  $\Delta\Omega/(2\pi) = 0.0, 0.1, 1.0$  and  $10.0$  kHz. Although the required RF amplitudes for the DANTE pulse and the parent pulse (without the delays  $\Delta t$ ) are identical, the excitation bandwidth of a DANTE pulse is proportional to  $(n\tau + (n - 1)\Delta t)^{-1}$  while the bandwidth of the parent pulse is proportional to  $(n\tau)^{-1}$ . The frequency profile of a DANTE pulse is identical to the parent pulse, except for the fact that the bandwidth is scaled by the delays  $\Delta t$ . Furthermore, when  $\Delta\Omega/(2\pi) = (\tau + \Delta t)^{-1}$ , the acquired phase shift is  $2\pi$  and another excitation frequency band is defined (Figure 5.15F). The separation between two excitation bandwidths equals  $(\tau + \Delta t)^{-1}$ . Figure 5.15B shows a simulation of the frequency profile of the DANTE pulse shown in Figure 5.15A. Besides the excitation profile of the combined  $n$  pulses (of duration  $\tau$ ) and the repeated excitation, the frequency profile is complicated due to the behavior of the individual  $n$  pulses. The frequency profile (or spectral density function) is given by the convolution of the individual pulses and the combined pulses. The spectral density function can analytically be described by [27,28]:

$$M_{xy}(\Delta\Omega) = \gamma M_0 \left( \frac{\sin(\Delta\Omega\tau/2)}{\Delta\Omega\tau/2} \right) \left( \frac{\sin(\Delta\Omega T_{\text{tot}}/2)}{\sin(\Delta\Omega T_{\text{tot}}/(2n))} \right) \int_{-\infty}^{+\infty} B_1(t) e^{-i\phi(t)} e^{-i\Delta\Omega t} dt \quad (5.33)$$

where the first term represents the Fourier transform of the individual square RF pulses of duration  $\tau$ . The last term represents the Fourier transformation of the overall shape of the DANTE pulse and the middle term determines the positions of the multiple excitation

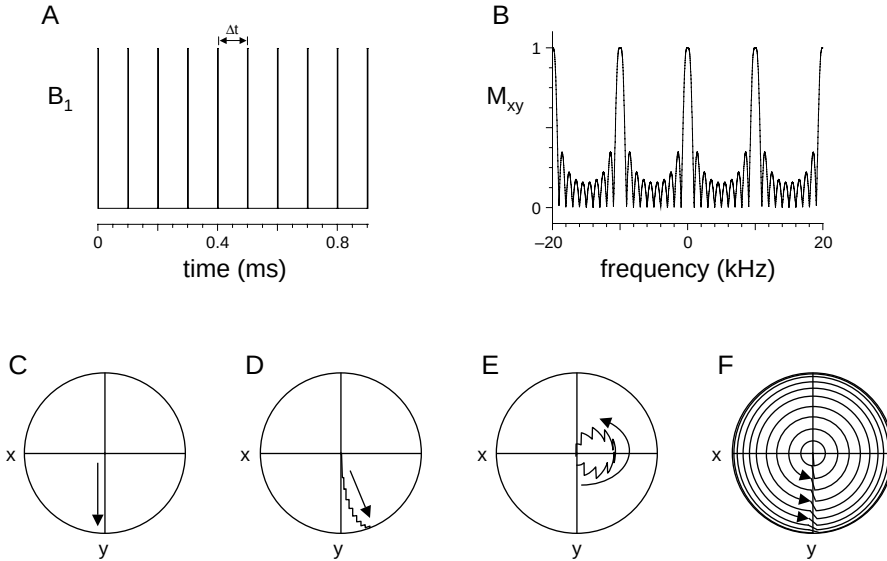


**Figure 5.15** (A) A 902  $\mu$ s DANTE pulse consisting of ten 2  $\mu$ s square RF pulses interleaved by 98  $\mu$ s delays. (B) Excitation profile for a 90° DANTE pulse. Rotations of the thermal equilibrium magnetization vector as projected onto the x-y plane during a DANTE pulse are shown for frequency offsets of (C) 0.0 kHz, (D) 0.1 kHz, (E) 1.0 kHz and (F) 10.0 kHz.

bands. Equation (5.33) predicts frequency bands at frequencies  $n(\tau + \Delta t)^{-1}$  ( $n = \pm 0, 1, 2, \dots$ ). However, the frequency profile is also influenced by the individual short pulses. When  $\tau$  is short enough, all frequency bands are of equal amplitude and phase. However, when  $\tau$  gets longer, the outer frequency bands are degraded according to the sinc function in Equation (5.33).

DANTE pulses are not limited to series of equal amplitude pulses. According to Equation (5.33) the frequency profile of the individual bands is determined by the Fourier transformation of the total pulse envelope. Therefore, by segmenting shaped pulses like sinc, Gaussian or any other pulse, as shown in Figure 5.16 the frequency profile can be made more selective.

## 5.6 Composite RF Pulses

For all RF pulses discussed in the previous paragraphs, the on-resonance nutation angle is given by Equation (5.1) and is linearly dependent of the RF amplitude  $B_1$ . When the RF field generated by the coil is spatially inhomogeneous, this immediately translates to the fact that a range of different nutation angles will be produced. Besides the signal loss, the variation in nutation angles can also lead to artifacts when accurate nutation angles are required. For this purpose, so-called composite RF pulses have been developed which have a certain degree of insensitivity to the RF amplitude [29–36]. Composite pulses are, as the name suggests, composed of several closely spaced RF pulses with variable nutation

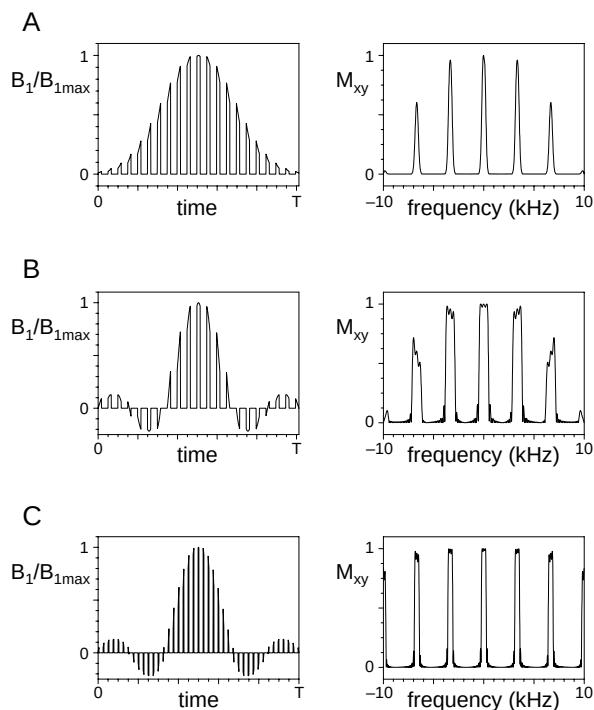


**Figure 5.16** Amplitude modulation and excitation profile of several shaped DANTE RF pulses. (A) 6.1 ms Gaussian shaped and (B) 6.1 ms sinc shaped DANTE pulses consisting of 21 RF pulses of 100  $\mu$ s each, interleaved by 200  $\mu$ s delays. (C) 12.05 ms sinc shaped DANTE RF pulse consisting of 41 RF pulses of 50  $\mu$ s each, interleaved by 250  $\mu$ s delays.

angles and/or phases. The overall effect of a composite RF pulse is identical to a single hard pulse, except that it is less sensitive to imperfections. The first composite pulses were developed intuitively (assisted by computer simulations) by considering the trajectory of magnetization at different RF amplitudes. The degree of compensation for imperfections was therefore determined by the limited human ability to visualize 3D rotations. As a consequence, many of the earlier developed composite pulses compensated for one imperfection (e.g. variation in RF amplitude), while the performance towards another parameter (e.g. resonance offset) degraded. Nevertheless, some of these efforts provided composite pulses with dual compensation. For example, the  $180^\circ$  composite pulse  $90^\circ_x 180^\circ_y 90^\circ_x$  is widely used for wideband inversion with insensitivity to variations in RF amplitude and resonance offset. Figure 5.17A shows a simulation of the longitudinal magnetization after this composite pulse as a function of the RF amplitude, while Figure 5.17B gives the longitudinal magnetization as a function of frequency offset. In both figures, the performance of a conventional  $180^\circ$  pulse is also shown (gray line). Even though the composite pulse is twice as long as the conventional  $180^\circ$  pulse, it inverts a much wider bandwidth with more insensitivity to RF amplitude variations. Figure 5.17D–F shows trajectories of the magnetization (initially along  $z$ ) as a function of the RF amplitude. When the RF amplitude

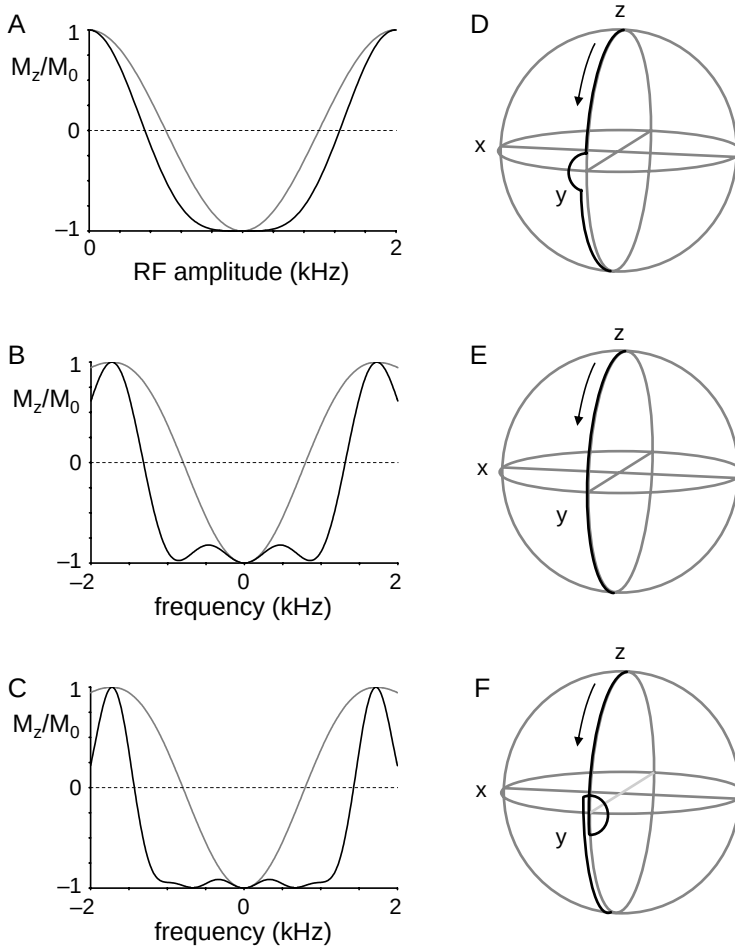


**Figure 5.17** Performance of  $180^\circ$  square and composite RF pulses. (A) On-resonance inversion efficiency as function of the RF field strength for square (gray line) and composite  $90^\circ_x 180^\circ_y 90^\circ_x$  (black line) RF pulses. Frequency inversion profiles for (B) square (gray line) and composite  $90^\circ_x 180^\circ_y 90^\circ_x$  (black line) RF pulses and for (C) square (gray line) and composite  $90^\circ_x 180^\circ_{-x} 270^\circ_x$  (black line) RF pulses. For all pulses in (B) and (C) the RF field strength was calibrated to give complete inversion on-resonance. (D–F) Rotations during the composite  $90^\circ_x 180^\circ_y 90^\circ_x$  pulse when the RF amplitude is (D) 10 % lower than, (E) at and (F) 10 % higher than the nominal RF field strength.

is 10 % below the nominal value for  $90^\circ$  and  $180^\circ$  pulses, the composite pulse is given by  $81^\circ_x 162^\circ_y 81^\circ_x$ . Although the first pulse does not properly excite the magnetization onto the transverse plane (Figure 5.17D), the following  $162^\circ_{+y}$  pulse rotates it through the transverse plane, after which the last  $81^\circ_{+x}$  pulse will rotate the magnetization close to the  $-z$  axis. When the RF amplitude is too high (Figure 5.17F), the opposite will happen, the first pulse will nutate the magnetization too far, after which the second pulse will reverse the

imperfection so that the final nutation angle will be close to the  $-z$  axis. The overall nutation angle of the composite pulse is therefore largely determined by the outer two pulses, whereas the inner pulse provides the compensation for imperfections. Similar arguments can also be used to explain the compensation for magnetization vectors at different frequency offsets. Figure 5.17C shows the off-resonance performance of another important composite pulse,  $90^\circ_x 180^\circ_{-x} 270^\circ_x$ . The composite pulses  $90^\circ_x 180^\circ_y 90^\circ_x$  (MLEV) and  $90^\circ_x 180^\circ_{-x} 270^\circ_x$  (WALTZ) pulses are very popular for heteronuclear broadband decoupling applications and are discussed in greater detail in Section 8.9. Note that while the off-resonance performance of WALTZ is superior to that of MLEV (compare Figure 5.17B and C), the sensitivity of WALTZ towards  $B_1$  magnetic field inhomogeneity is identical to that of a conventional square pulse.

A more systematic procedure in the development of composite RF pulses was proposed by Levitt and Ernst [33]. Their recursive expansion procedure allows the generation of composite RF pulses with arbitrary nutation angle which compensate to any desired degree for the effects of RF inhomogeneity. Unfortunately, the compensation towards RF inhomogeneity is achieved at the expense of severe bandwidth reduction. Garwood and Ke [35] have shown that symmetrization of the composite pulses obtained with the recursive expansion procedure restores the off-resonance characteristics, while the compensation with respect to RF inhomogeneity is retained. The composite pulse  $90_x 180_y 90_x$ , developed based on intuitive arguments, is part of the family of pulses obtained through the symmetrical recursive expansion procedure. In principle, the optimized composite pulses could be expanded for further improvements. However, this quickly leads to very long and complex pulse trains. A more attractive method for further improvement on the compensation for imperfections is to use adiabatic RF pulses as parent pulses. Adiabatic RF pulses are amplitude- and frequency-modulated pulses with an inherent insensitivity to imperfections and will be discussed in the next section.

## 5.7 Adiabatic RF Pulses

Surface coils are often used with *in vivo* NMR spectroscopy, either to enhance sensitivity or achieve a rough localization. The structure and characteristics of surface coils are discussed in Chapter 10. Besides the favorable characteristics of surface coils, the main disadvantage is that the  $B_1$  magnetic field generated by surface coils is extremely inhomogeneous. This will consequently lead to signal loss and artifacts when conventional RF pulses are transmitted with a surface coil, since the nutation angle  $\theta$  is proportional to the RF amplitude  $B_1$  [Equation (5.1)]. This problem could be solved by using a combination of a homogeneous volume coil for pulse excitation and a surface coil for sensitive signal reception (see Chapter 10). Besides the more difficult experimental set-up of the coils, this combination is bound to cause some signal loss due to residual inductive coupling when the coils are not perfectly orthogonal and because the (phase of the)  $B_1$  flux of transmission differs from that of reception (see Chapter 10).

Composite pulses have a certain degree of insensitivity to variations in RF amplitude. However, the RF amplitude generated by a surface coil can vary by a factor of 10 over the sensitive volume. Composite pulses have at most a compensation to RF amplitude of a factor three, such that composite pulses are inadequate to achieve optimal sensitivity when



used in combination with surface coils. Alternatively, RF pulses which operate according to adiabatic principles can be used [35, 37–50]. In principle, adiabatic pulses can be seen as the ultimate composite pulses, consisting of hundreds of hard pulses with different amplitude and phase. However, in general, adiabatic pulses are being referred to as amplitude- and frequency (or phase)-modulated pulses. Adiabatic pulses generate a uniform nutation angle independent of the RF amplitude, provided that the RF amplitude is above a certain threshold value. Therefore, surface coil experiments can be executed in a single-coil mode (with a constant phase relation between transmission and reception). Furthermore, adiabatic pulses eliminate time-consuming calibrations of RF power or pulse lengths (e.g. as shown in Figures 5.5 and 5.8) as is the case with conventional pulses. This is because adiabatic pulses require a one-time experimental determination of the minimum threshold amplitude at which the pulse becomes operational with a particular RF coil. Using a slightly higher RF amplitude than the minimum threshold value will eliminate the need for calibration in subsequent experiments, since effects of different samples are negligible when compared with the  $B_1$  insensitivity of adiabatic pulses. Figure 5.18 shows an example of the typical gain achieved when adiabatic RF pulses are employed in combination with a surface coil.

### 5.7.1 Rotating Frames of Reference

Because of the complex nature of adiabatic pulses, their principles are best illustrated with a single set of spins with Larmor frequency  $\omega_0$ . The motion of these spins are best visualized in a reference frame  $x'y'z'$  that rotates about  $z'$  (which is collinear with the direction of the main magnetic field  $B_0$ ) at the instantaneous frequency  $\omega(t)$  of the pulse, i.e. the  $x'y'z'$  frame is the conventional rotating frame of reference as used with the previously described RF pulses. The only difference being that with adiabatic pulses the frequency of this rotating frame varies as a function of time. In this rotating frame, which will be referred to as the frequency frame,  $B_1(t)$  does not precess in the transverse plane.  $B_1(t)$  has a constant orientation (arbitrary chosen along  $x'$ , Figure 5.19A). As adiabatic pulses have amplitude and frequency modulation, the frequency of the pulse  $\omega(t)$  deviates from the Larmor frequency  $\omega_0$  as a function of time. Consequently, the spins will encounter an additional magnetic field along  $z'$  with magnitude  $\Delta\omega(t)/\gamma$ , where  $\Delta\omega(t)$  is defined as  $\omega(t) - \omega_0$ . The appearance of  $\Delta\omega(t)/\gamma$  is identical to the presence of a vector  $\Delta\Omega/\gamma$  along  $z'$  in the case of off-resonance spins during a conventional RF pulse. Therefore, in analogy to Equation (5.2), the magnitude of the time-dependent, effective magnetic field  $B_e(t)$  in the frequency frame becomes:

$$B_e(t) = |\mathbf{B}_e(t)| = \sqrt{B_1^2(t) + \left(\frac{\Delta\omega(t)}{\gamma}\right)^2} \quad (5.34)$$

For conventional pulses, the effective field is a static vector, but for adiabatic pulses, the orientation and magnitude of  $\mathbf{B}_e$  changes in time as  $\mathbf{B}_1$  and  $\Delta\omega/\gamma$  are modulated. For a more convenient description of the motion of the magnetization in the presence of a time-dependent effective field, the magnetization can be divided in two categories, i.e. components that are collinear with or are perpendicular to  $\mathbf{B}_e(t)$  at the onset of the pulse. Both types of motion can be described best in a second rotating frame  $x''y''z''$ . In the second rotating frame, the orientation of  $\mathbf{B}_e(t)$  is constant [just as the orientation of  $\mathbf{B}_1(t)$  became

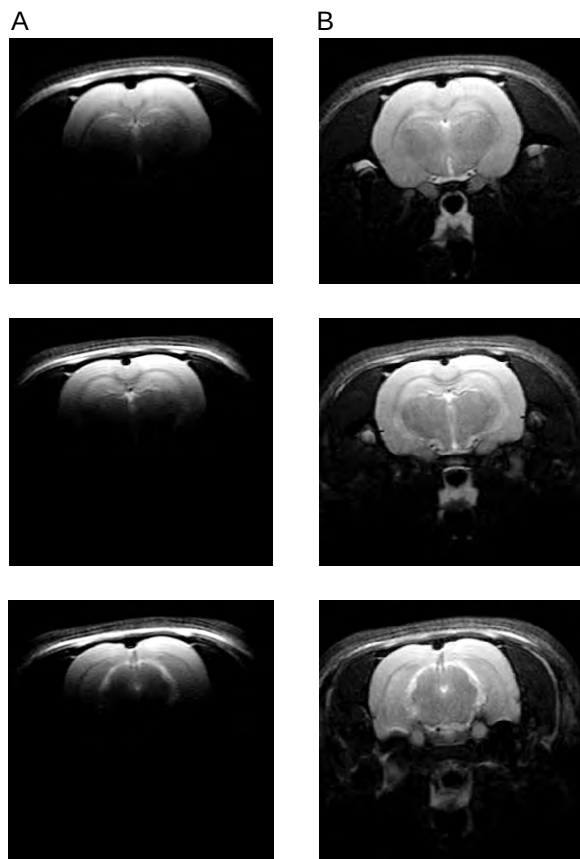


**Figure 5.18** Surface coil magnetic resonance images of rat brain (1.5 mm slice thickness) as acquired with (A) conventional sinc and (B) adiabatic RF pulses. The 20 mm diameter surface coil was positioned on top of the rat head, which corresponds to the top of the presented coronal images. The  $B_1$  insensitivity of adiabatic RF pulses eliminates all  $B_1$ -related signal losses during RF transmission.

fixed in the frequency frame]. Magnetization vectors which are initially parallel to  $\mathbf{B}_e(t)$  will remain like that throughout the pulse, while magnetization vectors perpendicular to the  $\mathbf{B}_e(t)$  will precess about  $\mathbf{B}_e(t)$ , but will remain perpendicular to  $\mathbf{B}_e(t)$ . However, although this description can provide a wealth of information about the rotations involved during adiabatic pulses, it is an incomplete description. This is because the second rotating frame changes its orientation with  $\mathbf{B}_e(t)$  relative to the frequency frame at a instantaneous angular velocity of  $d\alpha(t)/dt$ , where  $\alpha(t)$  is given by:

$$\alpha(t) = \arctan\left(\frac{\Delta\omega(t)}{\gamma B_1(t)}\right) \quad (5.35)$$

As a result, there is an additional contribution in the second rotating frame  $x''y''z''$  to the effective field along  $y''$  of magnitude  $d\alpha(t)/dt/\gamma$ , in analogy to the existence of  $\Delta\omega(t)/\gamma$

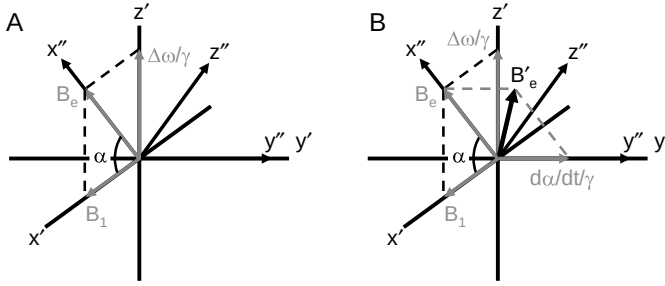


**Figure 5.19** Magnetic field vectors in rotating frames used to describe adiabatic RF pulses. (A) The frequency frame  $x'y'z'$  precesses at the instantaneous frequency of the RF pulse making the  $B_1$  orientation (arbitrarily chosen along  $x'$ ) stationary. (B) The second rotating frame  $x''y''z''$  rotates about  $y'$  with angular velocity  $d\alpha(t)/dt$  of the  $B_e$  rotation in the frequency frame. In the second rotating frame, the  $B_e$  orientation is static, leading to an additional vector  $(d\alpha(t)/dt)/\gamma [y'']$  along  $y' (=y'')$  as  $B_e$  is rotating.

in the frequency frame  $x'y'z'$ . Consequently, the two vectors in the second rotating frame,  $B_e(t)$  and  $((d\alpha(t)/dt)/\gamma)[y'']$  (where  $[y'']$  is a unit vector along  $y''$ ), form a new effective field vector  $B'_e(t)$ . The magnitude of  $B'_e(t)$  is given by:

$$B'_e(t) = |B'_e(t)| = \sqrt{B_e^2(t) + \left(\frac{d\alpha(t)}{\gamma dt}\right)^2} \quad (5.36)$$

The additional contribution of  $d\alpha(t)/dt/\gamma$  to  $B'_e(t)$  complicates matters in that magnetization vectors initially parallel with  $B_e(t)$  will no longer remain like that throughout the pulse, but will rotate about  $B'_e(t)$  instead, leading to incoherent rotations as a function of frequency offset and RF amplitude. However, provided that the magnetic field component that can be attributed to the angular velocity of  $B_e(t)$  is much smaller than the amplitude of  $B_e(t)$ , i.e.:

$$\left|\left(\frac{d\alpha(t)}{\gamma dt}\right)\right| \ll |B_e(t)| \quad (5.37)$$

then the additional term  $d\alpha(t)/dt/\gamma$  can be neglected and  $B'_e(t) \approx B_e(t)$ . This requirement, described by Equation (5.37), is known as the adiabatic condition. When the adiabatic condition is satisfied, the previous description can be further employed, i.e. magnetization initially parallel and perpendicular to  $B_e(t)$  will remain like that throughout the pulse. Furthermore, magnetization vectors perpendicular to  $B_e(t)$  will precess about  $B_e(t)$  though an angle  $\beta(t)$  given by:

$$\beta(t) = \gamma \int_0^t B_e(t') dt' = \gamma \int_0^t \sqrt{B_1^2(t') + \left(\frac{\Delta\omega(t')}{\gamma}\right)^2} dt' \quad (5.38)$$

Note the analogy of Equation (5.38) with the generation of a nutation angle  $\theta$ . For an on-resonance ( $\Delta\Omega = 0$ ), constant RF field  $B_1$  of length  $T$ , Equation (5.38) reduces to Equation (5.1). With the described formalism of the effective field in a second rotating frame, the performance of all adiabatic pulses can be described in a graphical manner.

### 5.7.2 Adiabatic Half and Full Passage Pulses

Adiabatic pulses are characterized by specific RF amplitude  $B_1(t)$  and frequency  $\omega(t)$  modulation functions. The magnetic field vectors encountered in the frequency frame can be written as:

$$\mathbf{B}_1(t) = B_{1\max} f_B(t) [\mathbf{x}'] \quad (5.39)$$

$$\Delta\omega(t) = \Delta\omega_{\max} f_\omega(t) [\mathbf{z}'] \quad (5.40)$$

$f_B(t)$  and  $f_\omega(t)$  are unitless, normalized modulation functions,  $B_{1\max}$  and  $\Delta\omega_{\max}$  are the modulation amplitudes of  $B_1(t)$  and  $\Delta\omega(t)$ , and  $[\mathbf{x}']$  and  $[\mathbf{z}']$  are unit vectors that identify the  $x'$  and  $z'$  axes of the frequency frame, respectively. Some commonly used combinations for  $f_B(t)$  and  $f_\omega(t)$  are  $\sin/\cos$ ,  $\tan/\tanh$  and  $\tanh/\text{sech}$ . Figure 5.20 shows the  $B_1(t)$  and  $\Delta\omega(t)$  modulations functions for  $f_B(t)/f_\omega(t) = \text{sech}/\tanh$ , or more specific  $f_B(t) = \text{sech}[\beta(1 - (2t/T))]$  and  $f_\omega(t) = \tanh[\beta(1 - (2t/T))]$ , with  $t$  varying from 0 to  $T$  and  $\beta$  defining the cut-off points for the infinite sech and tanh functions.  $\beta$  is typically chosen as  $\text{sech}(\beta) = 0.01$  (i.e. 1 % cut-off level). The pair of modulation functions defines the well-known Silver–Hoult hyperbolic secant pulse [37,38], the only pulse to date (except for the square pulse) which is obtained by analytical inversion of the Bloch equations.

On many NMR spectrometers, (adiabatic) RF pulses need to be implemented with phase modulation instead of frequency modulation. The time-dependent  $B_1$  phase  $\phi(t)$  during an

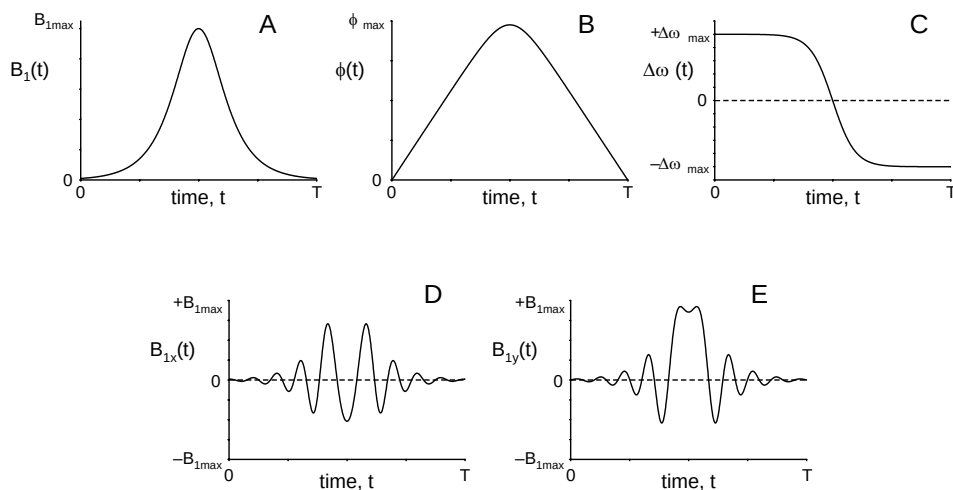


**Figure 5.20** Modulation functions of a hyperbolic secant adiabatic full passage. (A) RF amplitude modulated  $B_1(t)$  and (B) phase modulation  $\phi(t)$ . (C) Frequency modulation  $\Delta\omega(t)$  can be calculated as the time derivative of the phase modulation. The  $R$  value is defined as  $2\Delta\omega_{\max}T$ , where  $\Delta\omega_{\max}$  is the maximum frequency modulation and  $T$  is the pulse length. The (D) real  $B_{1x}(t)$  and (E) imaginary  $B_{1y}(t)$  RF amplitudes can be used to describe the RF pulse in the standard rotating frame of reference.

adiabatic RF pulse can be calculated as:

$$\phi(t) = \int_0^t \Delta\omega(t') dt' \quad (5.41)$$

The phase modulation of the hyperbolic secant pulse is shown in Figure 5.20B. An alternative (and somewhat inconvenient) visualization of the magnetic field vectors encountered during an adiabatic RF pulse can be represented in a frame  $xyz$  rotating with a constant frequency about  $z$  (which is collinear with the main magnetic field  $B_0$ ) at, for instance, the Larmor frequency  $\omega_0$  of the spins. In this rotating frame the combination of RF amplitude  $B_1(t)$  and phase  $\phi(t)$  modulation in the frequency frame constitute two new magnetic field vectors, the real and imaginary  $\mathbf{B}_1(t)$  given by:

$$\begin{aligned} \mathbf{B}_1(t) &= B_{1\max} f_B(t) e^{i\phi(t)} \\ &= B_{1\max} f_B(t) [\cos \phi(t) [\mathbf{x}] + i \sin \phi(t) [\mathbf{y}]] \\ &= B_{1x}(t) [\mathbf{x}] + i B_{1y}(t) [\mathbf{y}] \end{aligned} \quad (5.42)$$

where  $B_{1x}(t)$  and  $B_{1y}(t)$  are the real and imaginary RF fields along  $x$  and  $y$ , respectively. These modulations are shown for a hyperbolic secant pulse in Figure 5.20D and E. Although these modulation functions give an indication of the complex rotations involved with adiabatic RF pulses, they do not give any insight in the principles and applications (e.g. insensitivity to  $B_1$  field inhomogeneity) of adiabatic RF pulses. Therefore, the formalism for the  $B_1(t)$  and  $\Delta\omega(t)$  modulation functions given by Equations (5.39) and (5.40) [or the phase modulation of Equation (5.41)] is preferred.

The same modulation functions can be used for adiabatic half (AHP) and full passage (AFP) pulses, simply by varying  $t$  from 0 to  $+T/2$  (AHP) or  $+T$  (AFP), respectively. AHP and AFP pulses can be used for excitation and inversion, respectively. Figure 5.21 shows the

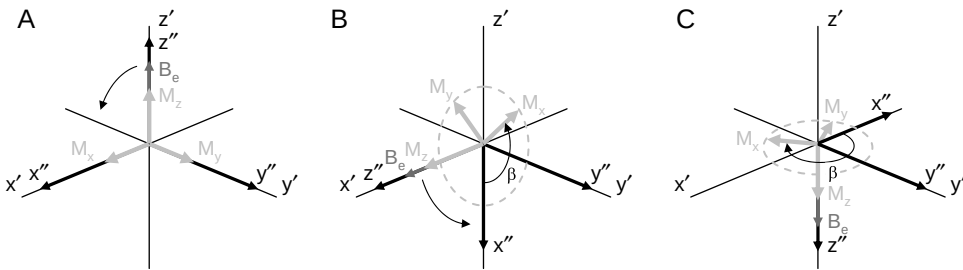


**Figure 5.21** On-resonance rotations of the effective field  $B_e$  (and the thermal equilibrium magnetization  $\mathbf{M}$  which is parallel with  $B_e$ ) during an AFP pulse. Halfway through the pulse (B) the  $B_1(t)$  and  $\Delta\omega(t)$  modulations could be terminated, resulting in an excitation of  $\mathbf{M}$  (AHP). When the AFP pulse is completed (C)  $\mathbf{M}$  is inverted. Since  $B_1(t)$  merely determines the rate at which  $B_e(t)$  changes orientation, the inversion is relatively insensitive to the RF amplitude. The rotations of  $M_x$  and  $M_y$  will be discussed in more detail in the text and Figure 5.23.

rotations of the longitudinal magnetization vector during a nonspecified adiabatic passage pulse. It is assumed that the pulse obeys the adiabatic condition at all times, such that the magnetization remains along the effective field  $\mathbf{B}_e$ . Furthermore,  $\mathbf{B}_e(0) = +\Delta\omega_{\max}/\gamma[\mathbf{z}']$ ,  $\mathbf{B}_e(T/2) = B_{1\max}[\mathbf{x}']$ , and  $\mathbf{B}_e(T) = -\Delta\omega_{\max}/\gamma[\mathbf{z}']$  (i.e. the transmitter frequency equals the Larmor frequency, such that  $\Delta\Omega = 0$  and  $\Delta\omega = \Delta\omega_{\max}$ ). At the beginning of the pulse, the magnetization is at thermal equilibrium along the positive  $z'$  axis parallel to  $\mathbf{B}_e$  (which is, according to definition of the second rotating frame  $x''y''z''$  parallel with  $z'$ ). During the pulse, the effective field  $\mathbf{B}_e$  starts to rotate towards the  $x'$  axis as the magnetic field vector  $\Delta\omega/\gamma$  along the  $z'$  axis and  $\mathbf{B}_1$  along the  $x'$  axis steadily decrease and increase, respectively. As the adiabatic condition is fulfilled throughout the pulse ( $|\mathrm{d}\alpha(t)/\mathrm{d}t| \ll |\gamma B_e(t)|$ ), the magnetization remains parallel with the effective field  $\mathbf{B}_e$ . Halfway through the AFP pulse ( $t = T/2$ ), the magnetic field vector along  $z'$  has reduced to zero and the effective field  $\mathbf{B}_e$  equals  $\mathbf{B}_1$  along  $x'$ , resulting in a complete excitation of the longitudinal magnetization. At this point the pulse could be terminated (as in the case of an AHP pulse) after which signal acquisition or other pulse sequence elements can follow. During an AFP pulse, the frequency modulation is decreased further, resulting in a magnetic field vector along  $-z'$  and a consequent rotation of  $\mathbf{B}_e$  towards the  $-z'$  axis. At the end of the pulse the effective field will be along  $-z'$ , giving an inversion of the longitudinal magnetization. The RF amplitude  $B_{1\max}$  is not very critical during an adiabatic pulse, since it merely determines the rate at which the effective field is rotating. As long as the rate of change of  $\mathbf{B}_e$  (i.e.  $\mathrm{d}\alpha/\mathrm{d}t$ ) is much smaller than the amplitude of  $\mathbf{B}_e$  (i.e. when the adiabatic condition is fulfilled), the inversion of longitudinal magnetization is independent of the RF amplitude.

The calibration of adiabatic RF pulses is normally performed in an identical manner to that of shaped RF pulses (i.e. Figure 5.8). The pulse length is kept constant, while the transmitter power is increased in units of dB. However, the calibration curves will appear completely different. Figure 5.22 shows the calibration curves for AHP and AFP pulses. The insensitivity to  $B_1$  magnetic field inhomogeneity is immediately apparent from the fact that the signal intensity remains constant above a minimum threshold RF amplitude. Although adiabatic RF pulses also have an upper threshold RF amplitude value, this is under practical circumstances not observed.

Off-resonance, the vector diagram of Figure 5.21 becomes slightly more complicated. Figure 5.23 shows the rotations during an AFP pulse at two different frequency offsets relative to the transmitter frequency,  $|\Delta\Omega| < |\Delta\omega_{\max}|$  and  $|\Delta\Omega| > |\Delta\omega_{\max}|$ . The rotations for  $|\Delta\Omega| < |\Delta\omega_{\max}|$  are nearly identical to those in Figure 5.21. First consider the rotations of  $\mathbf{M}_z$ . For frequency offset  $+\Delta\Omega$ , the total magnetic field vector at the onset of the pulse equals  $\Delta\omega = (\Delta\omega_{\max} + \Delta\Omega)[\mathbf{z}']$ . By analogy with the on-resonance situation, the effective field starts to rotate towards the  $x'$  axis. Halfway through the pulse ( $t = T/2$ ), the longitudinal magnetic field  $\Delta\omega/\gamma$  has been reduced to  $\Delta\Omega/\gamma$ , but is nevertheless along the  $+z'$  axis.  $\mathbf{M}_z$  is along the effective field which equals the vector sum of  $\mathbf{B}_1$  and  $\Delta\Omega/\gamma$ . In the second half of the AFP pulse,  $\Delta\omega/\gamma$  is reduced further and when  $|\Delta\Omega| < |\Delta\omega_{\max}|$ , the effective field will finish along the  $-z'$  axis [i.e.  $\Delta\omega(T) = (\Delta\Omega - \Delta\omega_{\max})$ ], giving an inversion of  $\mathbf{M}_z$ . However, when  $|\Delta\Omega| > |\Delta\omega_{\max}|$  (Figure 5.23B), the total longitudinal magnetic field at the end of the pulse will still be along the  $+z'$  axis, so that  $\mathbf{M}_z$  will be returned to its initial orientation. In summary, it can be concluded that for  $|\Delta\Omega| < |\Delta\omega_{\max}|$ , an AFP pulse

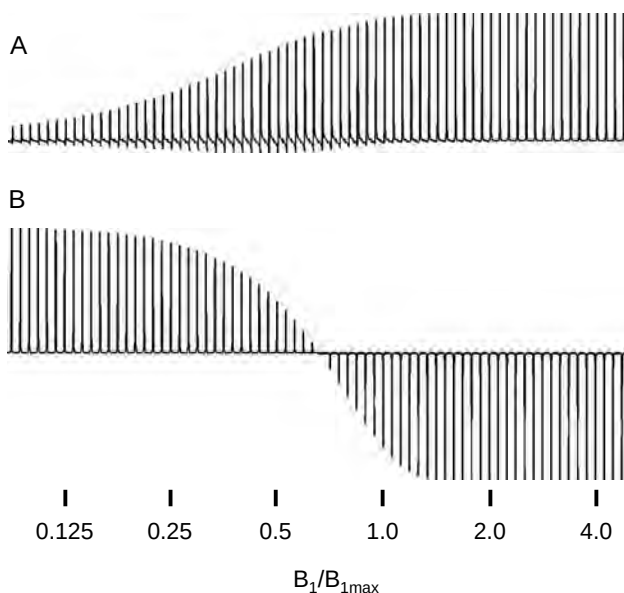


**Figure 5.22** RF field calibration of amplitude and frequency modulated adiabatic RF pulses. (A) Observed signal intensity following an AHP excitation pulse and (B) signal intensity which is observed when an AFP pulse is calibrated in an inversion recovery experiment with a very short recovery time. Note that the RF amplitude scale is not linear due to the nonlinear output of the RF amplifier. Both the AHP excitation and the AFP inversion pulse require a minimum RF amplitude to satisfy the adiabatic condition. Once the adiabatic condition is satisfied, the signal remains constant independent of the RF amplitude.

achieves complete inversion of longitudinal magnetization, while for  $|\Delta\Omega| > |\Delta\omega_{\max}|$ , the longitudinal magnetization is returned to its initial orientation.

For AHP pulses, there is no clear frequency selective excitation band. Halfway through the pulse ( $t = T/2$ ), the orientation of the effective field  $\mathbf{B}_e$  is determined by the relative amplitudes of the  $\mathbf{B}_1$  and  $\Delta\omega/\gamma$  magnetic field vectors. On-resonance,  $\mathbf{B}_e = \mathbf{B}_1$ , giving complete excitation independent of the RF amplitude. Off-resonance,  $|\mathbf{B}_e| = \sqrt{B_1^2 + (\Delta\Omega/\gamma)^2}$ , making the effective nutation angle  $\alpha [= \arctan(\Delta\Omega/\gamma B_1)]$ ,  $B_1$  and  $\Delta\Omega$  dependent. However, for the typical chemical shift ranges encountered with *in vivo*  $^1\text{H}$  and  $^{31}\text{P}$  MRS, the RF amplitude is often sufficient to make  $\mathbf{B}_1 \gg \Delta\Omega/\gamma$  and  $\mathbf{B}_e \approx \mathbf{B}_1$ . Figure 5.24 shows simulations based on the Bloch equations of the excitation and inversion performance for AHP and AFP pulses, respectively, as a function of RF amplitude  $B_{1\max}$  and frequency offset  $\Delta\Omega$ . Note that the frequency selective inversion of an AFP pulse is virtually independent of the RF amplitude. The effective bandwidth of an AHP pulse is not frequency selective and is dependent on the RF amplitude. At  $\Delta\Omega = -\Delta\omega_{\max}$ , the frequency modulation at the onset of the pulse has been reduced to zero, such that the AHP pulse can not coherently rotate the longitudinal magnetization onto the transverse plane. It has been proposed to use these ( $B_1$  dependent) incoherent rotations for selective resonance suppression [51].

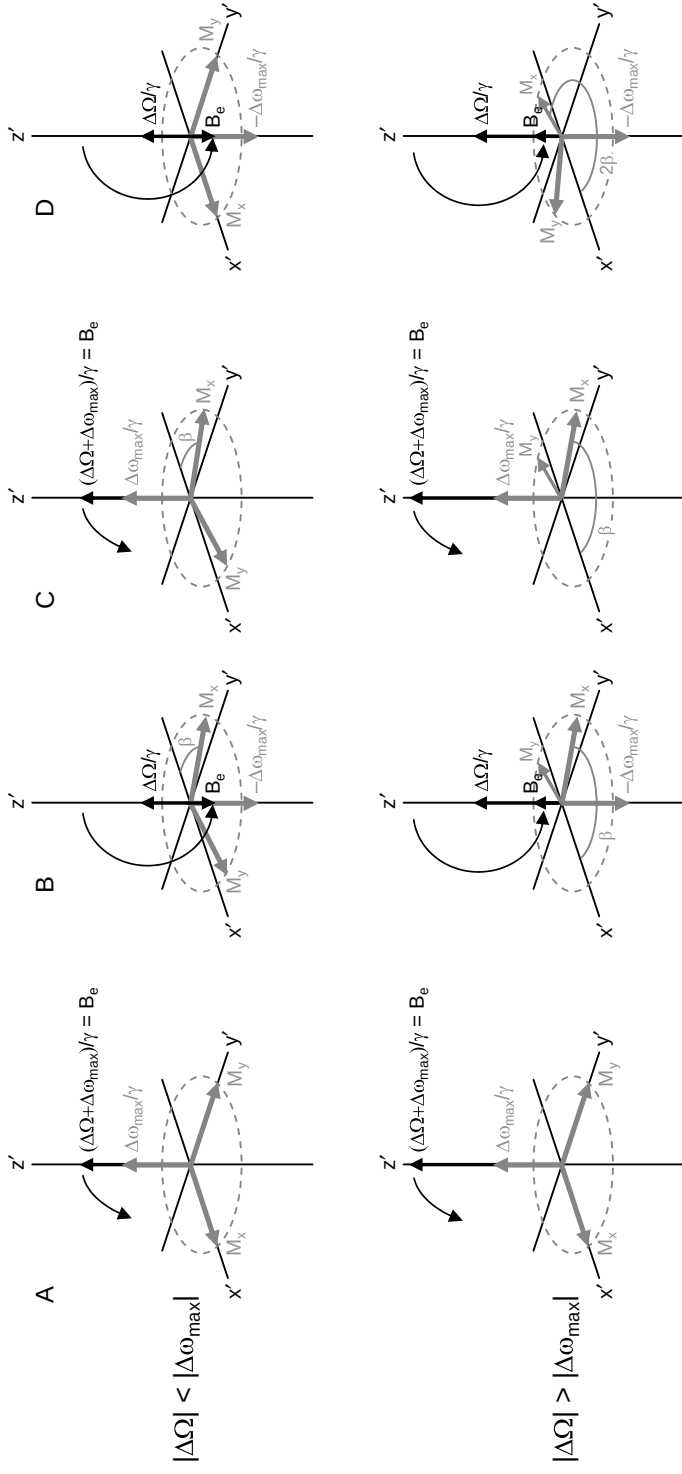


**Figure 5.23** Off-resonance rotations of the magnetization vectors  $\mathbf{M}_x$  and  $\mathbf{M}_y$ .  $\mathbf{M}_z$  is not shown because it follows  $\mathbf{B}_e$  throughout the pulse. (A, B) Within the frequency range,  $|\Delta\Omega| < |\Delta\omega_{\max}|$ ,  $\mathbf{M}_z$  inverts and  $\mathbf{M}_x$  and  $\mathbf{M}_y$  are dephased over an angle  $\beta$  given by Equation (5.38). Outside the frequency band,  $|\Delta\Omega| > |\Delta\omega_{\max}|$ ,  $\mathbf{M}_z$  returns to its initial orientation at the end of the pulse, while  $\mathbf{M}_x$  and  $\mathbf{M}_y$  are again dephased over an angle  $\beta$ . This indicates that an AFP pulse is not a plane rotation pulse. It also indicates that an AFP pulse is capable of frequency-selective inversion. (C, D) When a second, identical AFP pulse is executed following the first AFP pulse,  $\mathbf{M}_z$  returns to its initial orientation for all frequencies. More interestingly,  $\mathbf{M}_x$  and  $\mathbf{M}_y$  also return to their initial orientations for  $|\Delta\Omega| < |\Delta\omega_{\max}|$ . This makes the combination of two AFP pulses suitable for frequency-selective refocusing. More details can be found in the text and Figure 5.25.



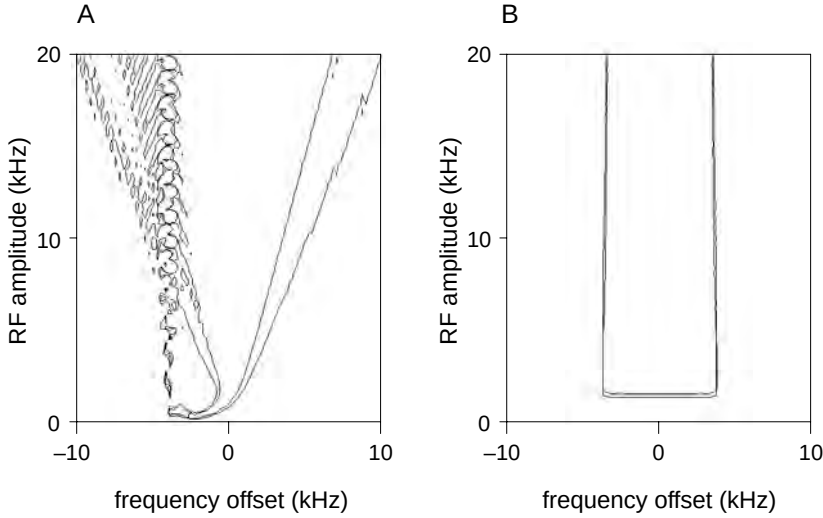


**Figure 5.24** Frequency profile of adiabatic passage pulses as a function of the RF amplitude. (A)  $M_{xy}$  for a 1 ms AHP pulse (contour levels are shown for  $0.90$  and  $0.95 M_{xy}$ ) and (B)  $M_z$  for a 2 ms AFP pulse (contour levels are shown for  $-0.90$  and  $-0.95 M_z$ ). Both pulse were sech/tanh modulated with  $2\Delta\omega_{\max}T = 10$ . Note that an AFP pulse achieves frequency-selective inversion, independent of the RF amplitude above a minimum threshold RF amplitude.

In Section 5.3 it was shown that each RF pulse has its own characteristic R value, i.e. product of bandwidth and pulse length. For adiabatic RF pulses, the R value can be varied by changing the maximum frequency modulation  $\Delta\omega_{\max}$  (or equivalently the maximum phase modulation). The maximum frequency modulation  $\Delta\omega_{\max}$  of an AFP pulse is directly related to the bandwidth as can be seen from Figure 5.24B where the inversion bandwidth equals  $2\Delta\omega_{\max}$ . For AHP pulses it is still useful to define a R value, even though  $\Delta\omega_{\max}$  is not directly related to the effective excitation bandwidth.

Another feature which can be deduced from Figures 5.21 and 5.23 is that AHP and AFP pulses are not plane or universal rotation pulses. A plane rotation pulse rotates all magnetization through a specific nutation angle irrespective of the initial orientation of the magnetization. At the start of an AFP (or AHP) pulse,  $\mathbf{M}_x$  and  $\mathbf{M}_y$  are perpendicular to the effective field  $\mathbf{B}_e$ . During the pulse,  $\mathbf{M}_x$  and  $\mathbf{M}_y$  remain perpendicular to  $\mathbf{B}_e$  and will simultaneously rotate about  $\mathbf{B}_e$  through an angle  $\beta$  given by Equation (5.38). Therefore,  $\mathbf{M}_x$  and  $\mathbf{M}_y$  will be dephased in the transverse plane as a function of RF amplitude and frequency offset. This has consequences for the practical utility of adiabatic passage pulses, in that an AHP pulse can only be used for excitation, i.e.  $(0, 0, M_z) \rightarrow (0, 0, M_x)$  and an AFP pulse only for inversion  $(0, 0, M_z) \rightarrow (0, 0, -M_z)$ . Rotations of the form  $(M_x, M_y, M_z) \rightarrow (M_x, -M_z, M_y)$  and  $(M_x, M_y, M_z) \rightarrow (M_x, -M_y, -M_z)$ , which are necessary for polarization transfer, stimulated-echo and spin-echo experiments are corrupted by the nonlinear phase shift  $\beta$ . A simple solution to the inability of an AHP pulse to execute the rotation  $(M_x, 0, 0) \rightarrow (M_z, 0, 0)$ , is to time-reverse the modulation functions, giving rise to a time-reversed AHP pulse (RAHP). At the beginning of a RAHP pulse,  $\mathbf{B}_e = B_{1\max}[\mathbf{x}']$ ,

such that  $\mathbf{M}_x$  is parallel with  $\mathbf{B}_e$  and  $\mathbf{M}_y$  and  $\mathbf{M}_z$  are perpendicular to  $\mathbf{B}_e$ . During the pulse,  $\mathbf{B}_e$  (and consequently  $\mathbf{M}_x$ ) rotates from  $x'$  to  $z'$ .  $\mathbf{M}_z$  and  $\mathbf{M}_y$  are dephased in the transverse plane by the  $B_1$  and  $\Delta\Omega$  dependent phase angle  $\beta$  at the end of the pulse. Even though a RAHP pulse is not a plane rotation pulse, it can be used in specialized applications to achieve the particular rotation  $\mathbf{M}_x \rightarrow \mathbf{M}_z$ .

### 5.7.3 Plane Rotations and Refocused Component

Before the refocusing capabilities of AFP pulses are discussed it is informative to define the refocused component. Suppose that a given RF pulse induces the rotations  $M_x \rightarrow -M_x$  and  $M_z \rightarrow -M_z$ . The RF pulse can then certainly be classified as an inversion pulse, but the pulse does not have to be a refocusing pulse per se. A refocusing pulse requires that only one component of the transverse magnetization changes sign. When the given RF pulse simultaneously induced the rotation  $M_y \rightarrow -M_y$  no refocusing is achieved as the net rotation  $(M_x, M_y) \rightarrow (-M_x, -M_y)$  simply describes a  $180^\circ$  phase shift of the transverse magnetization. The refocusing capabilities of a  $180^\circ$  pulse can be calculated by determining the so-called refocused component. Using the rotation matrix formalism given in Section 5.2, the refocused component can be calculated by performing four experiments in which the refocusing pulse is cycled along the  $+x$ ,  $+y$ ,  $-x$  and  $-y$  axes, together with a receiver phase along  $+x$  (add),  $-x$  (subtract),  $+x$  (add) and  $-x$  (subtract) axes, respectively. After this phase cycle the rotation matrix is given by:

$$\mathbf{R}(\alpha, \theta, [\phi \pm x, \phi \pm y]) = \cos^2 \alpha \sin^2(\theta/2) \begin{pmatrix} \cos 2\phi & -\sin 2\phi & 0 \\ -\sin 2\phi & -\cos 2\phi & 0 \\ 0 & 0 & 0 \end{pmatrix} \quad (5.43)$$

where  $\cos^2 \theta \sin(\alpha/2)$  is the refocused component and  $2\phi$  the corresponding phase. However, for a composite, adiabatic or any other complex pulse, the parameters  $\alpha$ ,  $\theta$  and  $\phi$  are not readily known, since for instance  $\theta$  is dependent on the RF amplitude and frequency offset. A more convenient method is to calculate the refocused component according to:

$$I = \frac{1}{2} \sqrt{(f_{xx} - f_{yy})^2 + (f_{xy} + f_{yx})^2} \quad (5.44)$$

with the corresponding phase given by:

$$\phi = \frac{1}{2} \arctan \left( \frac{f_{xy} + f_{yx}}{f_{yy} - f_{xx}} \right) \quad (5.45)$$

$f_{ab}$  is the fraction of initial  $M_a$  ( $a = x$  or  $y$ ) magnetization prior to the refocusing pulse which is converted to  $M_b$  ( $b = x$  or  $y$ ) magnetization after the pulse. For a perfect  $180^\circ$  refocusing pulse the refocusing component will be 1 [i.e. for  $(M_x, M_y, M_z) \rightarrow (M_x, -M_y, -M_z)$ ,  $f_{xx} = -f_{yy} = 1$  and  $f_{xy} = f_{yx} = 0$ ].  $f_{xx}$ ,  $f_{yy}$ ,  $f_{xy}$  and  $f_{yx}$  are readily obtained by performing two successive calculations of the Bloch equations with starting conditions  $(M_x, 0, 0)$  and  $(0, M_y, 0)$ . As will be shown in the next section, even though AFP pulses have a refocused component of 1, they do not belong to the class of universal or plane rotation pulses since the refocused phase is not uniform across the frequency profile. Universal or plane rotation

pulses are essential in a wide variety of applications, including polarization transfer and stimulated echo experiments.

#### 5.7.4 Adiabatic Full Passage Refocusing

Figure 5.25 shows simulations based on numerical integration of the Bloch equations to demonstrate the refocusing capabilities of a single AFP pulse. As indicated in Section 5.7.3, the calculation of only one starting condition [e.g. ( $M_x$ , 0, 0) as shown in Figure 5.25B3] is insufficient to characterize the refocusing capabilities of a RF pulse. Figure 5.25B2 shows the refocused component as calculated from Equation (5.44) using the simulation results from Figure 5.25B3 and B4. It follows that an AFP pulse refocuses all magnetization inside the slice ( $I = 1$ ), while no magnetization is refocused outside the slice ( $I = 0$ ). However, despite the excellent refocused component profile, an AFP pulse introduces a phase across the profile according to Equation (5.38). This would lead to a strong nonlinear phase roll across the NMR spectrum. When the AFP pulse is executed in concert with a magnetic field gradient in order to select a spatial slice, the phase across the profile would lead to signal loss.

The nonlinear phase shift induced by one AFP pulse can be cancelled by the application of a second identical AFP pulse [45, 46] as shown in the sequence of Figure 5.25C. For  $|\Delta\Omega| < |\Delta\omega_{\max}|$ , the effective field is inverted and  $M_x$  and  $M_y$  will experience a total rotation of  $\beta - \beta = 0$ , where  $\beta$  is the  $B_1$  and  $\Delta\Omega$  dependent phase shift given by Equation (5.38) (see Figure 5.23B for the corresponding rotations). In other words,  $M_x$  and  $M_y$  return to their initial orientations without a nonlinear phase shift. For  $|\Delta\Omega| > |\Delta\omega_{\max}|$ , the effective field is not inverted and the rotations of  $M_x$  and  $M_y$  during the two pulses coherently add to give a final rotation of  $\beta + \beta = 2\beta$ . The two AFP pulses can not be grouped together as one pulse (making it an effective  $2\pi$  or  $360^\circ$  pulse), since this would lead to a corruption of the acquired signal by phase shifts due to  $B_0$  inhomogeneity, magnetic field gradients and frequency offsets (i.e. the total refocused component of the pulses is zero, see Figure 5.25D2). The required arrangement of AFP pulses as shown in Figure 5.25C puts a lower limit on the minimum attainable echo-time. Therefore, AFP pulses with an effective nutation angle of  $3\pi$  have been developed in which the phase shift  $\beta$  generated by the first AFP pulse is compensated by a second  $2\pi$  pulse. However, the frequency profile of these  $3\pi$  pulses is not as selective as that obtained with the combination of Figure 5.25.

The applications of a double spin-echo pulse sequence employing an AHP excitation pulse and multiple AFP pulses for refocusing are widespread. The sequence has been used for 1D slice selection in imaging and spectroscopic imaging, for spectral editing and water suppression (see Chapter 6). Three pairs of AFP pulses can be used to achieve complete 3D spatial localization in a single acquisition [52, 53], as is discussed in detail in Chapter 6.

#### 5.7.5 Adiabatic Plane Rotation Pulses

AHP and AFP pulses have been very popular for *in vivo* surface coil experiments, due to their relative simplicity and excellent performance. AHP pulses are most commonly used for signal excitation in  $^1\text{H}$  and  $^{31}\text{P}$  MRS, while AFP pulses have been used for

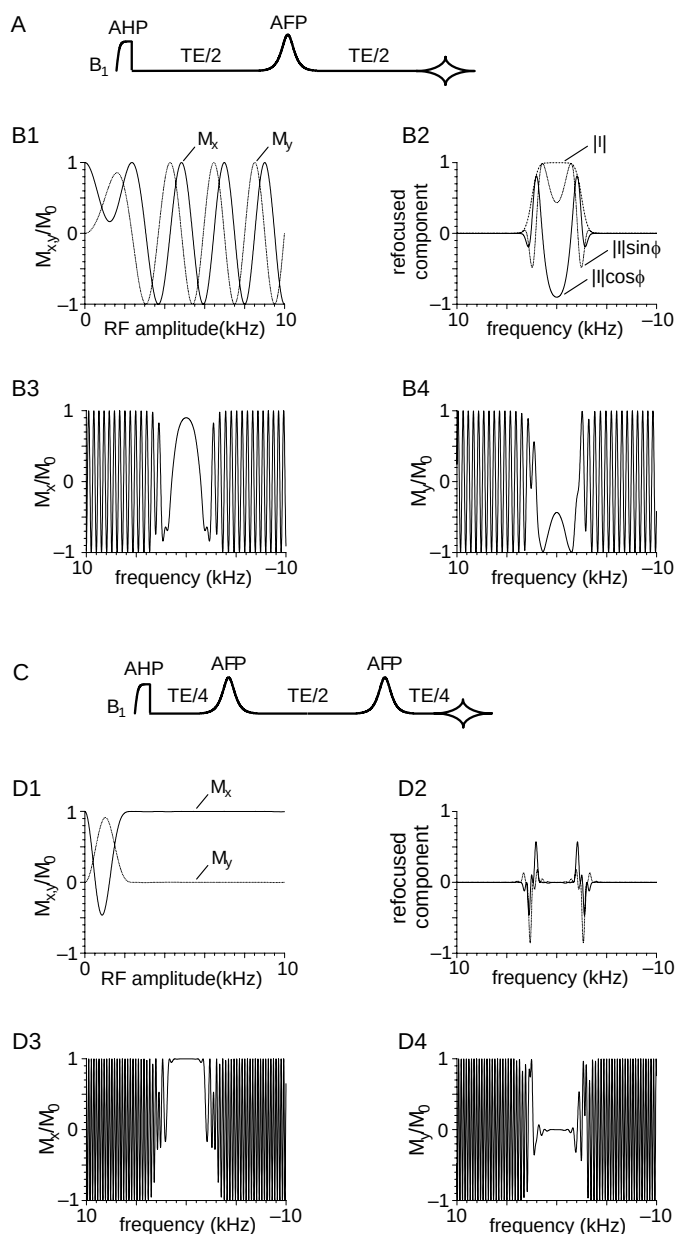


**Figure 5.25** Theoretical verification of the refocusing capabilities of an AFP pulse. A single AFP pulse as in (A) does not achieve acceptable refocusing, as the phase of the transverse magnetization is dependent on the RF amplitude (B1) and frequency offset (B2–B4). The dependence upon the frequency offset can be judged from the individual components  $M_x$  (B3) and  $M_y$  (B4) or from the refocused component (B2). The combination of two AFP pulses as in (C) achieves frequency-selective refocusing independent of the RF amplitude (D1) or frequency offset (D2–D4). The elimination of any frequency offset dependent phase dispersion can be deduced from (D2) the refocused component, (D3)  $M_x$  or (D4)  $M_y$ .

inversion in 3D ISIS localization (see Chapter 6) and  $T_1$  relaxation measurements with inversion recovery. The refocusing capabilities of AFP pulses have been used in a variety of imaging and spectroscopic imaging experiments. Despite this wide field of applications, their inability to achieve plane rotations makes them unsuitable for many other applications like polarization transfer and stimulated-echo experiments. Ugurbil *et al.* [41] made the first attempt to generate adiabatic plane rotation pulses, basically following the arguments presented for the refocusing capabilities of AFP pulses: For all plane rotation pulses, the transformation of any magnetization vector in the second rotating frame must be an identity transformation, i.e. the net rotation in the second rotating frame at the end of the pulse must be zero ( $\sum \beta = 0$ ). Furthermore, the desired plane rotation (and thus nutation angle) will be achieved by rotating the second rotating frame relative to the frequency frame through the desired nutation angle.

The first principle is satisfied by executing one or more  $\mathbf{B}_e$  inversions, as was demonstrated by Ugurbil *et al.* [41] for the so-called BIR-1 ( $B_1$  insensitive rotation) plane rotation pulse. The phase rotation  $\beta_1$  created during the first segment of the pulse can be cancelled during an identical, second segment in which the direction of rotation has been reversed by an inversion of the effective field  $\mathbf{B}_e$  (i.e.  $\beta_1 - \beta_1 = 0$ ). The desired nutation angle can be generated by phase shifting the  $\mathbf{B}_1$  field following a  $\mathbf{B}_e$  inversion. Unfortunately, since the effective field during BIR-1 is inverted when  $|\mathbf{B}_e| = B_{1\max}$ , the pulse is extremely sensitive to off-resonance effects. This is because off-resonance,  $\beta_1 \neq \beta_2$ , resulting in a nonzero rotation at the end of the pulse and a consequent dephasing of magnetization. More complex pulses, consisting of more segments are not sensitive to off-resonance effects for  $|\Delta\Omega| < |\Delta\omega_{\max}|$  as will be described for BIR-4.

### 5.7.6 Variable Angle Adiabatic Plane Rotation Pulse, BIR-4

The adiabatic plane rotation pulse BIR-4 [35, 48, 54] is composed of four adiabatic segments (Figure 5.26). Segments 2 and 4 equal AHP pulses, while segments 1 and 3 consist of time-reversed AHP pulses. Alternatively, BIR-4 can be seen as an AFP pulse, flanked by RAHP and AHP pulses, respectively. The pulse exhibits two  $\mathbf{B}_e$  inversions when  $\mathbf{B}_e = +\Delta\omega_{\max}/\gamma[\mathbf{z}']$  at  $t = T/4$  and  $3T/4$ , where  $T$  equals the pulse length. For all adiabatic RF pulses, frequency modulation is equivalent to phase modulation, since the phase  $\phi(t)$  equals the time integral of frequency  $\Delta\omega(t)$ . An additional feature of BIR-4 is that segments 2 and 3 are phase-shifted relative to segments 1 and 4.

The nutation angle generated by BIR-4 is determined by the values of two discontinuous phase shifts  $\Delta\phi_1$  and  $\Delta\phi_2$ , which occur between the first and second ( $t = T/4$ ) and between the third and fourth segment ( $t = 3T/4$ ) of the pulse. (Alternatively it can be stated that the nutation angle is determined by the phase shift of the middle two segments relative to the outer two segments.) To induce an arbitrary nutation angle  $\theta$ , the discontinuous phase shifts are set to  $\Delta\phi_1 = 180^\circ + \theta/2$  and  $\Delta\phi_2 = -(180^\circ + \theta/2) = -\Delta\phi_1$ . More general, BIR-4 achieves a plane rotation through an angle  $(\theta_1 + \theta_2)$  when the discontinuous phase shifts are set to  $\Delta\phi_1 = 180^\circ + \theta_1$  and  $\Delta\phi_2 = -(180^\circ + \theta_2)$ .

Figure 5.27 shows the rotations of the thermal equilibrium magnetization vector during BIR-4 in the second rotating frame and of the second rotating frame relative to the frequency

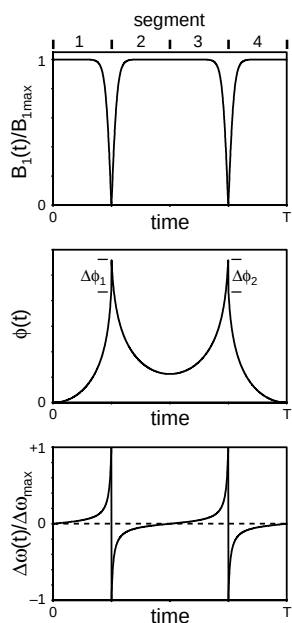


**Figure 5.26** RF amplitude  $B_1(t)$ , phase  $\phi(t)$  and frequency  $\Delta\omega(t)$  modulations of BIR-4. Two discontinuous phase shifts,  $\Delta\phi_1$  and  $\Delta\phi_2$ , are executed between segments 1 and 2 and between segments 3 and 4.

frame. Although only the thermal equilibrium vector is considered ( $\Delta\Omega = 0$ ), the rotations are valid for any  $\mathbf{M}$  perpendicular to  $\mathbf{B}_e(0)$  (e.g.  $\mathbf{M}_y$  when  $\mathbf{B}_e(0) = B_1[\mathbf{x}']$ ). Magnetization vectors parallel to  $\mathbf{B}_e(0)$  (e.g.  $\mathbf{M}_x$  when  $\mathbf{B}_e(0) = B_1[\mathbf{x}']$ ) are not considered since they remain parallel with  $\mathbf{B}_e(t)$  throughout the pulse (at least when the adiabatic condition is satisfied). At the onset of BIR-4 (Figure 5.27A), the laboratory, frequency and second rotating frames all coincide. Furthermore,  $\mathbf{B}_e(0) = B_1(0)[\mathbf{x}']$  and  $\mathbf{M}$  is along  $z'$  (which is collinear with  $z''$ ). The  $B_1$  and  $\Delta\omega$  modulations during the first segment of BIR-4, i.e.  $\mathbf{B}_e$  rotates from  $x'$  to  $z'$ , result in complete excitation of  $\mathbf{M}$ . However, since  $\mathbf{M}$  is perpendicular to  $\mathbf{B}_e$  at the onset of the pulse (and throughout the remainder of BIR-4),  $\mathbf{M}$  has also rotated about  $\mathbf{B}_e$  through a  $B_1$  and  $\Delta\omega$  dependent angle  $\beta$  given by Equation (5.38). The following two segments (i.e. a phase-shifted AFP pulse) rotate the entire second rotating frame  $x''y''z''$  about an axis with phase  $180^\circ + \theta/2$ , relative to the phase of the first segment. Simultaneously, the  $B_1$  and  $\Delta\omega$  dependent phase angle  $\beta$  of  $\mathbf{M}$  is reset to  $-\beta$  by the action of the AFP pulse. The final AHP pulse reduces the phase angle  $-\beta$  to zero, such that  $\mathbf{M}$  experiences an identity transformation in the second rotating frame. The nutation angle  $\theta$  is generated by the rotation of the second rotating frame  $x'y'z'$  relative to the frequency frame  $x''y''z''$ . Although Figure 5.27 only shows the on-resonance generation of an arbitrary nutation angle  $\beta$  by BIR-4, the principles also hold for off-resonance magnetization vectors (i.e.  $\Delta\Omega \neq 0$ ) provided that  $|\Delta\Omega| < |\Delta\omega_{max}|$ . For the on-resonance case (Figure 5.26), the phase angles  $\beta_i$  ( $i = 1, 2, 3$  or  $4$ ) generated by the four individual adiabatic segments are equal in magnitude and their absolute sum is zero ( $+\beta_1 - \beta_2 - \beta_3 + \beta_4 = 0$ ) resulting in an identity transformation in the second rotating frame. Note that the sign of  $\beta_i$  is inverted when a  $\mathbf{B}_e$  inversion occurs. The sign of  $\beta$  in the following segment(s) does not change until  $\mathbf{B}_e$  is inverted again. For off-resonance magnetization vectors, the magnitudes of  $\beta$  vary among the different segments, since  $\Delta\omega(t)/\gamma$  is offset by a constant amount. However,

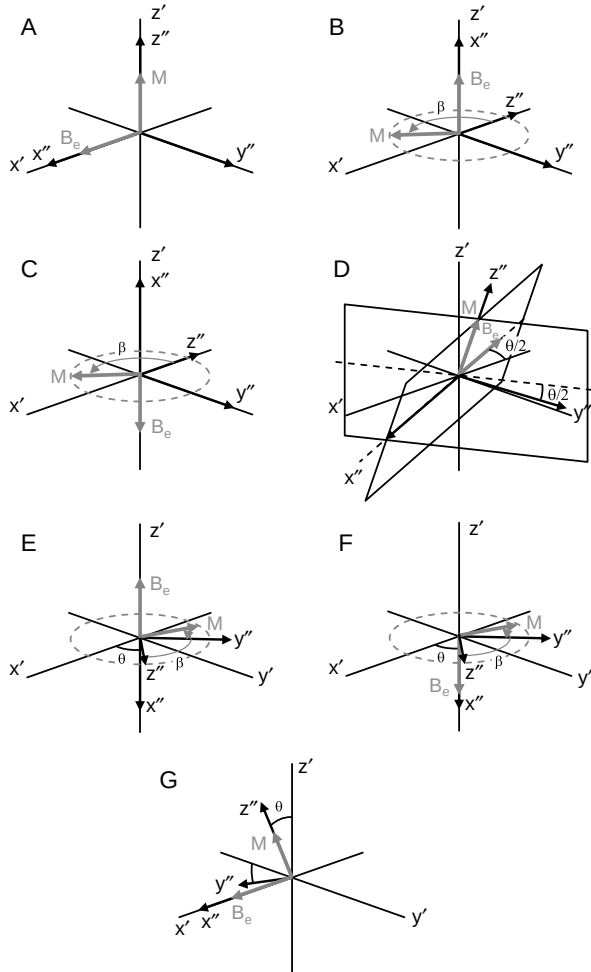


**Figure 5.27** Rotations of the thermal equilibrium magnetization during a BIR-4 pulse generating a nutation angle  $\theta$ . Magnetization vectors are shown at times (A)  $t = 0$ , (B)  $t = T/4$  prior to  $\mathbf{B}_e$  inversion, (C)  $t = T/4$  following  $\mathbf{B}_e$  inversion, (D)  $t = T/2$ , (E)  $t = 3T/4$  prior to  $\mathbf{B}_e$  inversion, (F)  $t = 3T/4$  following  $\mathbf{B}_e$  inversion and (G)  $t = T$ .

their sum is still zero, since  $\beta_1 = \beta_3$  and  $\beta_2 = \beta_4$ . When  $|\Delta\Omega| > |\Delta\omega_{\max}|$ ,  $\mathbf{B}_e$  can not be inverted such that  $\beta_i$  all have equal signs, making an identity transformation in the second rotating frame impossible.

BIR-4 can generate any arbitrary nutation angle simply by changing the phase shifts  $\Delta\phi_1$  and  $\Delta\phi_2$  within the pulse. A particularly rigorous and special pulse is generated when  $\theta = 0^\circ$ . This so-called identity BIR-4 does not directly excite spins (since the nutation angle is  $0^\circ$ ), but nevertheless has found widespread applications. An identity BIR-4 pulse is especially insensitive to  $B_1$  inhomogeneity, since it not only inverts  $\mathbf{B}_e$  at  $t = T/4$  and  $3T/4$ , but it inverts  $\mathbf{B}'_e$  as well. Therefore, the adiabatic condition can be satisfied less

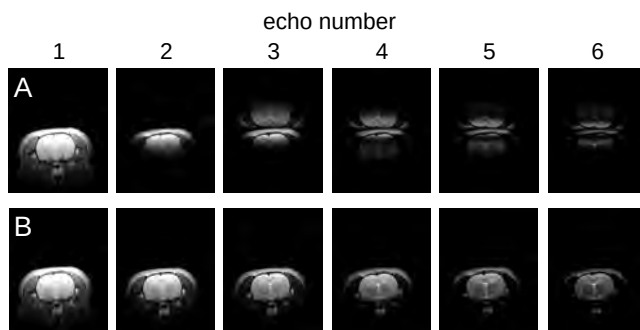


**Figure 5.28** Multi-echo imaging on rat brain at 4.7 T using (A) square and (B) adiabatic  $180^\circ$  BIR-4 RF pulses for refocusing. RF pulse irradiation and signal acquisition were performed with the same 20 cm diameter surface coil placed horizontally on top of the rat head with a sensitivity profile perpendicular to the coronal image orientation. For both types of RF pulses, the first image (echo number  $n = 1$ ) was generated with the adiabatic slice-selective pulse sequence of Figure 5.25C ( $TR/TE = 3000/35$  ms, 2 mm slice thickness). The subsequent echoes are sampled at  $TE = (35 + (n - 1)15)$  ms, with  $n = 2$  to 6. (A) The  $B_1$ -sensitivity of square refocusing pulses lead to signal loss (for  $n \geq 2$ ) and image artifacts (mirror images for  $n \geq 3$ ). Although the signal loss is inevitable when using square RF pulses, the artifacts may be removed with phase cycling and/or magnetic field crusher gradients. However, these solutions lead to increased measurement times and diffusion weighting, respectively. (B) The  $B_1$ -insensitivity and refocusing capabilities of  $180^\circ$  BIR-4 pulses give artifact-free images without the need for phase cycling or magnetic field gradients, in which the signal decrease in subsequent images is purely caused by  $T_2$  relaxation (ignoring diffusion).

stringent for  $\theta \sim 0^\circ$ . Even for  $\theta = \pi/2$ , some compensation due to partial  $B'_e$  inversion occurs, which results in similar power requirements of BIR-4 and AHP pulses even though BIR-4 is composed of four adiabatic passage segments [35]. Incorporation of symmetrical delays into an identity BIR-4 (at  $t = T/4$  and  $3T/4$ ) allows the evolution of a coupled spin-system from in-phase to anti-phase coherence, which can be excited to multiple quantum coherences by the last AHP [55]. This is extremely useful for  $B_1$  insensitive spectral editing as is discussed in Chapter 8. Incorporation of asymmetric magnetic field gradients into the delays allows for spatial tagging with complete  $B_1$  insensitivity [56]. Finally, an identity BIR-4 may be converted to an adiabatic analog of a jump-return pulse for solvent suppression [57,58] as is discussed in Chapter 6.

An example of the  $B_1$ -insensitivity and plane rotation capabilities of BIR-4 is shown in Figure 5.28, where  $180^\circ$  BIR-4 pulses are used in surface coil multi-echo imaging to get artifact-free  $T_2$ -sensitized images.

### 5.7.7 Modulation Functions

Adiabatic RF pulses can, in principle, be executed with a wide range of  $B_1$  and  $\Delta\omega$  modulation functions. However, the choice of modulation functions will strongly determine to what extent the adiabatic condition is satisfied and thereby the RF amplitude and frequency offset ranges over which the pulse executes the desired rotation. Quantitative information about the effective RF amplitude and frequency offset ranges can be



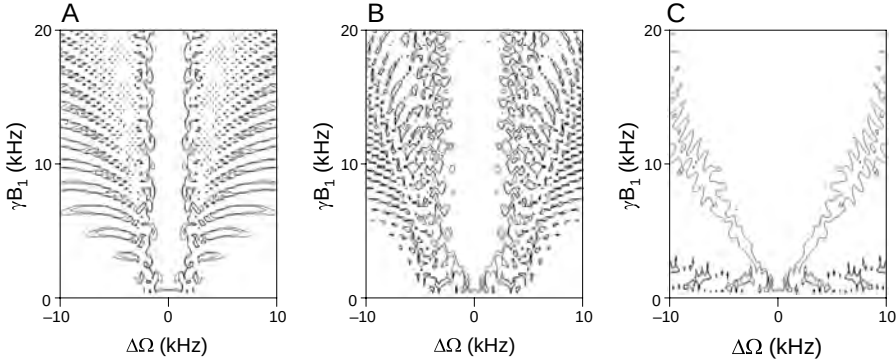


**Figure 5.29** Frequency profiles for 90° BIR-4 pulses as a function of the RF amplitude. BIR-4 pulses are executed with (A) sech/tanh, (B) sin/cos and (C) tanh/tan modulation functions for  $B_1(t)$  and  $\Delta\omega(t)$ , respectively. The maximum frequency modulation of the pulses was adjusted to give the same minimum RF amplitude for on resonance excitation. The sech/tanh modulated BIR-4 pulse is suitable for frequency selective excitation with dephasing outside the frequency band. The tanh/tan modulated BIR-4 pulse closely approximates a pulse which is optimized to excite over large RF amplitude and frequency offset ranges.

obtained by calculating the Bloch equations for a particular pair of modulation functions. Figure 5.29 shows simulations based on the Bloch equations for 90° BIR-4 pulses executed with (A) sech/tanh, (B) sin/cos and (C) tanh/tan modulation functions [following convention, the adiabatic pulses are specified according to the modulation functions for  $f_B(t)$  and  $f_\omega(t)$ , respectively]. The frequency modulation  $\Delta\omega_{\max}$  was adjusted for each pulse to give the same minimum RF amplitude on-resonance to execute the rotation  $M_z \rightarrow M_{xy}$  for  $\geq 95\%$ . (The minimum RF amplitude corresponded to 1.5 kHz for a 4 ms pulse.) Figure 5.29 clearly illustrates that the particular modulation functions used have strong effects on the performance of the pulse. The sech/tanh based pulse achieves approximately frequency-selective excitation independent of the RF amplitude and has been used for spatial localization [58]. The excitation bandwidth of a tanh/tan based BIR-4 increases with increasing RF amplitude, a favorable characteristic when uniform (nonselective) excitation is required. The sin/cos based BIR-4 pulse has intermediate characteristics.

Adiabatic modulation functions can be optimized to achieve certain characteristics like minimum RF power or improved frequency selectivity. Among many methods, analytical and numerical evaluation of the adiabatic condition [43, 59–62], and the procedure of offset-independent adiabaticity (OIA) [49] has produced excellent adiabatic pulses. Since the theory of offset-independent adiabaticity is very general and the OIA-produced AFP pulses become important for wide-band decoupling (see Chapter 8) the principles are briefly outlined.

### 5.7.8 Offset-independent Adiabaticity

In the presence of a frequency offset  $\Delta\Omega$ , the general frequency modulation function during any AFP pulse can be expressed as:

$$\Delta\omega(t) = (\Delta\Omega - \Delta\omega_{\max} f_\omega(t))[\mathbf{z}'] \quad (5.46)$$

In combination with Equation (5.39) for the RF modulation, Equation (5.46) can be used to derive a general expression for the adiabatic condition, expressed as a ratio  $K$ , for any time during the pulse  $t$  at any frequency offset  $\Delta\Omega$ :

$$K(\Delta\Omega, t) = \left| \frac{\gamma B_1(t)}{(d\alpha(t)/dt)} \right| = \frac{\Delta\omega_{\max}^2}{\gamma B_{1\max}} \frac{\left[ \left( \frac{\gamma B_{1\max}}{\Delta\omega_{\max}} f_B(t) \right)^2 + \left( \frac{\Delta\Omega}{\Delta\omega_{\max}} - f_\omega(t) \right)^2 \right]^{3/2}}{\left| \left( \frac{\Delta\Omega}{\Delta\omega_{\max}} - f_\omega(t) \right) \left( \frac{df_B(t)}{dt} \right) - f_B(t) \left( \frac{df_\omega(t)}{dt} \right) \right|} \gg 1 \quad (5.47)$$

The method of OIA states that the adiabatic condition  $K(\Delta\Omega, t)$  must be equally satisfied for all frequency offsets  $\Delta\Omega$  inside a given bandwidth. In other words,  $K(\Delta\Omega, t)$  needs to be constant for  $|\Delta\Omega| < |\Delta\omega_{\max}|$ . Using this requirement, the adiabatic condition can be calculated during the entire pulse when an isochromat at frequency  $\Delta\Omega$  is on-resonance, i.e.  $f_\omega(t) = \Delta\Omega/\Delta\omega_{\max}$  since the requirements on the adiabatic conditions are most stringent when the pulse traverses through the transverse plane (i.e.  $\Delta\omega = 0$ ). At time  $t_{\Delta\Omega}$ , i.e. when the isochromat  $\Delta\Omega$  is on-resonance, the adiabatic condition reduces to:

$$K(t_{\Delta\Omega}) = \frac{(\gamma B_{1\max} f_B(t_{\Delta\Omega}))^2}{\Delta\omega_{\max} \left( \frac{df_\omega(t_{\Delta\Omega})}{dt} \right)} \gg 1 \quad (5.48)$$

Modulation functions that satisfy the offset-independent adiabaticity can now be calculated with the equality specified by Equation (5.48), i.e.:

$$\left( \frac{df_\omega(t_{\Delta\Omega})}{dt} \right) = \frac{(\gamma B_{1\max} f_B(t_{\Delta\Omega}))^2}{\Delta\omega_{\max} K(t_{\Delta\Omega})} \quad (5.49)$$

Since the factor  $(\gamma B_{1\max}/\Delta\omega_{\max} K(t_{\Delta\Omega}))$  is simply a scaling factor, Equation (5.49) can be simplified to the following condition for OIA:

$$\left( \frac{df_\omega(t)}{dt} \right) = C f_B^2(t) \quad \text{or} \quad f_\omega(t) = C \int_0^t f_B^2(t') dt' \quad (5.50)$$

Many pairs of  $(f_B, f_\omega)$  functions satisfy Equation (5.50), including the well-known hyperbolic secant [37, 38] and chirp [63] modulation functions. Figure 5.30 shows the off-resonance performance of several useful modulation functions. It has been shown that while the peak power requirements ( $B_{1\max}$ ) vary widely for different modulation functions, the mean or average power ( $B_{1\text{rms}}$ ) is roughly the same. As pointed out by Tannus and Garwood [49], the peak power is determined by the  $f_B$  modulation function, while the average power is determined by the inversion bandwidth, provided that it is larger than the intrinsic bandwidth of the  $f_B$  modulation function.

## 5.8 Pulse Imperfections and Relaxation

The experimental performance of RF pulses can often substantially deviate from that predicted by theoretical calculations. This can typically be attributed to experimental imperfections like  $B_1$  magnetic field inhomogeneity and RF amplifier nonlinearity or intrinsic NMR parameters like  $T_1$  and  $T_2$  relaxation.

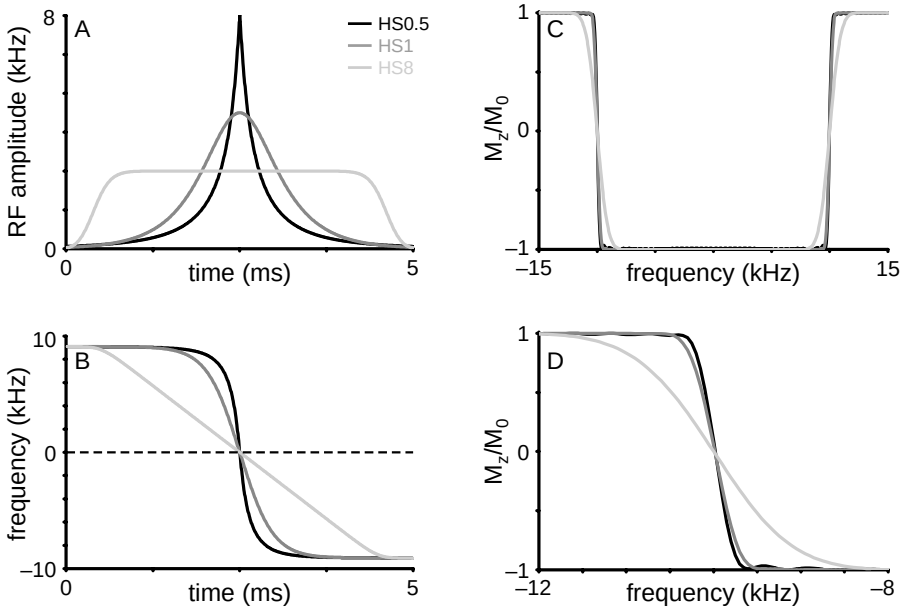


**Figure 5.30** Modulation functions and simulations of adiabatic inversion pulses which satisfy the condition for OIA. (A) RF amplitude functions given by  $B_1(t) = B_{1\max}\text{sech}(\beta t^n)$  with  $n = 0.5, 1$  or  $8$ , where  $n = 1$  corresponds to the classical hyperbolic secant pulse. (B) Frequency modulation functions calculated from (A) with Equation (5.50) for  $\Delta\omega_{\max} = 10$  kHz and  $T = 5.0$  ms. The RF amplitude  $B_{1\max}$  is scaled to the lowest amplitude that produces a  $>99\%$  inversion on-resonance. Simulated  $M_z/M_0$  frequency profiles for (C)  $-15$  kHz  $\leq \Delta\Omega \leq 15$  kHz and (D)  $8$  kHz  $\leq \Delta\Omega \leq 12$  kHz. Note that while the average powers of all pulses are roughly identical, the higher peak power for HS0.5 pulse results in a greatly reduced transition width.

Incorrect calibration of the RF power or inhomogeneity of the transmit  $B_1$  magnetic field are the most commonly encountered effects that can lead to unexpected results, artifacts and signal loss. Figure 5.31A shows the inversion profile of a 1 ms Gaussian RF pulse for  $B_1$  amplitude of 80, 100 and 120 % of the nominal amplitude to produce an on-resonance  $180^\circ$  rotation. When the  $B_1$  field deviates from the nominal amplitude the inversion is incomplete, leading to signal loss. Figure 5.31B shows the inversion profile for a 1 ms optimized sinc pulse for the same range of  $B_1$  amplitudes. In this case, a deviation of the nominal nutation angle has a number of effects. When the nutation angle is too low, the frequency profile is significantly broader. In case the magnetization is over rotated, the frequency profile gets narrower, while at the same time signal outside the band is affected. These effects can lead to signal loss and artifacts and can become a dominant factor when the nutation angles deviate even further from the nominal value. For certain applications the problem of incorrect nutation angles can be largely alleviated through the use of adiabatic RF pulses. For those applications where adiabatic RF pulses can not be used, the user

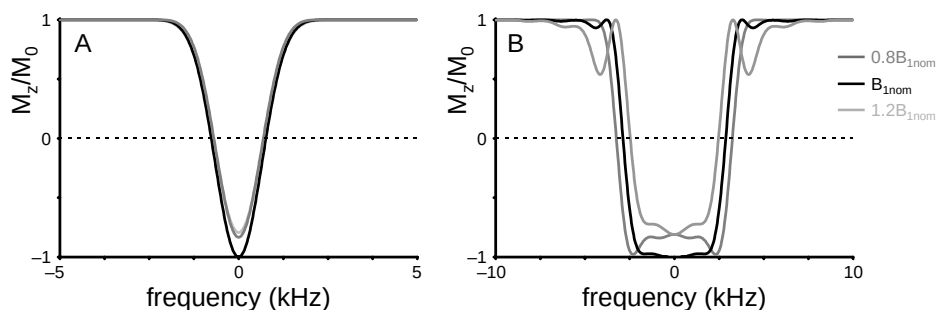


**Figure 5.31** Effect of incorrect RF power calibration on the frequency profile of (A) 1 ms Gaussian and (B) 1 ms optimized sinc inversion pulses. Profiles are shown for a nominal  $B_1$  magnetic field strength,  $B_{1nom}$ , that produces an on-resonance  $180^\circ$  rotation (black lines), as well as for  $0.8B_{1nom}$  and  $1.2B_{1nom}$ . While the frequency profile for a Gaussian pulse is relatively insensitive to variations in the  $B_1$  magnetic field strength, the frequency profile of the optimized sinc pulse shows strong deviations, especially for higher  $B_1$  magnetic fields.

should be aware of the problem and, when required, take steps ( $B_1$  mapping, simulation) to take the effects into account.

Other imperfections which lead to distorted frequency profiles may arise from the hardware employed and especially the RF amplifier. Nonlinearities and unwanted phase modulation can significantly degrade the frequency profile. Figure 5.32 shows the effect of RF amplifier imperfections on the performance of an optimized sinc pulse [8]. Figure 5.32A shows the RF shape serving as the input to the RF amplifier. Due to input power dependent amplitude and phase modulations (Figure 5.32B), the RF pulse transmitted by the RF coils is distorted (Figure 5.32C), leading to an imperfect frequency profile with significant unwanted signal excitation outside the desired frequency band (Figure 5.32D). However, in most cases these imperfections can be alleviated by calibrating the amplitude and phase responses of the RF amplifier [64, 65] and pre-distorting the input RF waveform (Figure 5.32E). The pre-distorted RF wave form is distorted by the nonlinearities of the RF amplifier (Figure 5.32B) given a waveform that is close to the ideal, desired RF waveform (Figure 5.32F and G). The obtained frequency profile has greatly improved and is close to the theoretical profile. Another potential source of artifacts may be an insufficient digital resolution of the RF and gradient waveforms employed [65].

For nonselective excitation with square RF pulses, ignoring relaxation is a valid approximation, since the length of the RF pulse (10–1000  $\mu$ s) is much smaller than typical  $T_1$  and  $T_2$  relaxation times (10–3000 ms). However, if the pulse length of (selective) RF pulses are in the same range as  $T_1$  and  $T_2$ , relaxation during the pulse may become significant. This may lead to degraded frequency profiles which can cause imperfections for several applications like water suppression and spatial localization.

Relaxation is readily incorporated into the Bloch equations [see Equations (5.17)–(5.19)], such that relaxation during RF pulses can be quantitatively calculated. Figure 5.33 shows the effect of short  $T_1$  and  $T_2$  values on the performance of a 5 ms AFP inversion pulse ( $R = 10$ ). Short  $T_2$  relaxation times lead to two effects, namely that (1) the magnetization within the slice is no longer perfectly inverted and (2) the longitudinal magnetization outside the slice no longer returns to its initial orientation. The second effect can

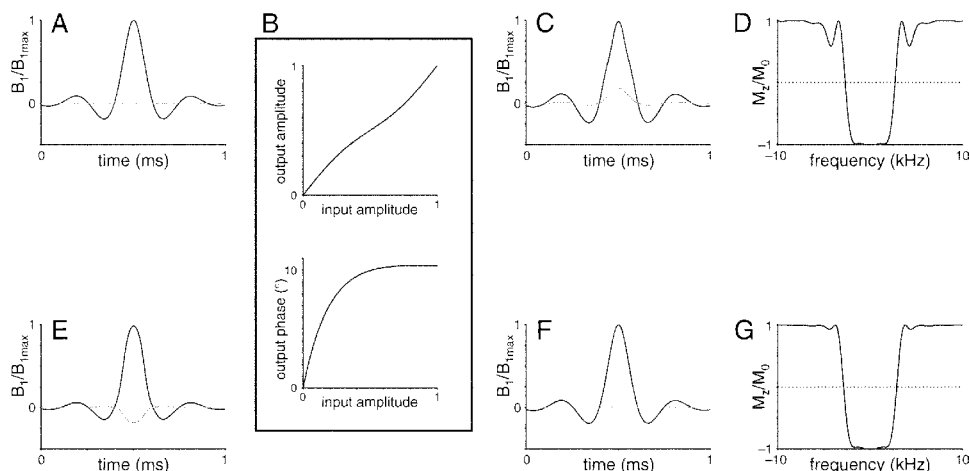


**Figure 5.32** Effect of RF amplifier nonlinearity and phase modulation. In the presence of (B) RF amplifier nonlinearity and phase modulation (C) the amplified output signal does not match (A) the optimized sinc input signal, leading to (D) distortions in the frequency inversion profile. When the RF amplifier nonlinearity and phase modulation are quantitatively known, (E) the input signal can be pre-distorted or pre-warped such that (F) the amplified output signal corresponds to the desired optimized sinc function which then (G) produces a greatly improved frequency inversion profile.

have dramatic effects when the AFP pulse is used in ISIS localization (see Chapter 6). Subtraction of experiments with and without an AFP inversion pulse will, in the absence of relaxation, lead to cancellation of all signal outside the inversion slice. In the presence of short  $T_2$  relaxation times, the subtraction will be imperfect such that the localized volume will be contaminated. The degradation of the inversion profile can easily be understood when the evolution of the magnetization during the pulse is calculated for  $\Delta\Omega = 0$  (Figure 5.33B) and  $\Delta\Omega = 3$  kHz (Figure 5.33C). On-resonance, the magnetization traverses the transverse plane during a significant part of the pulse, where it is subject to  $T_2$  relaxation. Outside the inversion band, the longitudinal magnetization returns to its initial orientation at the end of the pulse. However, during the pulse the magnetization is partially rotated to the transverse plane, leading to incomplete signal recovery in the presence of short  $T_2$  relaxation.

The presence of short  $T_1$  relaxation also has two effects. First, the inversion efficiency within the slice is further reduced and second the inversion profile becomes asymmetrical. The loss of symmetry within the inversion band can be derived from Figure 5.33. For  $0 < \Delta\Omega < \Delta\omega_{\max}$ , the magnetization is rotated to the  $-z'$  axis at a later stage during the RF pulse than for  $-\Delta\omega_{\max} < \Delta\Omega < 0$ . This will lead to different longitudinal relaxation behavior for different frequencies.

In Section 5.7.2 it was shown that, in the absence of relaxation, the inversion band generated by an AFP pulse is virtually independent of the RF amplitude. In the presence of short  $T_1$  and  $T_2$  relaxation times, this is not entirely true. Because the rotations in the laboratory frame and the magnitude of the effective field in the frequency frame increase with increasing RF amplitude, relaxation (and especially  $T_2$  relaxation) has a

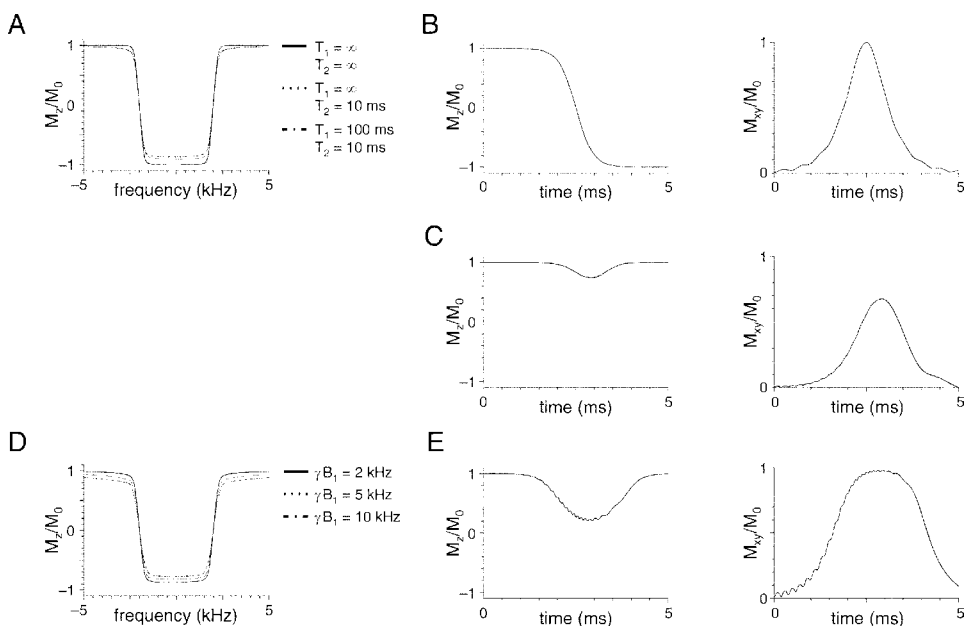


**Figure 5.33** Effect of relaxation on the frequency profile of a 5 ms hyperbolic secant adiabatic inversion pulse ( $2\Delta\omega_{\max}T = 20$ ). (A) Frequency profiles for three different combinations of  $T_1$  and  $T_2$  relaxation times, as indicated [ $(\gamma B_1/2\pi) = 2.0$  kHz]. (B) On-resonance evolution of longitudinal and transverse magnetization during an AFP pulse [ $T_1 = \infty$ ,  $T_2 = \infty$ ,  $(\gamma B_1/2\pi) = 2.0$  kHz,  $T = 5.0$  ms]. (C) Evolution of longitudinal and transverse magnetization during an AFP pulse 3.0 kHz off-resonance. (D) Frequency profiles for three different RF amplitudes in the presence of relaxation ( $T_1 = 100$  ms,  $T_2 = 10$  ms). (E) Off-resonance (3.0 kHz) evolution of longitudinal and transverse magnetization during an AFP pulse for  $(\gamma B_1/2\pi) = 10$  kHz. Clearly, relaxation has a more pronounced effect at higher RF amplitudes.

larger effect. Figure 5.33D shows the inversion profile for a 5 ms AFP pulse (hyperbolic secant modulation with  $R = 10$ ) in the presence of  $T_1 = 100$  ms and  $T_2 = 10$  ms at different RF field strengths. Figure 5.33E shows the evolution of (initially longitudinal) magnetization 3 kHz off-resonance during an AFP pulse at a RF field strength of 10 kHz. When compared with the same situation at a RF field strength of 2 kHz (Figure 5.33C), it is clear that  $T_2$  (and  $T_1$ ) relaxation leads to more signal loss at higher RF field strengths.

For conventional, nonadiabatic RF pulses, the effect of short  $T_1$  and  $T_2$  relaxation times is often less pronounced and often simply leads to a reduction of the in-slice magnetization without significantly affecting the overall slice profile.

## 5.9 Power Deposition

The magnetic energy absorbed by nuclear spins is, through relaxation pathways, ultimately dissipated as heat in the sample. Since this amount is only a small fraction of the thermal energy present at ambient temperatures it does not pose a safety consideration. However,

due to the electrical conductivity of biological tissue, the transmitted RF magnetic field induces electrical currents in the tissue, of which the majority is transformed to heat by Ohmic heating. This RF power deposition can heat biological tissues to the point where it becomes a dominant safety consideration. The guidelines for exposure to RF magnetic fields in NMR offered by several safety agencies are based upon the assumption of such heating effects. The power deposited by RF pulses is most commonly quantified by determining the so-called specific absorption rate (SAR), which is the mass- and time-normalized rate at which RF power is deposited in biological tissue. The specific absorption rate is typically expressed in units of watts per kilogram. It can be shown that the deposited power  $P$  during a RF pulse of length  $T$  is proportional to the square of the main magnetic field strength and the irradiating RF field according to:

$$P \propto \omega_0^2 \int_0^T B_1^2(t) dt \quad (5.51)$$

making the SAR over the repetition time  $TR$  of the sequence equal to:

$$SAR = \frac{t_{RF}}{TR} \times \frac{P}{m} \quad (5.52)$$

where  $m$  equals the mass and  $(t_{RF}/TR)$  is the duty cycle for the RF pulses. While Equations (5.51) and (5.52) appear relatively straightforward, the actual calculation is complex [66,67] due to spatial variations in tissue resistivity and  $B_1$  magnetic field strength, which is especially apparent at high magnetic fields (see Chapter 10). However, Equations (5.51) and (5.52) do give a qualitative insight into means of RF power management. For instance, increasing the pulse length of a RF pulse while keeping the nutation angle constant reduces the power deposition. Furthermore, decreasing the RF duty cycle, for instance by increasing the repetition time  $TR$ , reduces the SAR. The mass in Equation (5.52) should be used with care, as it is the mass that is 'seen' by the RF coil. Therefore, when determining the RF power deposition for a head volume coil, the effective mass of the head, rather than the total body mass, should be used.

Current American (FDA) safety guidelines specify limits on the specific absorption rate of less than  $4 \text{ W kg}^{-1}$  for whole body over 15 min,  $3 \text{ W kg}^{-1}$  averaged over the head for 10 min,  $8 \text{ W kg}^{-1}$  in any gram of tissue in the head or torso for 15 min, and  $12 \text{ W kg}^{-1}$  in any gram of tissue in the extremities for 15 min. Especially for high-field human applications this puts limits on the minimum attainable repetition time, the maximum number of RF pulses and the type of RF pulses. Since direct calculation of the amount of deposited RF power is difficult on a subject-specific basis, the current method of monitoring and limiting SAR is to measure the power output of the RF amplifier. A conservative approach would then be to assume that all the power coming from the RF amplifier is deposited into the tissue. However, the amount of power transferred to the biological tissue inside the coil can be readily determined by measuring the loaded and unloaded quality factors,  $Q_{\text{loaded}}$  and  $Q_{\text{unloaded}}$ , of the RF coil (see Chapter 10 for more information on quality factors), respectively. The amount of power absorbed by the tissue is then given by:

$$P_{\text{tissue}} = P_{\text{amplifier}} \left( 1 - \frac{Q_{\text{loaded}}}{Q_{\text{unloaded}}} \right) \quad (5.53)$$

where  $P_{\text{amplifier}}$  is the power output of the RF amplifier, corrected for power losses between the amplifier and RF coil. Therefore, only when the RF coil is significantly loaded with the sample (i.e. large drop in  $Q$  upon loading) will the majority of RF power be deposited into the tissue. One caveat with Equation (5.53) is that it only predicts the total amount of power deposited into the tissue, it does not provide any information on where the power is deposited. Therefore even though the total power may be well below safety guidelines it is possible that ‘local hotspots’ may receive RF power in excess of regulations. This is especially the case at higher magnetic fields, where the  $B_1$  magnetic field becomes spatially heterogeneous (see Chapter 10). ‘Local hotspots’ can be determined through  $B_1$  mapping. While most MR systems take a conservative approach to RF power management, the drive towards higher magnetic fields will fuel the development of more accurate means of determining the spatial distribution of SAR (see also Exercise 5.5).

One of the most effective ways of reducing RF power is to minimize the average power of a RF pulse by distributing the RF pulse amplitude more uniformly over the pulse length. Conolly *et al.* [68] have presented a procedure which recalculates the pulse profile of spatially selective pulse in order to reduce the SAR. Their procedure of variable-rate selective excitation (VERSE) is based on the fundamental Larmor equation, which indicates that the rotation of magnetization about a magnetic field is proportional to the magnetic field strength and the duration. Therefore, the same on-resonance rotation can be achieved by many different combinations of magnetic field strength and duration, as long as their product is constant. This principle can be derived more quantitatively by considering the Bloch equations (ignoring relaxation):

$$\frac{d\mathbf{M}(\mathbf{r}, t)}{dt} = \mathbf{M}(\mathbf{r}, t) \times \gamma \mathbf{B}(\mathbf{r}, t) \quad (5.54)$$

where  $\mathbf{B}(\mathbf{r}, t) = (B_{1x}(t), B_{1y}(t), G(\mathbf{r}))$  and  $B_{1x}(t)$  and  $B_{1y}(t)$  are the real and imaginary components of the RF field and  $G(\mathbf{r})$  is the magnetic field gradient. Next define an arbitrary function of time  $\tau(t)$  and let  $\mathbf{M}^*(\mathbf{r}, t) = \mathbf{M}(\mathbf{r}, \tau(t))$ .  $\mathbf{M}^*(\mathbf{r}, t)$  is the magnetization at any time  $t$  during the recalculated RF (and gradient) pulse. Conolly *et al.* [68] have assumed a constant-time VERSE algorithm, which implies  $\tau(T) = T$  and  $\mathbf{M}^*(\mathbf{r}, T) = \mathbf{M}(\mathbf{r}, \tau(T)) = \mathbf{M}(\mathbf{r}, T)$ , i.e. identical slice profiles reached at different time traversal schedules. The Bloch equations are now given by:

$$\frac{d\mathbf{M}^*(\mathbf{r}, t)}{dt} = \frac{d\mathbf{M}(\mathbf{r}, \tau(t))}{d\tau} \left( \frac{d\tau(t)}{dt} \right) = \mathbf{M}^*(\mathbf{r}, t) \times \gamma \mathbf{B}(\mathbf{r}, \tau(t)) \left( \frac{d\tau(t)}{dt} \right) \quad (5.55)$$

From Equation (5.55) it follows that identical slices are excited when the new RF and gradient waveforms  $B_1^*(t)$  and  $G^*(\mathbf{r}, t)$  are calculated from the original waveforms  $B_1(t)$  and  $G(\mathbf{r}, t)$  according to:

$$\mathbf{B}_1^*(t) = \mathbf{B}_1(\tau(t)) \left( \frac{d\tau(t)}{dt} \right) \quad (5.56)$$

$$\mathbf{G}^*(\mathbf{r}, t) = \mathbf{G}(\mathbf{r}, \tau(t)) \left( \frac{d\tau(t)}{dt} \right) \xrightarrow{\mathbf{G}(\mathbf{r}, \tau(t))=1.0} \tau(t) = \int_0^T \mathbf{G}^*(\mathbf{r}, t) dt \quad (5.57)$$

By taking  $G(\mathbf{r}, t) = \text{constant}$  (e.g. = 1.0), which is normally the situation, the function  $\tau(t)$  can be calculated as the integral of the new gradient waveform. Conolly *et al.* [68] have



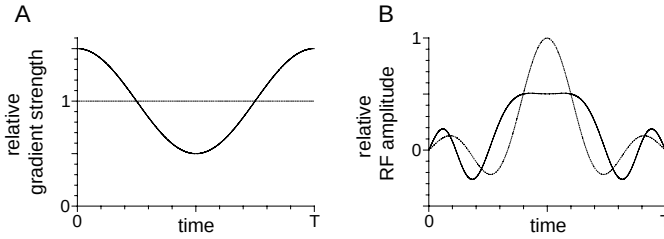


**Figure 5.34** Example of VERSE optimization. (A) The original sinc pulse excites a spatial slice in the presence of a constant magnetic field gradient (dotted line). The VERSE gradient waveform was chosen as  $G(t) = G_{\max}(0.5\cos(2\pi t/T) + 1)$  with  $0 \leq t \leq T$  (solid line). Note that the VERSE gradient has an average value which equals that of the original gradient waveform. (B) Using Equations (5.56) and (5.57) the VERSE  $B_1$  modulation can be recalculated. Because the RF amplitude is more uniformly spread over the pulse duration, the average and peak power of the VERSE pulse (solid line) are only 26 and 64% of the original pulse (dotted line), respectively.

derived algorithms to evaluate Equations (5.56) and (5.57) in order to obtain a minimum SAR RF pulse [i.e. minimize Equation (5.52)]. However, significant SAR reductions can also be obtained by simple trial-and-error methods as shown in Figure 5.34. By executing relatively high and low gradients when the RF pulse has relatively low and high amplitudes, respectively, the RF amplitude is more uniformly distributed over the pulse length leading to a reduction in peak power, but also of the average power. Note, that the continuous-time variable-rate theorem given above is only valid for on-resonance magnetization vectors. Off-resonance, the slice profile is slightly degraded, but acceptable for typical frequency offsets encountered in *in vivo*  $^1\text{H}$  MRS. An advantage of gradient modulation is that the displacement of the slice due to off-resonance effects (i.e. the chemical shift artifact, see Chapter 6) is reduced.

The VERSE algorithm is readily applied to adiabatic RF pulses as demonstrated by Conolly *et al.* [68, 69] and Slotboom *et al.* [52, 70]. Others methods to reduce the power deposition of RF pulses include the use of phase-modulation [19, 21] and numerical optimization [43].

## 5.10 Multidimensional RF Pulses

The concept of multidimensional spatially selective RF pulses was proposed by Bottomley and Hardy [71, 72]. Two years later, Pauly *et al.* [73] provided a convenient notation based on the k-space formalism used in MRI (see Chapter 4) for the description and further development of multidimensional RF pulses.

The theory of multidimensional RF pulses is based on the small nutation angle approximation as discussed in Section 5.3. In analogy with Equation (5.21), the transverse magnetization at spatial position  $\mathbf{r}$  at the end of a (time-dependent) RF pulse in the presence

of a time-varying magnetic field gradient can be described by:

$$M_{xy}(\mathbf{r}) = \gamma M_0 \int_0^T B_1(t) e^{i\phi(t)} e^{-i\gamma \mathbf{r} \cdot \int_t^T \mathbf{G}(t') dt'} dt \quad (5.58)$$

where  $T$  is the pulse length. The  $k$ -space formalism is readily incorporated into Equation (5.58), since the spatial frequency variable  $\mathbf{k}(t)$  is defined as:

$$\mathbf{k}(t) = \gamma \int_t^T \mathbf{G}(t') dt' \quad (5.59)$$

$\mathbf{k}(t)$  parametrically describes a 'path' through spatial frequency space (or  $k$ -space). Incorporation of Equation (5.59) into Equation (5.58) leads to:

$$M_{xy}(\mathbf{r}) = \gamma M_0 \int_0^T B_1(t) e^{i\phi(t)} e^{-i\mathbf{k}(t) \cdot \mathbf{r}} dt \quad (5.60)$$

Since Equation (5.60) is rather difficult to interpret, Pauly *et al.* [73] have rewritten it to a conceptually more useful expression given by:

$$M_{xy}(\mathbf{r}) = \gamma M_0 \int_{\mathbf{k}} W(\mathbf{k}) S(\mathbf{k}) e^{-i\mathbf{k} \cdot \mathbf{r}} d\mathbf{k} \quad (5.61)$$

where

$$W(\mathbf{k}(t)) = \frac{B_1(t) e^{i\phi(t)}}{|\gamma \mathbf{G}(t)|}$$

and

$$S(\mathbf{k}) = \int_0^T \left\{ \delta(\mathbf{k}(t) - \mathbf{k}) \left| \frac{d\mathbf{k}(t)}{dt} \right| \right\} dt \quad (5.62)$$

Equation (5.61) expresses the fact that the transverse magnetization is the Fourier transform of a spatial frequency weighting function  $W(\mathbf{k})$  multiplied by a spatial frequency sampling function  $S(\mathbf{k})$ .  $S(\mathbf{k})$  may be thought of as a sampling path of  $k$ -space. It determines both the area and density of the sampled  $k$ -space. The requirements for the  $k$ -space trajectory are identical to those for MRI, i.e. the trajectory should uniformly cover the part of  $k$ -space where the spatial frequency weighting function has significant energy and it should cover that region with sufficient density to avoid aliasing. Most often, the  $k$ -space trajectory and desired excitation profile are known, such that the required RF waveform can be calculated as the inverse Fourier transform of the spatial excitation profile:

$$B_1(t) e^{i\phi(t)} = -i |\mathbf{G}(t)| \int_{\mathbf{r}} \frac{M_{xy}(\mathbf{r})}{M_0} e^{i\mathbf{k}(t) \cdot \mathbf{r}} d\mathbf{r} \quad (5.63)$$

One of the most commonly used k-space trajectories is a constant angular rate spiral trajectory, which is described by:

$$\begin{aligned} k_x &= k_{\max} \left( 1 - \frac{t}{T} \right) \cos \left( \frac{2\pi n t}{T} \right) \\ k_y &= k_{\max} \left( 1 - \frac{t}{T} \right) \sin \left( \frac{2\pi n t}{T} \right) \end{aligned} \quad (5.64)$$

which is graphically shown in Figure 5.35A. The parameter  $n$  determines the number of k-space cycles, typical values are 8 to 16. The discrete coverage of k-space will lead to radial sidelobes, analog to aliasing due to a limited sampling rate. The number of cycles  $n$  determines the position of the first aliasing sidelobe. The k-space trajectory described by Equation (5.64) is established by magnetic field gradients which are shaped according to:

$$\begin{aligned} G_x(t) &= -\frac{k_{\max}}{\gamma T} \left[ 2\pi n \left( 1 - \frac{t}{T} \right) \sin \left( \frac{2\pi n t}{T} \right) + \cos \left( \frac{2\pi n t}{T} \right) \right] \\ G_y(t) &= \frac{k_{\max}}{\gamma T} \left[ 2\pi n \left( 1 - \frac{t}{T} \right) \cos \left( \frac{2\pi n t}{T} \right) - \sin \left( \frac{2\pi n t}{T} \right) \right] \end{aligned} \quad (5.65)$$

Equation (5.65) is graphically shown in Figure 5.35A. To minimize gradient switching effects (i.e. eddy currents), the  $G_y$  gradient should be ramped to reach the maximum gradient strength of the first point. Assuming that k-space is covered uniformly,  $S(k)$  can be neglected and the required  $B_1$  profile for a given excitation profile can be calculated with Equation (5.63) [or alternatively Equation (5.62)]. The most simple 2D RF pulse generates a Gaussian excitation profile (since the Fourier transform of a Gaussian equals a Gaussian) and is given by:

$$\begin{aligned} B_1(t) &= W(k(t)) |\gamma G(t)| \\ &= C e^{-\beta^2(1-t/T)^2} \sqrt{\left[ 2\pi n \left( 1 - \frac{t}{T} \right) \right]^2 + 1} \end{aligned} \quad (5.66)$$

where  $\beta$  and  $C$  are constants. The  $B_1$  profile is drawn in Figure 5.35A for  $\beta = 2$ . Figure 5.35B shows simulations based on the Bloch equations for a  $90^\circ$  nutation angle. Even though the derived formalism is only valid within the small nutation angle regime (i.e.  $\theta < 30^\circ$ ), the excitation performs very well for nutation angles in the order of  $90^\circ$ . Pauly *et al.* [74] have given a formal description for the generation of larger nutation angle multidimensional RF pulses. Note that the excitation profile is inherently refocused.

Until this point it was assumed that k-space was sampled uniformly, such that  $S(k(t))$  could be ignored and  $W(k(t))$  would be an accurate representation of the desired excitation profile. However, if k-space is sampled nonuniformly (as is the case with almost all commonly used k-space trajectories) the observed profile will be a convolution of the desired profile  $W(k(t))$  with the k-space density sampling function  $S(k(t))$ . In general this will lead to a broadening ('smearing') of the observed profile. Hardy *et al.* [75] have proposed a method to analytically determine the k-space density of a particular k-space trajectory and derive a compensation factor  $\delta(k(t))$ . For a spiral trajectory,  $\delta(k(t))$  is given

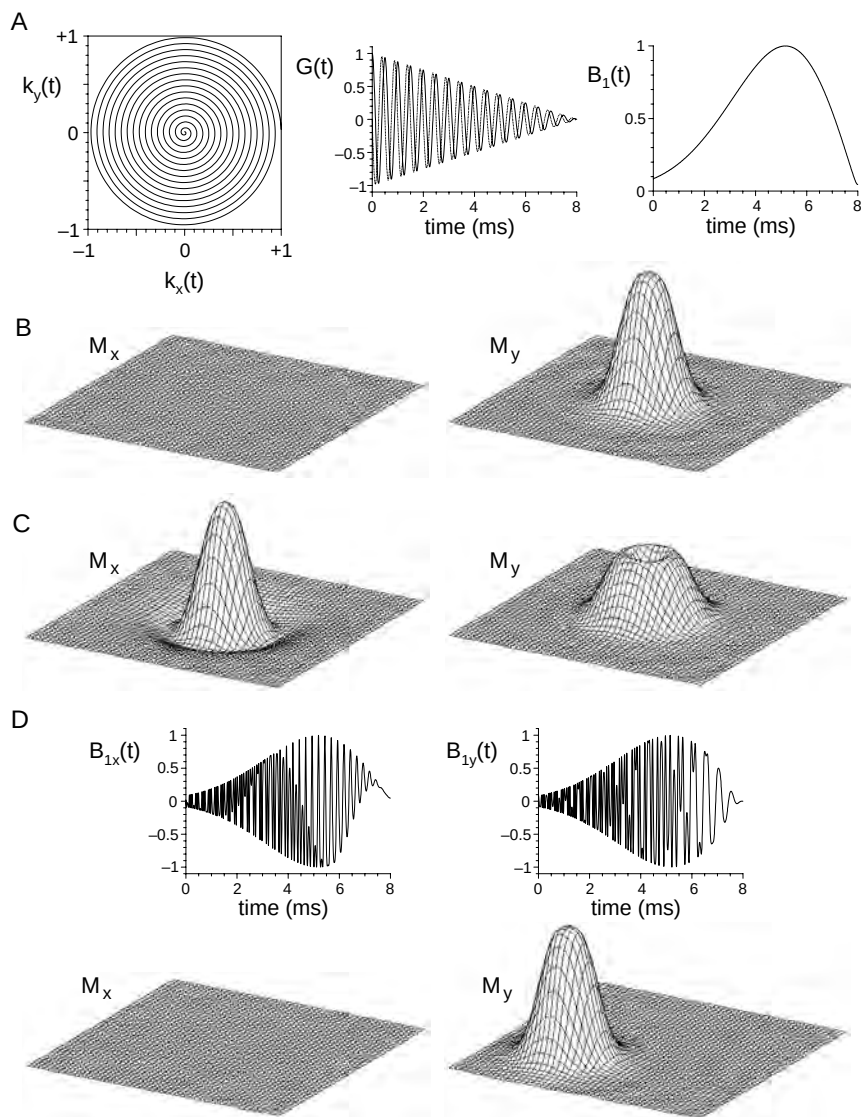


**Figure 5.35** Characteristics of 2D RF pulses. (A) Spiral ( $n = 16$ )  $k$ -space sampling scheme (left) and the corresponding constant angular rate magnetic field gradients (middle,  $G_x$  = solid line,  $G_y$  = dashed line). To excite a symmetrical, Gaussian profile in the gradient isocenter, the RF modulation is real-valued (right). (B) On-resonance excitation profile of the pulse as shown in (A). Note that the excitation profile is inherently refocused. (C) Excitation profile 100 Hz off-resonance. The profile is no longer refocused and is slightly broadened. (D) Excitation out of the gradient isocenter (or excitation of asymmetrical profiles) is accompanied by a complex RF modulation as described by Equation (5.68).

by:

$$\delta(k(t)) = \frac{\sqrt{[2\pi n(1 - t/T)]^2 + 1}}{2\pi n(1 - t/T)} \quad (5.67)$$

Correction factors for other k-space trajectories are described by Hardy *et al.* [75]. Because spiral k-space trajectories have a relatively uniform density distribution, the effect of Equation (5.67) is not very large for  $n > 8$ . However, for  $n < 8$  and for other k-space trajectories (i.e. radial, pinwheel, Lissajous) the compensation can significantly improve the observed excitation profile [75].

The position of the excited region can be shifted to a position  $(x_0, y_0)$  through the relationship:

$$B'_1(t) = B_1(t)e^{i\phi(t)}$$

where

$$\phi(t) = \gamma \int_0^t [x_0 G_x(t') + y_0 G_y(t')] dt' \quad (5.68)$$

i.e. for asymmetrical excitation profiles, the RF amplitude becomes complex. By linearly combining and scaling the two gradients, the excited region can be rotated and scaled [Figure 5.35D].

The above presented derivations and simulations did not take off-resonance effects into account. However, off-resonance effects have prevented the widespread implementation of multidimensional RF pulses in *in vivo* MRS applications. Figure 5.35C shows simulations of a Gaussian shaped 2D 90° excitation profile (compare Figure 5.35B) for a 8 ms RF pulse, 100 Hz off-resonance. The main effect for the relatively simple Gaussian RF pulse is that the excitation profile is no longer inherently refocused, i.e.  $M_x \neq 0$ , leading to a phase shift in the spectral dimension. However, for more complicated excitation profiles (e.g. Figure 5.36), off-resonance modulation may have a more detrimental effect in that the excitation profile is shifted and broadened, leading to significant localization errors.

The main reason for the strong deterioration of the excitation profile by off-resonance effects is the relatively long pulse length of multidimensional RF pulses. The pulse length is primarily determined by the maximum available gradient strengths and the maximum allowable gradient slew rates. Several methods have been designed to decrease the pulse length and hence the sensitivity to off-resonance effects [75, 76].

Hardy and Cline [76] have developed a method which optimizes the gradient waveform in terms of gradient slew rates. The slew rate of the commonly used gradient waveforms given by Equation (5.65) decreases as the gradient amplitude decreases. By numerically and analytically evaluating the gradient slew rate  $s(t) = (dG(t)/dt)$ , they were able to design a gradient waveform with a constant slew rate and with a circa 33 % reduction of the pulse length.

Another method to reduce the pulse length of multidimensional RF pulses is to divide the entire RF pulse into smaller segments [77, 78]. An identical end result, with improved off-resonance performance is then obtained by adding the NMR signal in successive excitations. Hardy and Bottomley have employed this principle to obtain  $^{31}\text{P}$  localization

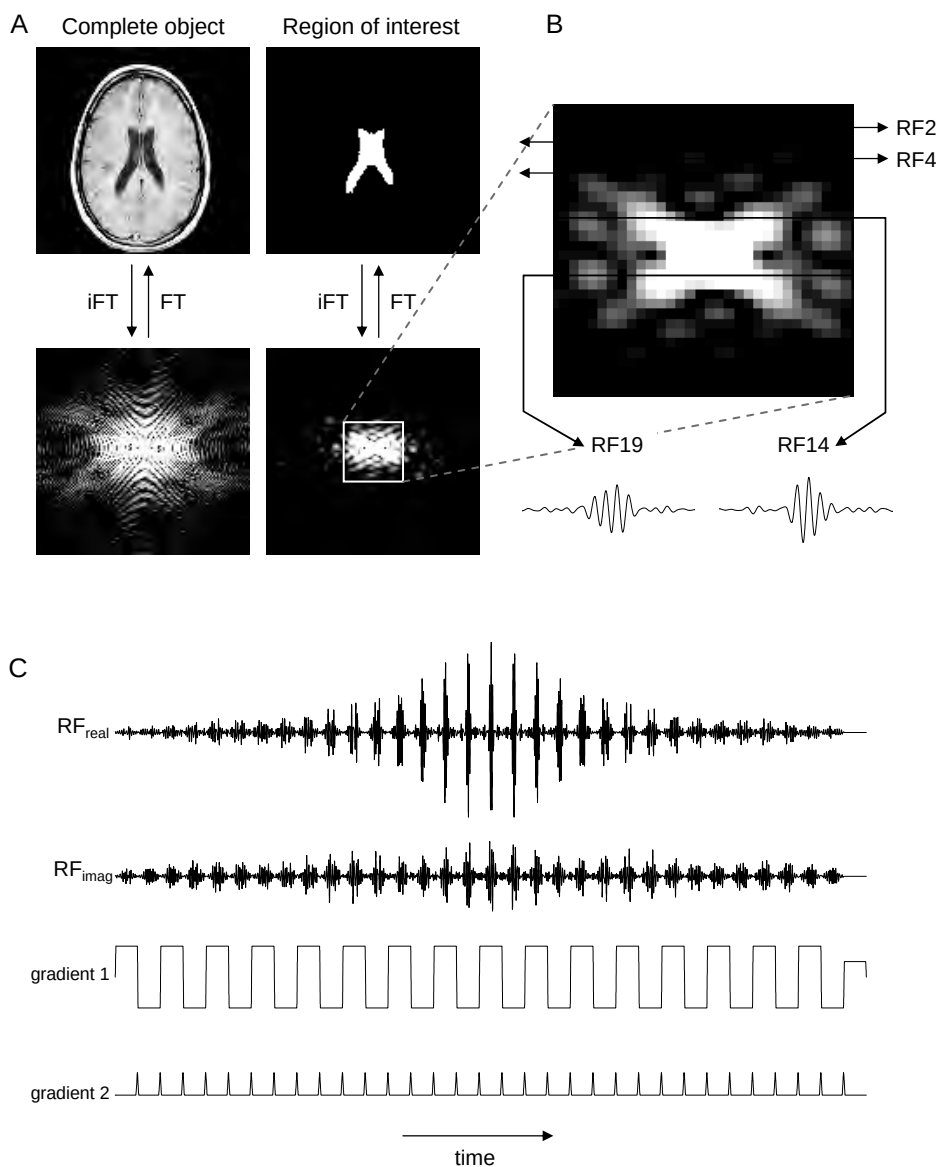


**Figure 5.36** Multidimensional RF pulse design using  $k$ -space analysis. (A) Image space and spatial frequency of  $k$ -space are related by Fourier transformations, similar to the relation between (low nutation angle) RF pulses and their frequency profile. (B) Excitation of arbitrary shapes, like the ventricles as shown in (A) can be achieved when the RF is modulated as the  $k$ -space intensity while  $k$ -space is traversed by magnetic field gradient modulation, as shown in (C). The complex RF modulation based on the  $k$ -space signals in (B) in concert with the EPI-like  $k$ -space trajectory will lead to the excitation of a 2D spatial area closely corresponding to the ventricles as shown in (A).

of the human heart on a clinical MR system [76]. They showed that the combination of slow rate maximization and RF pulse segmentation improves the effective (spectral) bandwidth from  $<1$  ppm to  $>20$  ppm. Qin *et al.* [78] have extended this principle to  $^1\text{H}$  NMR spectroscopy of arbitrarily shaped, localized volumes in rat brain at 4.0 T.

The Fourier relationship between the RF pulse shape/k-space sampling and the excitation profile is readily extended from simple excitation profiles as shown in Figure 5.35 to complex, arbitrarily shaped regions of interest (ROIs). Figure 5.36 shows the principle steps in the calculation of a 2D RF pulse aimed at the excitation of the ventricular compartment. From an anatomical MR image (Figure 5.36A) the ventricular ROI is selected, which can equally well be represented in k-space. The RF pulse shapes are directly proportional to the complex k-space signals when the k-space trajectory is given by a back-and-forth ‘blipped’ EPI trajectory (Figure 5.36B). Executing the RF in concert with oscillating magnetic field gradients as shown in Figure 5.36C excites the ROI shown in Figure 5.36A, convolved with the point spread function of the limited k-space sampling pattern.

### 5.11 Spectral-spatial RF Pulses

All conventional, amplitude modulated RF pulses exhibit some degree of spectral selectivity, the selectivity being proportional to the pulse length. When these RF pulses are executed in the presence of a magnetic field gradient the spectral selectivity is lost. Instead a frequency offset gives rise to a displacement of the spatial slice, the well-known chemical shift artifact (see Chapter 6).

Using a k-space interpretation of small nutation excitation as presented in Section 5.10, it is possible to design RF pulses that are both spatially and spectrally selective. These so-called spectral-spatial pulses eliminate the chemical shift artifact and can substitute combinations of conventional RF pulses which produce spatial and spectral selectivity (e.g. water suppressed, localized  $^1\text{H}$  MRS or fat/water imaging). A detailed theoretical description of spectral-spatial RF pulses will not be given since it largely follows the earlier discussed k-space excitation formalism. A concise theoretical description, as well as experimental applications, are given by Meyer *et al.* [79] and Spielman *et al.* [80]. Here the resulting RF amplitude and gradient modulation functions will be given. For a 1D spectral-spatial pulse, k-space should be sampled in one spatial dimension, e.g.  $k_z$ , and the spectral dimension,  $k_\omega$ . The spectral dimension is automatically sampled as the pulse progresses. An oscillating  $G_z$  gradient samples the spatial dimension of k space, while retaining the spectral selectivity. For a given gradient modulation, the RF modulation can be calculated in a similar manner as presented for multidimensional RF pulses. Gaussian profiles in the spectral and spatial dimensions are generated by the following the RF modulation:

$$B_1(t) = B_{1\max} e^{-\pi[\sin(2\pi n(t-T))/A]^2} e^{-\pi[(t-T/2)/B]^2} |\cos(2\pi n(t-T))| \quad (5.69)$$

in the presence of a sinusoidally modulated magnetic field gradient

$$G(t) = G_{\max} \cos(2\pi n(t-T)) \quad (5.70)$$

A and B are parameters which determine the spectral and spatial slice characteristics produced by the RF pulse. Figure 5.37A shows a spectral-spatial pulse for  $A = 0.8$  and

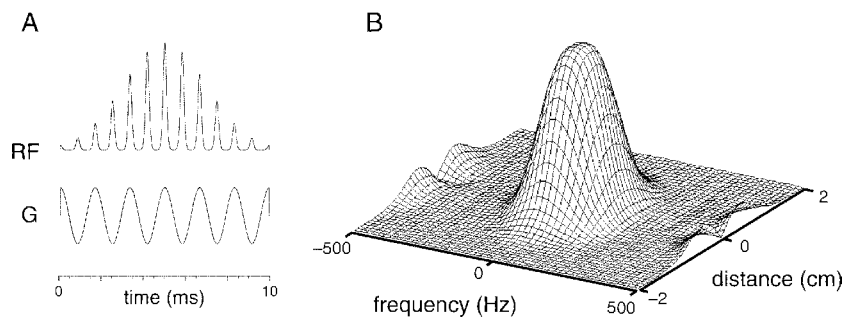


**Figure 5.37** (A) Spectral-spatial pulse as described by Equations (5.69) and (5.70) for  $A = 0.8$  and  $B = 0.5$ . Note that in practice gradient ramps need to proceed and follow the first and last points of the gradient, respectively. (B) Excitation profile of the pulse shown in (A). In the spectral domain aliasing sidelobes are observed, which are absent in the spatial domain.

$B = 0.5$ . Figure 5.37B shows the  $90^\circ$  excitation profile of the pulse shown in Figure 5.37A in both the spectral and spatial dimensions. To achieve more rectangular slice profiles, the RF pulse [i.e. Equation (5.69)] can be modulated according to a sinc function or other optimized functions. In the spectral dimension, several side lobes can be observed due to the discrete sampling of  $k_\omega$  (i.e. one pulse for each half cycle of the oscillating magnetic field gradient). Because  $k_z$  is sampled continuously, no spatial side lobes are observed in the spatial dimension. The spectral selectivity can be used for selective excitation of one resonance (e.g. NAA) while simultaneously suppressing another (e.g. water). Because of the narrow bandwidth in the spectral dimension, which is caused by the long pulse length dictated by the maximum achievable slew rate, only resonances which resonate close to each other (e.g. NAA and lactate) can be simultaneously excited. A sinusoidally modulated magnetic field gradient is not optimal in terms of slew rate characteristics. Shorter pulse lengths can be achieved by triangular modulated gradients.

Note that spectral-spatial pulses are not inherently refocused. In the limit of the small nutation approximation the acquired phase in both the spectral and spatial dimension is linear. Thus the spatial phase dispersion can be corrected by gradient reversal and the spectral response can be refocused by a  $180^\circ$  RF pulse.

## 5.12 Exercises

**5.1** Longitudinal magnetization can be ‘excited’ into the transverse plane by a  $90^\circ$  (or  $\pi/2$ ) pulse.

**A** If the pulse length of the square  $90^\circ$  pulse is 1.0 ms, what is the required  $B_1$  magnitude in  $\mu\text{T}$  to achieve excitation?

**B** How many Larmor precession cycles will occur in the laboratory frame at  $B_0 = 3.0$  T during the  $90^\circ$  excitation pulse?



- 5.2 A birdcage RF coil generates a  $B_1$  field of  $37.6 \mu\text{T}$  at the maximum amplifier output. Calculate the shortest possible pulse length to achieve signal inversion with a 'square' RF pulse.
- 5.3 Consider a multislice spin-warp sequence with 1 ms SLR90 linear ( $R = 6$ ) and 2 ms SLR180 ( $R = 12$ ) pulses for excitation and refocusing, respectively (see Table 5.1).
- A Calculate the slice selection gradient strengths (in  $\text{Hz cm}^{-1}$ ) during each pulse in order to select 2 mm thick slices.
  - B Assuming constant slope ( $150 \mu\text{s}$  from 0 to 100 %) gradients, calculate the  $90^\circ$  slice refocusing gradient strength for a plateau duration of 0.5 ms.
  - C Repeat the calculation under (B) when excitation is performed with a minimum-phase SLR 90 ( $R = 18$ ) excitation pulse. Hint: Use Figure 5.13 to estimate the net phase evolution during the pulse.
- 5.4 A Derive an expression for the on-resonance  $B_1$  dependence of the longitudinal magnetization following a composite MLEV pulse ( $90^\circ_x 180^\circ_y 90^\circ_x$ ).
- B Calculate the range of nutation angles (between  $0^\circ$  and  $180^\circ$ ) for which the longitudinal magnetization is at least 99 % inverted (i.e.  $M_z/M_0 < -0.98$ ) following a MLEV pulse.
  - C Explain the mechanism of compensation in terms of  $M_x$ ,  $M_y$  and  $M_z$  components following the MLEV pulse.
  - D Calculate the  $B_1$  insensitivity of MLEV relative to a square pulse for the 99 % inversion interval.
  - E Show that the on-resonance nutation angle generated by a composite WALTZ pulse ( $90^\circ_x 180^\circ_{-x} 270^\circ_x$ ) has the same sensitivity towards RF inhomogeneity as a regular  $180^\circ$  pulse.
- 5.5 Consider a PRESS sequence executed with 2 ms sinc-shaped RF pulses for excitation and refocusing.
- A Calculate the relative change in RF power deposition when the pulse lengths are increased to 4 ms.
  - B Calculate the relative change in RF power deposition when the pulses are changed to linear phase SLR excitation and refocusing pulses with  $R = 6$ .
  - C Given a maximum RF amplitude and pulse length of 1500 Hz and 5 ms, respectively, calculate the RF pulse combination (using the pulses tabulated in Table 5.1) that gives the smallest chemical shift displacement.
  - D Calculate the RF power deposition of the pulse combination obtained under C relative to the default pulses (i.e. 2 ms sinc).
  - E Calculate the relative change in RF power deposition when the echo- and repetition times are reduced and increased by a factor of two, respectively.
- 5.6 A A 2 ms square pulse was calibrated to produce a  $90^\circ$  nutation angle at a RF power of 23 dB. Calculate the RF power setting for a 1 ms sinc pulse producing a  $180^\circ$  nutation angle.
- B A given RF coil was calibrated to produce a 1310 Hz  $B_1$  magnetic field at a RF power setting of 10 dB. Calculate the RF power setting for 1 ms sinc inversion pulse.
- 5.7 Consider a 10 ms AFP pulse with sech/tanh modulation and  $R = 60$ . Over the  $B_1$  amplitude range in which the adiabatic condition is satisfied, qualitatively describe

what happens to the longitudinal and transverse magnetization during the pulse (starting from a thermal equilibrium state) for:

- A** a frequency offset of +30 kHz;
- B** a frequency offset of +3 kHz;
- C** a frequency offset of +0.3 kHz;
- D** a frequency offset of −3 kHz.
- E** Describe how the rotations at each frequency can be used for specific applications and discuss if the same could be achieved with AFP pulses based on tanh/tan modulation.

**5.8** Consider a 16.02 ms optimized SLR 90° pulse ( $R = 6.0$ ), segmented into 81 RF pulses of 20  $\mu$ s each interleaved with 180  $\mu$ s delays.

- A** Calculate the excitation bandwidth of the center excitation band.
- B** Calculate the frequency difference between adjacent excitation bands.
- C** Calculate the power deposition of the DANTE SLR pulse relative to a conventional SLR pulse of equal bandwidth.
- D** Calculate the timings/segments of a new pulse in which the excitation bandwidth is only half as wide, but with equal separation between the excitation bands.

**5.9** A PRESS sequence is executed with 3 ms optimized SLR90 ( $R = 12$ ) and SLR180 ( $R = 12$ ) excitation and refocusing pulses, respectively. Signal is acquired from a  $2 \times 2 \times 2$  cm = 8 ml volume in the human occipital cortex with TR = 1500 ms and TE = 20 ms.

- A** Given a maximum gradient amplitude of 40 mT m<sup>−1</sup> and constant-time gradient ramping of 500  $\mu$ s, calculate the amplitude of a trapezoidal slice selection refocusing gradient with a 1 ms plateau period. Note that the ramp time during a constant-time gradient is independent of the gradient amplitude.
- B** Recalculate the slice selection refocusing gradient amplitude when the sequence is executed with constant-slope gradient ramping of 80 mT m<sup>−1</sup> ms<sup>−1</sup>. Note that the ramp time during a constant-slope gradient scales linearly with the gradient amplitude.
- C** Repeat the calculations under (A) and (B) for a minimum phase SLR90 ( $R = 12$ ) pulse for which the effective dephasing pulse length equals T/10.

**5.10**

- A** Derive Equation (5.8) from Equations (5.5)–(5.7).
- B** Derive Equations (5.10)–(5.12) from Equations (5.5)–(5.9).
- C** Derive Equation (5.43) using Equations (5.5)–(5.9).

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# 6

## Single Volume Localization and Water Suppression

### 6.1 Introduction

Restricting signal detection to a well-defined region of interest (ROI) is crucial for meaningful *in vivo* NMR spectroscopy. First and foremost, spatial localization is used to remove unwanted signals from outside the ROI, like extracranial lipids in MRS applications of the brain. However, spatial localization has many additional beneficial effects, in particular in managing tissue and magnetic field heterogeneity. For example, at a macroscopic level the brain can be segmented into gray matter, white matter and CSF, each of which has unique metabolic profiles. Careful positioning of the localized volume can minimize ‘partial volume effects’ (i.e. contamination of signal from one compartment by signal from another compartment), thereby providing a more genuine tissue characterization. Additional benefits of spatial localization originate from the fact that variations in  $B_0$  and  $B_1$  magnetic fields are greatly reduced over the small localized volume, thereby providing narrower spectral lines and more uniform signal excitation and reception, respectively.

Figure 6.1A and B show unlocalized  $^1\text{H}$  and  $^{31}\text{P}$  NMR spectra from newborn piglet brain, respectively. The  $^1\text{H}$  NMR spectrum is acquired with a combination of water suppression techniques (as will be discussed in Section 6.3). Figure 6.1C and D show the corresponding localized  $^1\text{H}$  and  $^{31}\text{P}$  NMR spectra. Clearly, the unlocalized  $^1\text{H}$  NMR spectrum is contaminated by large signals from extracranial lipids, such that cerebral metabolite resonances can not be observed. Furthermore, because the  $B_0$  magnetic field homogeneity over a large unlocalized area is difficult to optimize, the resonances are broad, thereby further reducing the spectral resolution and degrading the water suppression. Upon spatial localization, the overwhelming lipid resonances are removed and the  $B_0$  magnetic field homogeneity has improved, given a recognizable  $^1\text{H}$  NMR spectrum of the brain. Besides the broad  $^{31}\text{P}$  NMR signal from bone, the unlocalized and localized  $^{31}\text{P}$  NMR spectra do not differ as

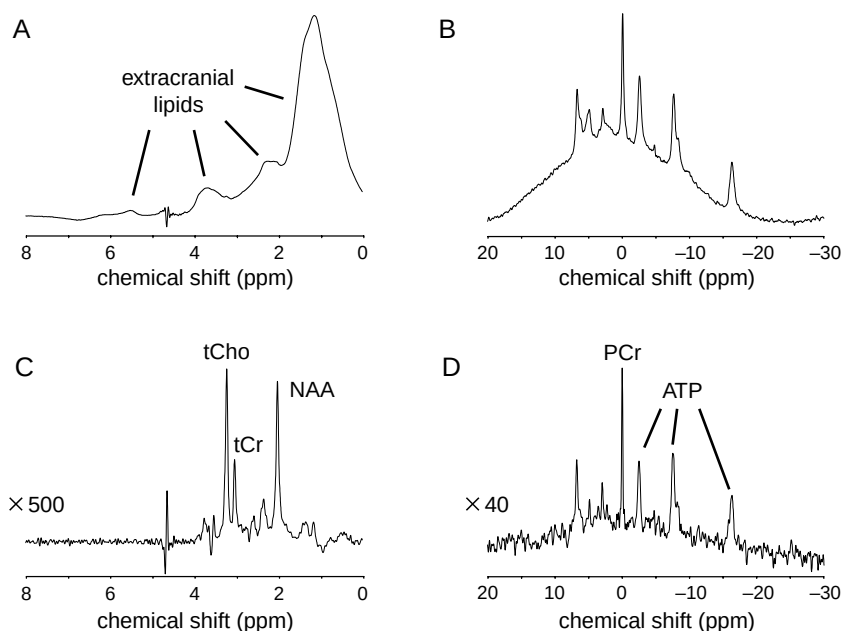


**Figure 6.1**  $^1\text{H}$  and  $^{31}\text{P}$  NMR spectra from newborn piglet brain in vivo. (A) Unlocalized  $^1\text{H}$  and (B)  $^{31}\text{P}$  NMR spectra. The presence of unwanted signal and the low magnetic field homogeneity over a large volume decrease the spectral resolution. (C) PRESS localization (Section 6.2.4,  $TE = 144$  ms,  $1000\ \mu\text{l}$ ) for  $^1\text{H}$  and (D) ISIS localization (Section 6.2.1,  $1000\ \mu\text{l}$ ) for  $^{31}\text{P}$  allow the unambiguous detection of metabolites with enhanced spectral resolution.

much as the corresponding  $^1\text{H}$  NMR spectra. However, the ratio between PCr and ATP has been reduced upon localization, indicating that  $^{31}\text{P}$  signals from extracranial muscle tissue (with a high PCr/ATP ratio) have been excluded. Furthermore, as in the case of  $^1\text{H}$  MRS, all the  $^{31}\text{P}$  NMR resonances are narrower in the localized spectrum.

It can be concluded from Figure 6.1 that spatial localization does not only allow the unambiguous detection of metabolites from a well-defined spatial volume, but also increases the spectral resolution by: (1) narrower resonances; (2) exclusion of broad unwanted resonances; and (3) improved water suppression (for  $^1\text{H}$  MRS).

In the last two decades, a wide variety of spatial localization techniques have been developed. To avoid getting lost in the maze of acronyms, they are divided into single- and multivoxel techniques. Single voxel techniques, in which signal is acquired from a single spatial volume will be described in this chapter, while multivoxel techniques are discussed in Chapter 7.

Spatially dependent magnetic field gradients are at the heart of spatial localization methods. Several of the earlier methods utilize magnetic field gradients in the excitation RF field  $B_1$  [1–9], based on the classical rotating frame zeugmatography experiment by Hoult [1]. However, the localized volume obtained with  $B_1$ -gradient-based localization

techniques is typically inferior to that obtained with  $B_0$ -gradient-based methods. As a result, almost all spatial localization requirements are currently addressed with  $B_0$ -gradient-based methods, as will be described in the first part of this chapter. For more information on  $B_1$ -based localization, the reader is referred to the literature [1–10].

## 6.2 Single Volume Localization

All localization methods based on  $B_0$  magnetic field gradients rely on the selection of a spatially selective slice by the application of a frequency-selective RF pulse in the presence of a  $B_0$  magnetic field gradient (see Section 4.2). Although many combinations of RF pulses and  $B_0$  magnetic field gradients exist to select a 3D volume, they can be divided into techniques that leave the magnetization in the volume of interest (VOI) unperturbed during the localization procedure (hereafter referred to as outer volume suppression) and those that selectively perturb the magnetization in order to remove unwanted magnetization outside the VOI (hereafter referred to as single voxel or single volume localization). Since outer volume suppression (OVS) is most commonly used for the removal of extracranial lipid signals in proton spectroscopic imaging, the principles of OVS will be discussed in Chapter 7.

### 6.2.1 Image Selected *In Vivo* Spectroscopy (ISIS)

The ISIS localization method, as first described by Ordidge *et al.* [11] achieves full 3D localization in eight scans. The basic ISIS sequence is depicted in Figure 6.2. It employs three frequency-selective inversion pulses in the presence of three orthogonal magnetic field gradients. The inversion pulses are turned on or off according to an encoding scheme as shown in Figure 6.2. When zero or an even number of  $180^\circ$  pulses are executed, the desired magnetization in the cross-section of the three selected slices ends up along the positive longitudinal axis and following a  $90^\circ_{-x}$  excitation pulse will end up along the positive y axis. During a scan with an odd number of  $180^\circ$  pulses, the desired magnetization ends up along the negative longitudinal axis and is excited to the negative y axis by a  $90^\circ_{-x}$  pulse. Adding and subtracting the individually stored scans with even and odd number of  $180^\circ$  pulses, respectively, will constructively accumulate signal from the desired location while destructively canceling signal from all other locations. Figure 6.3 shows a practical example of 2D ISIS on a spherical, water-filled phantom. In each scan signal from the entire sample is excited, whereby the ISIS inversion pulses change the phase of the excited signal. Note that in the scan with two  $180^\circ$  pulses, the magnetization in the cross-section experiences a double inversion and therefore ends up with a positive phase. The localized volume can be obtained (Figure 6.3E) by subtracting images B and C from images A and D (i.e.  $E = A - B - C + D$ ). It is easily verified that this addition/subtraction scheme cancels all signal, except the signal within the localized volume. By following the entire eight-step procedure shown in Figure 6.2, this is readily extended to complete 3D localization.

Even though ISIS can theoretically give perfect localization in eight scans, the localization accuracy can be compromised by a number of factors. When the *in vivo* MRS



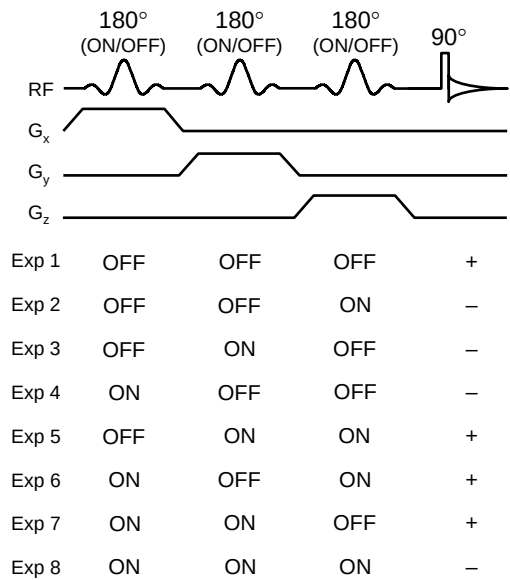


**Figure 6.2** Pulse sequence for ISIS localization. For 1D ISIS localization, two experiments are required, one with and one without a spatially selective inversion pulse prior to excitation. Subtraction of the two datasets will only give signal from the localized volume. For 3D localization, eight experiments ( $2 \times 2 \times 2$ ) are required as shown, whereby the right column indicates the relative receiver phase ( $+ = 0^\circ$ ,  $- = 180^\circ$ ).

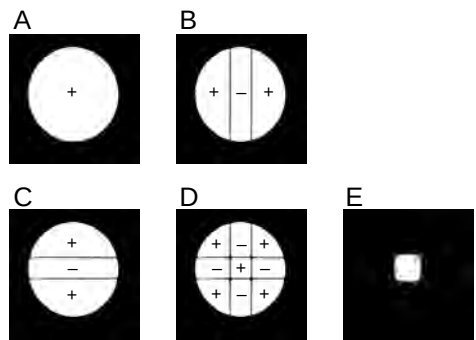


**Figure 6.3** Experimental evaluation of 2D ISIS localization by MRI. (A) Image of a water-filled sphere. No spatially selective (in-plane) inversion pulses were executed. (B and C) Images (in absolute value) in which one spatially selective inversion pulse has been executed. The  $+$  and  $-$  signs correspond to noninverted and inverted areas, respectively. (D) Image of the sphere after the execution of two spatially orthogonal selective inversion pulses. Due to the double inversion in the middle of the sphere, the magnetization resides along the positive longitudinal axis. (E) The localized volume is obtained according to the following add-subtract scheme:  $(E) = (A) - (B) - (C) + (D)$ .

experiment is executed with surface coils (for increased sensitivity),  $B_1$  magnetic field inhomogeneity can degrade the performance (i.e. slice profile) of the inversion and excitation pulses. While this problem can lead to greatly reduced sensitivity and increased contamination from unwanted signals, it is easily alleviated by using adiabatic RF pulses. ISIS is typically executed with AFP pulses for selective inversion (see Section 5.7.2) and AHP or BIR-4 pulses (see Section 5.7.6) for excitation.

Another potential source of signal loss and contamination is caused by so-called ‘ $T_1$  smearing’ [12–14]. When the longitudinal  $T_1$  relaxation is incomplete between subsequent acquisitions within the eight ISIS add–subtract acquisitions, the longitudinal magnetization prior to a given inversion (and acquisition) depends on the particular order in which the add–subtract scheme is executed. Because the longitudinal magnetization is not the same for all eight acquisitions, the cancellation of unwanted signals is incomplete. The degree of contamination due to ‘ $T_1$  smearing’ depends on the repetition time relative to the  $T_1$  relaxation time, the degree of  $B_1$  inhomogeneity and the order in which the eight ISIS acquisitions are performed [12–14].  $T_1$  smearing can be minimized, or even eliminated in several ways. In situations where signal averaging is required (as is usually the case), Ordidge *et al.* [11] originally proposed signal averaging each of the eight acquisitions separately, with a sufficiently long relaxation delay between different acquisitions to allow for complete signal recovery. Although theoretically a good approach, this method increases the sensitivity to motion artifacts and consequently contamination. Using amplitude-modulated RF pulses in an inhomogeneous  $B_1$  field will leave residual longitudinal magnetization after excitation as a function of spatial position and acquisition number. This problem can easily be solved by the application of adiabatic excitation pulses. In principle, the combination of adiabatic RF pulses and a long repetition time should completely eliminate effects of ‘ $T_1$  smearing’. When shorter repetition times are used in order to optimize the signal-to-noise ratio, ‘ $T_1$  smearing’ can be minimized by the application of a post-acquisition saturation pulse, such that the longitudinal magnetization is identical for all acquisitions.

Even though ISIS is the most popular localization technique for the observation of short  $T_2$  species (such as ATP with  $^{31}\text{P}$  NMR), transverse  $T_2$  relaxation during the inversion pulses can significantly degrade the localization performance. Figure 6.4 shows the 1D localization profiles for a 5 ms optimized amplitude-modulated inversion pulse (see Section 5.4) and a 5 ms hyperbolic secant AFP pulse (see Section 5.7.2,  $\Delta\omega_{\text{max}} = 1.0$  kHz) for the situation of infinite  $T_2$  and  $T_2 = 10$  ms. In all cases an infinite  $T_1$  was assumed. The amplitude-modulated pulse (Figure 6.4A) gives a moderate in-slice signal reduction, with a small increase in out-of-slice signal contamination. As was demonstrated in Section 5.8, the adiabatic inversion pulse has a greater sensitivity towards short  $T_2$  relaxation times, with a dependency on the  $B_1$  magnetic field strength. With short  $T_2$  relaxation times and high  $B_1$  amplitudes, the in-slice signal significantly decreases, while the out-of-slice signal contamination increases, thereby compromising the localization accuracy.

For  $^1\text{H}$  MRS, ISIS is rarely executed as the basic scheme depicted in Figure 6.2. Besides the requirements of water suppression, a potential problem inherent to ISIS is that in each acquisition signal from the entire sensitive coil volume is obtained. When the VOI is small compared with the entire excited volume, even small subtraction errors could lead to large contamination artifacts. Furthermore, in areas where the  $B_0$  magnetic field homogeneity,

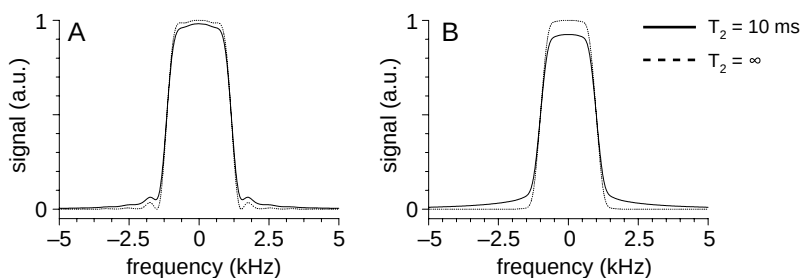


**Figure 6.4** Transverse relaxation during 1D ISIS localization. Selective inversion was achieved with (A) a 5 ms optimized, amplitude-modulated pulse [Section 5.4,  $(\gamma B_{1\max}/2\pi) = 1.09$  kHz] and (B) a 5 ms AFP pulse [ $\Delta\omega_{\max} = 1.0$  kHz,  $(\gamma B_{1\max}/2\pi) = 2.5$  kHz]. Localization in the presence of short  $T_2$  relaxation times (i.e.  $T_2 = 10$  ms) for both pulses leads to in-slice signal loss and out-of-slice signal contamination. However, due to the longer on- and off-resonance trajectories followed by magnetization during an AFP pulse, the effects are more pronounced and increase with increasing RF amplitude.

and hence the water suppression is poor, the large unsuppressed water resonance could lead to receiver dynamic range problems during the individual eight scans (even when the water is perfectly suppressed in the final spectrum). Finally, manual shimming on the localized volume is tedious, as complete 3D localization is only achieved after eight scans. All the potential problems, as well as signal contamination for compounds with short  $T_2$  relaxation times, can be significantly reduced when ISIS is combined with a (separate) method for OVS. Connelly *et al.* [15] originally proposed to combine ISIS with so-called noise pulses [16], giving rise to the OSIRIS (outer volume suppressed image related *in vivo* spectroscopy) technique. The frequency-domain excitation profile of a noise pulse consists of a selective null band in which magnetization remains unperturbed, together with noise (i.e. random dephasing of magnetization) outside this null band. This combination leads to a significant suppression of the magnetization outside the VOI *in a single scan*, such that receiver gain and motion problems are minimized and manual shimming becomes feasible. While noise pulses were initially proposed, essentially any kind of OVS can be used, including techniques that combine the ISIS and OVS principles into one pulse [17, 18].

## 6.2.2 Chemical Shift Displacement

The spatial position of the localized volume element obtained by a localization method that employs frequency selective RF pulses in the presence of magnetic field gradients will be linearly affected by the chemical shift of the compound under investigation. From Equation (4.3) it follows that the frequency of spins with Larmor frequency  $\omega_0$  in the presence of a magnetic field gradient  $G_x$  in the x direction is given by:

$$\omega(x) = \omega_0 + \gamma x G_x \quad (6.1)$$

or

$$x = \frac{\omega(x) - \omega_0}{\gamma G_x} \quad (6.2)$$

Therefore, a difference in Larmor frequency  $\omega_0$  between two different compounds results in a spatial displacement  $\Delta x$  of the localized volume for one compound relative to the other according to:

$$\Delta x = \frac{\Delta \omega}{\gamma G_x} = \frac{\Delta \omega}{\Delta \omega_{\max}} V_x \quad (6.3)$$

where  $\Delta \omega$  represents the difference in Larmor frequency. The right-hand equality is constructed based on the fact that the required gradient strength  $\gamma G_x$  to select a given voxel size  $V_x$  in the  $x$  direction is proportional to the spectral bandwidth of the RF pulse  $\Delta \omega_{\max}$ .

For instance, the difference in Larmor frequency between water and lipids at 4.0 T equals circa 580 Hz. For a 4 ms optimized sinc inversion pulse with a 1500 Hz inversion bandwidth, the required magnetic field gradient strength to select a  $3 \times 3 \times 3$  cm volume is  $0.117 \text{ G cm}^{-1}$  ( $= 1.17 \text{ mT m}^{-1}$ ). With these specific settings, the chemical shift displacement for the lipid resonances in one spatial direction equals 1.16 cm (Figure 6.5). For the complete 3D volume, the water and lipid resonances only have 23.1 % of their corresponding voxels in common. This could result in misinterpretations of absolute concentrations when tissue,  $B_0$  or  $B_1$  heterogeneity plays an important role. Furthermore, when performing signal

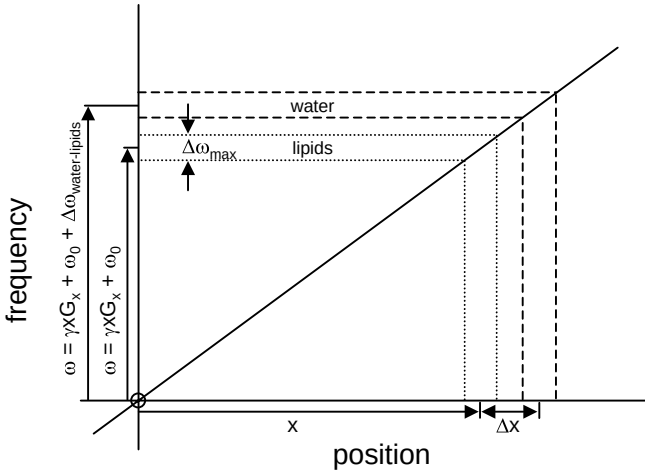


**Figure 6.5** Principle of chemical shift displacement. A frequency selective RF pulse executed at a frequency  $\omega_0 + \gamma x G_x$  during a magnetic field gradient  $G_x$  selects, for the lipid resonances, a spatial slice at position  $x$ . The frequency difference between water and lipids,  $\Delta \omega_{\text{water-lipids}}$ , leads to slice selection for the water at spatial position  $x + \Delta x$ , where  $\Delta x$  is given by Equation (6.3).

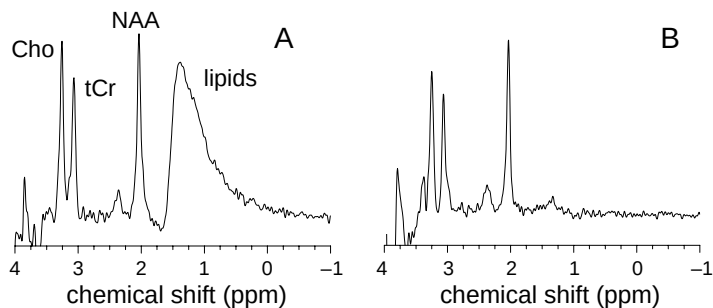


**Figure 6.6** Chemical shift displacement effects in localized  $^1\text{H}$  NMR spectra from newborn piglet brain in vivo at 4.7 T. (A) Localized  $^1\text{H}$  NMR spectrum obtained from a  $10 \times 10 \times 10 \text{ mm}^3$  voxel calculated and acquired relative to the water resonance. Because of the  $\sim 700 \text{ Hz}$  frequency difference between water and lipids, the chemical shift displacement causes contamination with extracranial lipid signals. (B)  $^1\text{H}$  NMR spectrum from the same localized volume as in (A), but now calculated and acquired relative to the NAA resonance. The reduced frequency difference between NAA and lipids ( $\sim 100 \text{ Hz}$ ) resulted in minimal lipid contamination.

localization in areas close to the skull, the chemical shift displacement artifact can lead to severe spectral lipid contamination even though the localization method works perfect for water and metabolites close to water. Figure 6.6 shows a practical example of the water–lipid chemical shift displacement in proton MRS of brain. When the localized volume is based on the water resonance frequency a significant amount of extracranial lipids is observed. When the volume placement is based on the NAA methyl resonance at 2.01 ppm, the chemical shift displacement for the lipids is much smaller and no significant extracranial lipid resonances can be observed.

Equation (6.3) reveals that the chemical shift displacement artifact gets more severe at higher magnetic fields (since  $\Delta\omega$  increases with magnetic field strength) and that the artifact can only be minimized by increasing the magnetic field gradient strength, which (for a constant voxel size) corresponds to an increase in spectral bandwidth of the RF pulse. Shortening the RF pulse length will increase the bandwidth, at the expense of an increased specific absorption rate (SAR). On most (human) systems one would typically encounter the SAR limitation well before any gradient strength limitations. This observation has led to the development of gradient-modulated RF pulses, which can achieve high magnetic field gradient amplitude at equal RF peak amplitude. The variable rate selective excitation (VERSE) principle was discussed in Chapter 5 to lower the average and peak RF power by increasing the gradient amplitude during periods of low RF amplitude in order to maintain the same nutation angle on-resonance. FOCI (frequency-offset corrected inversion) pulses were specifically designed to reduce the chemical shift displacement by increasing the effective spectral bandwidth through magnetic field gradient modulation. Starting with a regular AFP pulse executed with hyperbolic secant modulation, as described in Section 5.7.2, a FOCI pulse is created by multiplying the AFP RF, frequency and gradient modulation by a function  $A(t)$  which maximizes the RF amplitude modulation over the

range  $|t| > (1/\beta)\text{asech}(1/f)$  according to:

$$B_1(t) = A(t)B_{1\max}\text{sech}(\beta t) \quad (6.4)$$

$$\Delta\omega(t) = A(t)\Delta\omega_{\max}\tanh(\beta t) \quad (6.5)$$

$$G(t) = A(t)G_{\max} \quad (6.6)$$

with

$$A(t) = \begin{cases} \cosh(\beta t) & \text{for } |t| < \left(\frac{1}{\beta}\right)\text{asech}\left(\frac{1}{f}\right) \\ f & \text{for } \left(\frac{1}{\beta}\right)\text{asech}\left(\frac{1}{f}\right) \leq |t| \leq 1 \end{cases} \quad (6.7)$$

where  $-1 \leq t \leq 1$ . Figure 6.7 shows the AFP and corresponding FOCI modulation functions, as well as the inversion profile on- and off-resonance. It follows that the FOCI gradient modulation leads to (1) improved slice profiles and to (2) decreased chemical shift displacement.

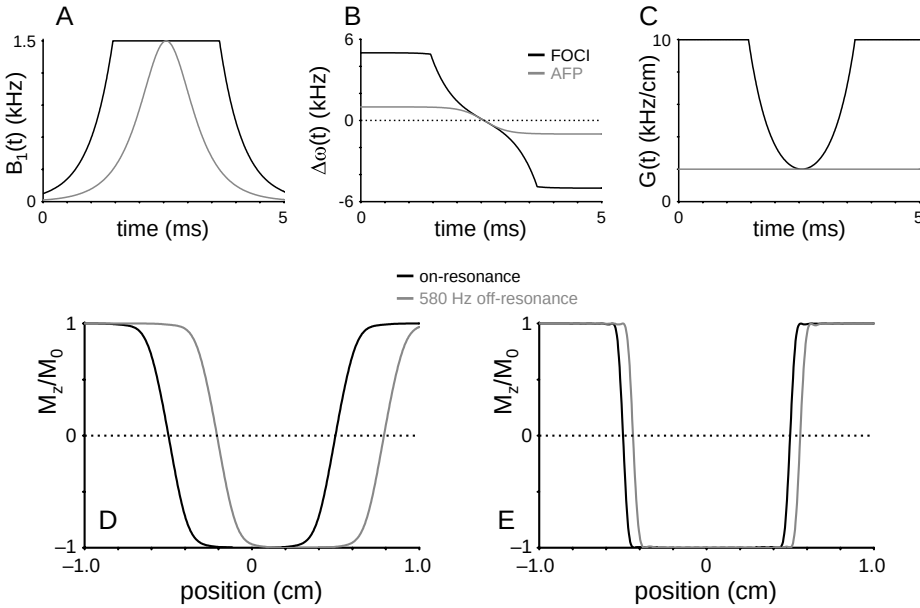


**Figure 6.7** Reduction of chemical shift displacement through the use of FOCI pulses. (A) Amplitude, (B) frequency and (C) magnetic field gradient modulation of AFP (gray lines) and FOCI (black lines) pulses. (D, E) Simulated spatial inversion profile of (D) an AFP pulse ( $\Delta\omega_{\max} = 1.0$  kHz,  $T = 5$  ms,  $G_{\max} = 2.0$  kHz  $\text{cm}^{-1}$ ) and (E) a FOCI pulse on-resonance (black lines) and 580 Hz off-resonance (gray lines). The increased frequency modulation range and associated magnetic field gradient amplitude of FOCI greatly reduces the chemical shift displacement.

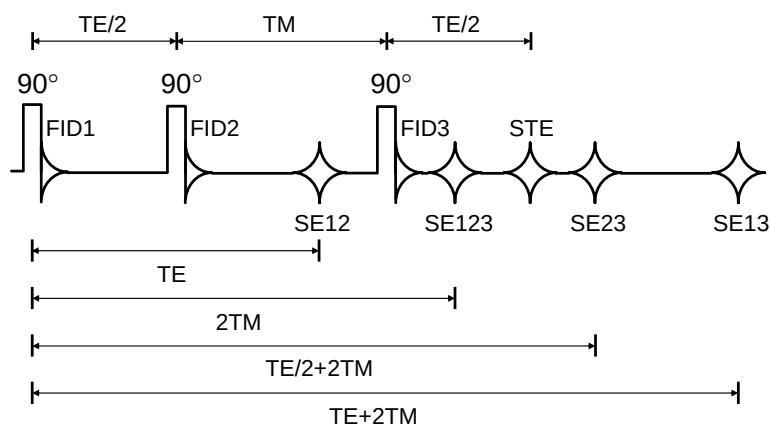


**Figure 6.8** Echo formation during a sequence containing three RF pulses. In principle, three FIDs (FID1, FID2, and FID3), four spin echoes (SE12, SE13, SE23, and SE123) and one stimulated echo (STE) can be generated. The echoes appear at distinct temporal positions, depending on the TE and TM periods. The relative amplitudes of the echoes are determined by the nutation angles of the RF pulses.

### 6.2.3 Stimulated Echo Acquisition Mode (STEAM)

STEAM is a localization technique [19–25] capable of complete 3D localization in a single acquisition (i.e. STEAM is a so-called single-shot or single-scan localization technique). Before describing the STEAM localization technique it is informative to consider the basic pulse sequence (without magnetic field gradients) consisting of three RF pulses, each with a nominal nutation angle of  $90^\circ$  (Figure 6.8). As early as 1950, Hahn described that this pulse sequence generates five echoes and three FIDs [26]. When the first two pulses are separated by a delay TE/2 and the last two pulses (i.e. pulses 2 and 3) are separated by a delay TM, then four spin-echoes can be formed. The spin echo formed by pulses 1 and 2 (SE12) occurs at a position TE after the initial excitation pulse, SE13 at TE + 2TM, SE23 at position TE/2 + 2TM and SE123 (due to refocusing by pulses 2 and 3 of magnetization excited by pulse 1) occurs at position 2TM. Besides these four spin-echoes a so-called stimulated echo (STE) is formed at position TE + TM (or after a delay TE/2 following the last  $90^\circ$  pulse). Figure 6.9A shows an experimental verification of the generated echoes on a sample containing water with TE = 200 ms and TM = 20 ms. The echoes were formed in the presence of a low-amplitude magnetic field gradient present during the entire pulse sequence.

The positions of the different echoes depend on the timing parameters TE and TM, while the relative amplitudes depend on the nutation angles of the RF pulses. In most imaging and spectroscopy experiments, the (STE) is the signal of interest. (The spin-echo SE123 is the signal of interest for PRESS, see next section.) Therefore, the FID and spin-echo signals need to be eliminated in order to obtain unambiguous information from the STE. This could be achieved by phase cycling the individual RF pulses as shown in

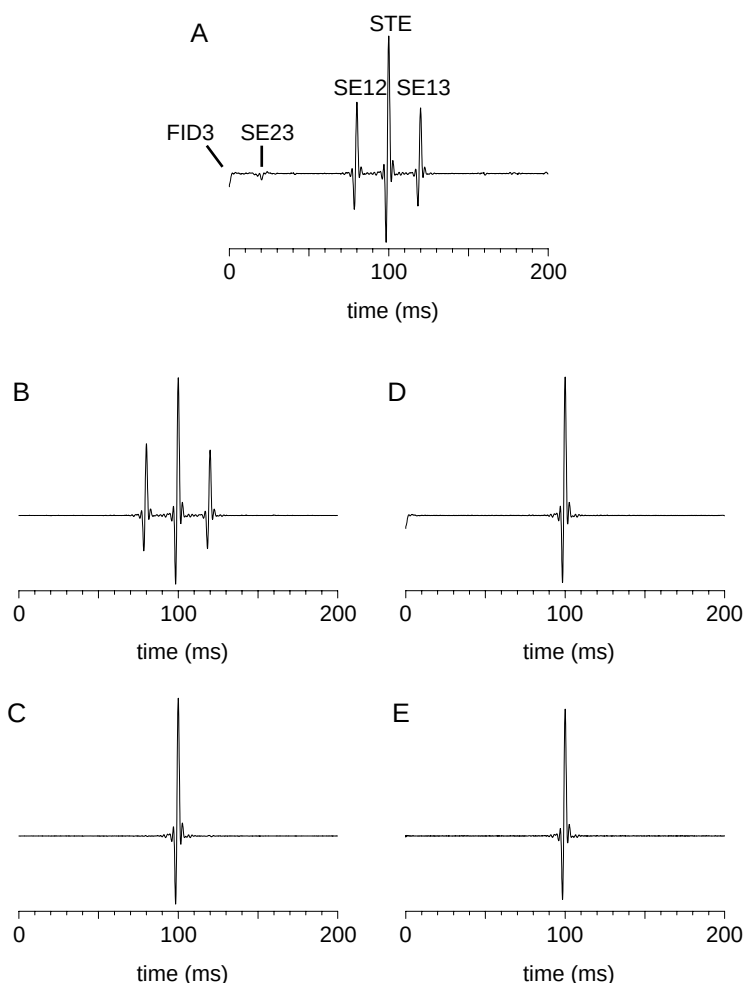


**Figure 6.9** Experimental verification of the echo formation as shown in Figure 6.8 on a water-filled sphere. For the particular sequence used,  $TE = 200$  ms and  $TM = 20$  ms with nominal nutation angles of  $60^\circ$  ( $20\ \mu\text{s}$  square RF pulses transmitted with a homogeneous solenoidal RF coil). The echoes were acquired immediately after the last RF pulse in the presence of a low-amplitude magnetic field gradient (which was present during the entire sequence). (A) Echoes without any phase cycling or gradient crushers. Besides the stimulated echo STE, the spin echoes SE12 and SE13 are especially intense. (B) After a two-step phase cycle of the last RF pulses [e.g.  $60^\circ (+x)$  and  $60^\circ (-x)$ ]. (C) Complete eight-step phase cycle of all three RF pulses. All unwanted echoes have been cancelled, leaving only the STE. (D) Echo formation in the presence of a TM gradient crusher. Besides the desired STE, only an unwanted FID arising from the last RF pulse remains. (E) Complete removal of all unwanted coherences by a combination of TE and TM crushers in a single acquisition.



Figure 6.9B and C. However, this will compromise the single-scan character of STEAM localization. Furthermore, phase cycling can only achieve good results on stable (i.e. motionless) systems, which is not always the case with *in vivo* NMR spectroscopy. As an alternative, the unwanted FID and spin-echo signals can be eliminated with the use of magnetic field gradients (Figure 6.9D and E). By placing a ‘crusher’ gradient in the TM period, all spin-echoes are eliminated leaving only the STE and a FID component arising from the last excitation pulse. The FID component can easily be eliminated by placing identical ‘crusher’ gradients in both TE/2 periods. With these additional TE and TM crusher gradients Figure 6.9E shows that a clean selection of the STE can be achieved in a single scan. In the presence of strong  $B_0$  magnetic field inhomogeneity at higher magnetic field strengths, it is possible to observe so-called multiple spin-echoes at multiples of TM [27,28]. Although these additional spin-echoes can be used for some applications, they are in general not desirable. Phase cycling may not be able to remove these unwanted echoes, but magnetic field gradients can still produce a clean selection of the STE.

Using the product operator formalism (see Appendix A4) it can be shown that with these TE and TM crushers only signal arises which was along the longitudinal axis during the TM period (provided that the TE and TM crushers are of sufficient strength to achieve complete dephasing). The amplitude of the STE equals:

$$M_{xy} = \frac{1}{2} M_0 \sin\theta_1 \sin\theta_2 \sin\theta_3 e^{-TM/T_1} e^{-TE/T_2} e^{-(4/\pi)^2 \gamma^2 G^2 \delta^2 (\Delta - \delta/4) D} \quad (6.8)$$

where  $\theta_i$  ( $i = 1, 2$  or  $3$ ) represents the nutation angle of RF pulse  $i$ . Equation (6.8) shows that the amplitude of a STE (and of STEAM) is theoretically only 50 % of a spin-echo. Later it will be shown that in practice this does not always hold due to the different sensitivities of the localization sequence to relaxation, scalar coupling and diffusion. The 50 % signal reduction originates from the fact that the second  $90^\circ$  pulse only rotates half of the transverse magnetization to the longitudinal axis, while the other half is dephased by the following TM crusher. Figure 6.10 graphically demonstrates the formation of a STE. Equation (6.8) further shows that longitudinal  $T_1$  relaxation only affects the final STE during the TM period (assuming a long repetition time), while transverse  $T_2$  relaxation only plays a role during the TE period. The last term in Equation (6.8) describes the effect of diffusion on the STE (a sinusoidal gradient was assumed, but this expression can be replaced with the expression for any arbitrary shaped gradient). The separation between the two diffusion gradients that are placed in the first and second TE/2 delay periods, is at least equal to TM, making the ‘b-factor’ large even when  $G$  or  $\delta$  are small. Since the magnetization during TM is not influenced by  $T_2$  relaxation, STEs offer a good method of measuring diffusion constants of short  $T_2$  species (for which the longitudinal  $T_1$  relaxation time is relatively long). In Chapter 3 it was discussed how a STE sequence can be used to study diffusion restriction in skeletal muscle by varying the TM period. In this way, the diffusion weighting increases, while the signal loss due to  $T_2$  relaxation remains constant.

The response of scalar-coupled spins to the STEAM sequence is substantially more complicated than for the ISIS or PRESS methods, because the multiple  $90^\circ$  pulses can create multiple quantum coherences and induce polarization transfer effects. Using the product operator formalism (see Appendix A4) it can be shown (see also Exercise 6.11)

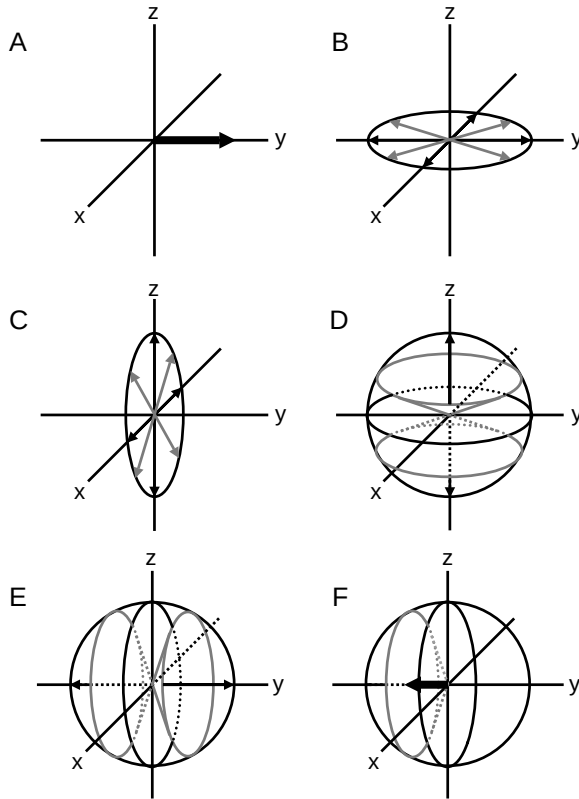


**Figure 6.10** Stimulated echo formation by three  $90^\circ$  RF pulses in combination with TE and TM crusher gradients. (A) Excitation of magnetization by a  $90^\circ$  RF pulse. (B) During the first TE/2 period transverse magnetization is completely dephased by the TE crusher gradient (and chemical shifts and magnetic field inhomogeneity). For illustration purposes, eight individual magnetization vectors have been drawn (at multiples of  $45^\circ$ ), but in reality there will be a continuous dephasing. (C) The second  $90^\circ$  RF pulse rotates the entire xy plane to the xz plane. Note that for proper formation of a stimulated echo, it is essential to execute a true plane rotation (universal rotation) pulse, like square, sinc or BIR-4 pulses. (D) Application of a TM crusher gradient leads to a complete dephasing of all transverse components. This can be represented as a randomization of magnetization among cones, in which there remains a net coherent longitudinal component [except for magnetization which was completely in the transverse plane during (C)], but in which there is no net transverse magnetization. (E) The last RF pulse rotates the cones by  $90^\circ$  such that the coherent longitudinal components in (D) are rotated to the transverse plane. (F) In the last TE/2 period, the magnetization in the transverse plane is rephased by a second, matched TE crusher gradient (and chemical shifts and magnetic field inhomogeneity). At the top of the echo, the coherent transverse magnetization is maximized. However, this is only half of the initially excited magnetization, because the magnetization is completely dephased and present among an infinite number of cones filling the hemisphere along the negative y axis.

that the STEAM signal for the A spins in a  $A_nX$  ( $n = 1, 2$  or  $3$ ) spin system is given by:

$$\begin{aligned}
 S(\text{TE} + \text{TM}) = & \frac{1}{2} \left\{ \cos^2 \left( \frac{\pi J_{AX} \text{TE}}{2} \right) - \frac{1}{2} \sin^2 \left( \frac{\pi J_{AX} \text{TE}}{2} \right) \right. \\
 & \times \left[ 1 - \cos^{n-1} \left( \frac{\pi J_{AX} \text{TE}}{2} \right) \cos \left( \frac{\Delta \omega_{AX} \text{TE}}{2} \right) \right] \\
 & \left. \times \cos^{n-1}(\pi J_{AX} \text{TM}) \cos(\Delta \omega_{AX} \text{TM}) \right\} \quad (6.9)
 \end{aligned}$$

where  $J_{AX}$  and  $\Delta \omega_{AX}$  represent the scalar coupling (in Hz) and frequency difference (in  $\text{rad s}^{-1}$ ) between spins A and X, respectively. The first term in Equation (6.9) originates from A magnetization that was along the longitudinal axis during the TM period. The second, much more complicated term in Equation (6.9) originates from zero quantum coherences during the TM period and includes contributions from both the A and X spins (the X spin contribution appears due to polarization transfer during the final  $90^\circ$  pulse).

Figure 6.11 shows simulated STEAM signal for lactate (an  $A_3X$  spin system) according to Equation (6.9). Figure 6.11A shows the signal intensity as a function of the echo-time TE for a constant TM and Figure 6.11B shows the lactate signal dependence on the TM duration (for a constant TE). The calculations were performed for a magnetic field strength of 4.7 T, such that  $\Delta \omega_{AX} = 556$  Hz. For lower magnetic field strengths, the high-frequency signal modulation apparent in Figure 6.11 will appear at a proportionally lower frequency.

The preceding discussion has been limited to the basic three pulse STE sequence. The STEAM localization sequence is a direct derivation of the basic pulse sequence in which the nonselective square RF pulses have been replaced by frequency-selective RF pulses in the presence of magnetic field gradients (Figure 6.12). Since transverse magnetization is dephased during half of the pulse length, gradient refocusing lobes are required following slice selection. To reduce the minimum attainable echo time, inherently refocused  $90^\circ$  RF pulses can be employed. On a state-of-the-art MR system with fast and strong magnetic field gradients it has been shown that excellent proton NMR spectra can be obtained at  $\text{TE} = 1$  ms [29].

## 6.2.4 Point Resolved Spectroscopy (PRESS)

The PRESS localization method [30–33] is a so-called double spin-echo method, in which slice-selective excitation is combined with two slice-selective refocusing pulses (Figure 6.13). When the first  $180^\circ$  pulse is executed after a time  $t_1$  following the excitation pulse, a spin-echo is formed at time  $2t_1$ . The second  $180^\circ$  pulse refocuses this spin-echo during a delay  $2t_2$ , such that the final spin-echo is formed at time  $2t_1 + 2t_2$  (which equals the echo-time of PRESS, i.e.  $\text{TE} = 2t_1 + 2t_2$ ). The first echo contains signal from a column which is the intersection between the two orthogonal slices selected by the  $90^\circ$  pulse and the first  $180^\circ$  pulse. The second spin-echo only contains signal from the intersection of the three planes selected by the three pulses resulting in the desired volume. Signal outside the VOI is either not excited or not refocused, leading to rapid dephasing of signal by the ‘TE crusher’ magnetic field gradients (see Figure 6.13).

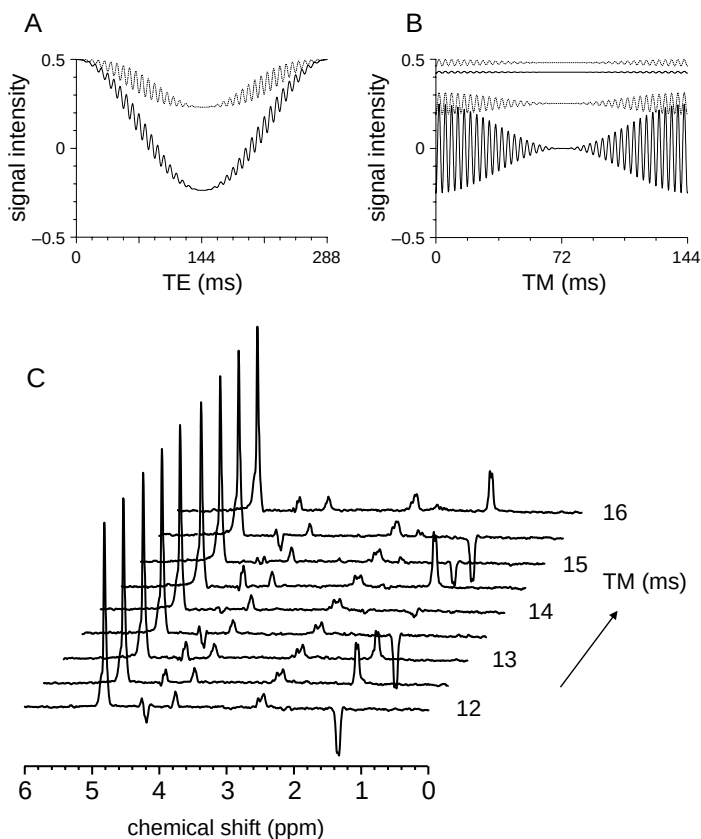


**Figure 6.11** Signal intensity of the lactate methyl protons ( $A_3X$  spin system) as a function of TE and TM during a STE sequence. (A) TE dependence of the signal intensity for TM = 10.8 ms (lower trace) and TM = 12.6 ms (upper trace). (B) TM dependence of the signal intensity for TE = 18, 36, 72, 144 ms (top to bottom traces, respectively). In both figures, the fast oscillations arise from chemical shift evolution during TM, which is dependent upon the magnetic field strength. (C) Experimental spectra of lactate (1.31 and 4.10 ppm) and glutamate (~2.2 ppm and 3.75 ppm) in D<sub>2</sub>O acquired with a STE sequence with TE = 144 ms. Especially the lactate resonances show a strong TM dependence.

## 6.2.5 Signal Dephasing with Magnetic Field Gradients

The primary reason for the appearance of spectral artifacts in single voxel localization, like spurious and variable signals, incomplete water suppression and lipid contamination, is given by an incomplete dephasing of unwanted coherences. As was shown for a STE pulse sequence (Figure 6.12), a three RF pulse sequence can generate three FID signals, four spin-echoes and a STE. During a STEAM experiment all coherences, except the STE, must be destroyed (e.g. by magnetic field gradient dephasing), while in a PRESS sequence the spin echo arising from the center of the three intersecting, orthogonal planes must be

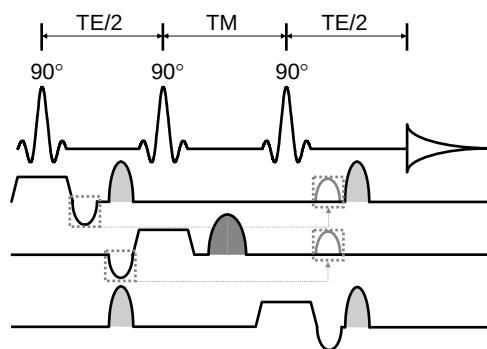


**Figure 6.12** STEAM pulse sequence for 3D spatial localization. Essentially the square RF pulses of Figure 6.8 have been replaced by frequency selective RF pulses (e.g. sinc) in the presence of magnetic field gradients. Refocusing gradients are required to rephase the phase evolution during the excitation pulses. These gradients may also be positioned with opposite sign on the other side of the TM period. When inherently refocused excitation RF pulses are used, the refocusing gradient lobes can be omitted or at least greatly reduced. However, for all STEAM sequences, TE and TM crusher gradients of sufficient strength are required to remove unwanted coherences and to achieve accurate localization.

selected. Moonen *et al.* [34] have analyzed the PRESS sequence by using the coherence pathway formalism (see Appendix A4). They have derived expressions for the intensity of all coherences in the presence of magnetic field gradients,  $T_1$ ,  $T_2$  and  $T_2^*$  relaxation and chemical shifts. To appreciate the importance of magnetic field gradients for dephasing of unwanted coherences, Figure 6.14 shows a qualitative overview (i.e. only including effects of magnetic field gradients) of the attenuation of observable magnetization in PRESS

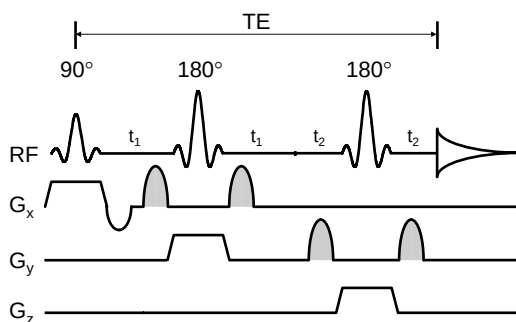


**Figure 6.13** PRESS pulse sequence for 3D spatial localization. Two pairs of crusher gradients (different in direction and/or amplitude) surrounding the  $180^\circ$  refocusing pulses ensure the selection of the desired coherences, while simultaneously destroying all others. One point of consideration is that it should be avoided that the second pair of TE crushers cancel the effect of the first pair. When sufficient gradient strength is available this can easily be accomplished by using one pair of TE crushers in two orthogonal directions as shown here. If gradient strength is limited, the amplitudes of the TE crushers in simultaneous orthogonal directions should be chosen differently.

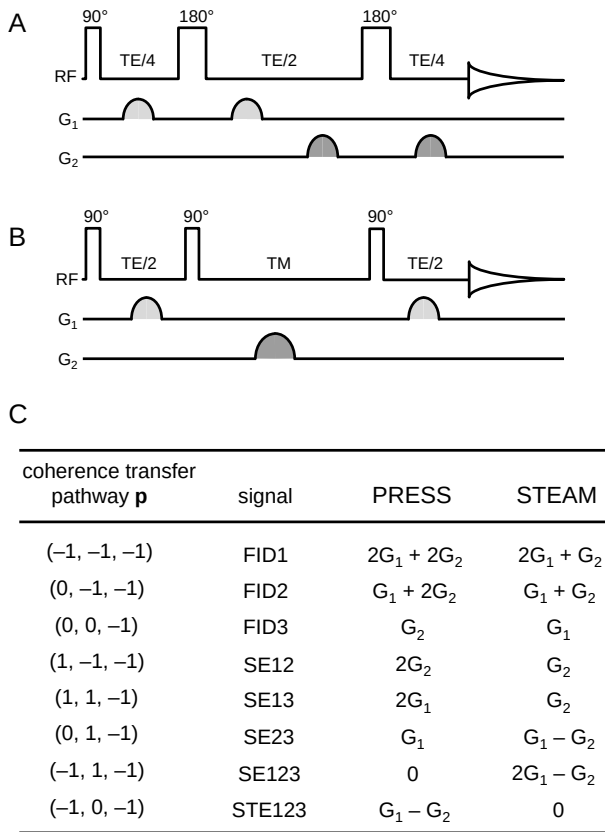


**Figure 6.14** Magnetic field gradient crusher efficiency for all SQCs in (A) PRESS and (B) STEAM. For simplicity only crusher gradients in one direction are shown. However, the  $B_0$  magnetic field crushing capacity may be increased by employing magnetic field gradients in orthogonal directions. (C) Suppression efficiency of different coherences encountered in PRESS and STEAM in terms of gradient areas  $G_1$  and  $G_2$ , which are related to the suppression factor  $f$  according to Equation (6.10).

and STEAM sequences. The suppression factor  $f$  of the coherences is proportional to the nonrefocused gradient areas given in Figure 6.14C according to:

$$f = \int_{x_1}^{x_2} \int_{y_1}^{y_2} \int_{z_1}^{z_2} \rho(x, y, z) e^{+i\gamma \left[ \int_0^T x G_x(t) dt + \int_0^T y G_y(t) dt + \int_0^T z G_z(t) dt \right]} dx dy dz \quad (6.10)$$

where  $\rho(x, y, z)$  is the spin density at spatial position  $(x, y, z)$ . For an object of constant spin density, Equation (6.10) evaluates to a sinc function, similar to the derivation of Equation (4.15) from Equation (4.14). From Figure 6.14C it follows that unwanted coherences experience different amounts of dephasing due to external applied magnetic field gradients.

$B_0$  magnetic field inhomogeneity may either support or oppose the dephasing introduced by the magnetic field gradient pulses. For example, signal from  $p = (-1, -1, -1)$ , i.e. the FID originating from the first  $90^\circ$  pulse, is continuously dephased by  $B_0$  magnetic field inhomogeneity that has the same sign as the applied magnetic field gradient. However, incomplete dephasing can result if the magnetic field inhomogeneity opposes the applied magnetic field gradient. The limited crushing capability for the FID originating from the last RF pulse is common to both STEAM and PRESS, whereas the low magnetic field gradient dephasing for the spin echo between RF pulses 2 and 3 is unique to the PRESS sequence. Note that the TM crushing gradient  $G_2$  in STEAM is extremely effective. All unwanted coherences, except the FID from the last pulse are suppressed. Note that some unwanted coherences are refocused by inappropriate  $G_1$  and  $G_2$  gradient combinations. For example, PRESS will detect an unwanted STE (STE123) on top of the spin echo (SE123) when  $G_1 = G_2$ . This problem is readily alleviated when the crusher gradient pairs are applied along different (orthogonal) spatial axes.

When used for localized MRS, the three RF pulses in STEAM or PRESS define three orthogonal slices such that unwanted coherences become associated with certain spatial positions. Figure 6.15 shows a 2D slice selected by a frequency selective  $90^\circ$  pulse in the presence of a magnetic field gradient. The desired coherences, i.e. SE123 or  $p(-1, 1, -1)$  for PRESS and STE123 or  $p(-1, 0, -1)$  for STEAM, originate from the intersection of slices selected by all three RF pulses. Other unwanted coherences are localized to specific spatial positions. Considering the dephasing efficiency of coherences in PRESS and STEAM (Figure 6.14C) and the specific localized origin of coherences, it may be expected that the slice selection gradient order should have considerable impact on the spectral quality when dephasing by magnetic field gradients is insufficient.

Because the  $B_0$  magnetic field distribution in the human head is highly heterogeneous, it is instructive to analyze the  $B_0$  magnetic field inhomogeneity in transverse (i.e. through both eyes, or slices from top to bottom), sagittal (i.e. between both eyes, or slices from left to right) and coronal slices of the brain in order to determine the optimal slice order. Note that a transverse orientation in animal studies is in the direction of a human coronal orientation and vice versa, due to the different positioning of animals in the magnet. In sagittal and coronal slices, motion artifacts due to swallowing can lead to  $B_0$  magnetic field inhomogeneity. Signal from the sinuses and mouth can be another source for artifacts in localized proton MRS. In transverse (or axial) slices through the human brain, internal motion is in general minimal. Recalling the low dephasing efficiency of the FID arising from the last RF pulse, the slice order is optimal when the last RF pulse selects a transverse plane. This also avoids signal from sinuses and mouth. The orientation of the slice selection gradients of the first two RF pulses is not very critical. Figure 6.15B shows a  $^1\text{H}$  NMR spectrum acquired with the PRESS sequence from human midfrontal gray matter [35] with a transverse–coronal–sagittal slice order. Clearly, significant artifacts are present due to incomplete dephasing of unwanted coherences from outside the VOI. The artifacts are readily removed by increasing the magnetic field ‘TE crusher’ gradient amplitudes in the PRESS sequence. However, when magnetic gradient field strength is limited, the spectral appearance may be improved by changing the slice order of the three pulses. Figure 6.15C shows the same  $^1\text{H}$  NMR spectrum, now acquired with a coronal–sagittal–transverse slice order. In this case, the slice with the smaller amount of  $B_0$  magnetic field inhomogeneity is selected last, thereby making optimal use of the magnetic crusher efficiency to dephase more inhomogeneous regions throughout the sequence. In addition, optimizing the sign of

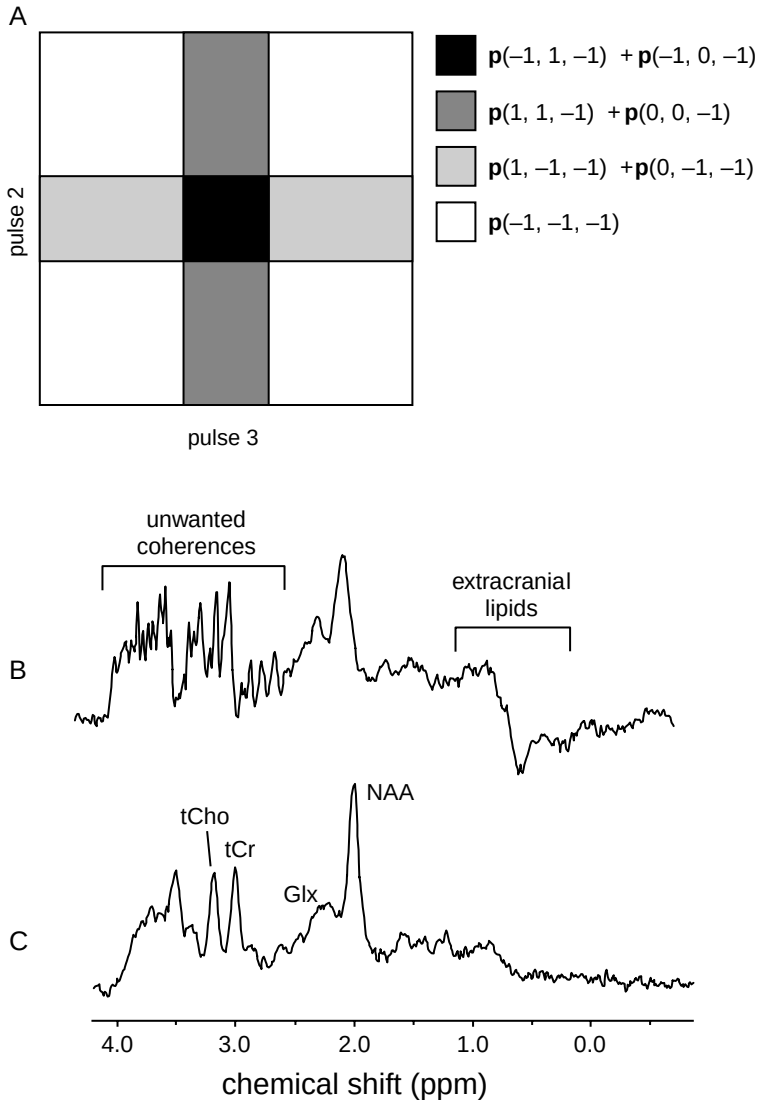


**Figure 6.15** (A) Spatial origin of different coherence transfer pathways. In the plane selected by the first  $90^\circ$  pulse, nine different areas can be recognized. Each area is the source for different coherence transfer pathways, such that suppression of unwanted coherences becomes synonymous with accurate spatial localization. (B, C) Midfrontal  $^1\text{H}$  NMR spectra of a normal volunteer acquired with PRESS localization ( $\text{TR}/\text{TE} = 3000/30$  ms). (B)  $^1\text{H}$  NMR spectrum acquired with an axial–coronal–sagittal slice order and (C) with a coronal–sagittal–axial slice order. The low dephasing efficiency of the FID arising from the last RF pulse leads, in spectrum (B) to significant artifacts, since the last RF pulse selects an area with significant susceptibility changes (i.e. sinuses and basal skull). With the rearranged slice order of spectrum (C), the last RF pulse selects a more homogeneous area which has lower dephasing requirements and hence leads to reduced artifacts. (Reproduced with permission from Ernst and Chang [35].)



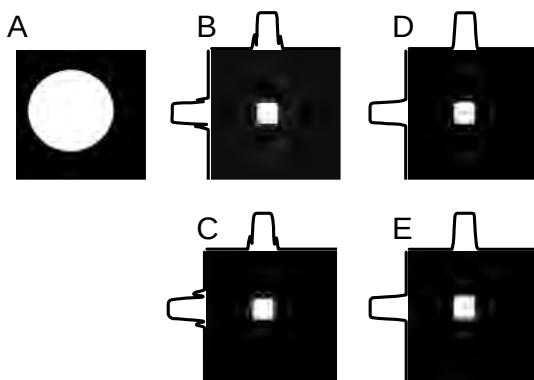


**Figure 6.16** Experimental evaluation of STEAM and PRESS localization by MRI on a water-filled sphere. (A) Image of the sphere without single voxel localization. Images of the localized volume selected by the first and second RF pulses of (B) STEAM and (C) PRESS. Note that the imperfect frequency profile of conventional sinc pulses leads to significant sidebands. However, besides these sidebands, which could be taken into account, the localization accuracy is excellent. Further note that the signal intensity of the STEAM localized volume is 50 % of that from the PRESS volume. The localization sidebands can be significantly reduced with optimized RF pulses. Superb localization can be achieved for (D) STEAM and (E) PRESS by replacing the  $90^\circ$  RF pulses with SLR optimized pulses and the  $180^\circ$  RF pulses by optimized RF pulses described by Mao et al. [37]. For details on RF pulse design see Chapter 5.

the magnetic field crusher gradient such that it dephases in concert with the magnetic field inhomogeneity gradient may further improve the suppression of unwanted coherences.

A useful method to determine the localization accuracy of STEAM and PRESS (or other localization techniques) is to visualize the localized volume by imaging techniques. Since STEAM and PRESS manipulate the transverse magnetization during voxel selection, the localized volume can be visualized simply by direct incorporation of imaging (i.e. frequency- and phase-encoding) gradients into the localization sequence. Figure 6.16A shows a 2D image of a spherical phantom. Figure 6.16B and C shows 2D images of the localized volumes of STEAM and PRESS, respectively (excitation and refocusing was achieved with regular sinc pulses). The PRESS image shows the selections of the  $90^\circ$  and the first  $180^\circ$  pulses. The localization accuracy of both STEAM and PRESS is excellent, aside from small sidelobes arising from the truncated sinc pulses. Note that the signal intensity of the STEAM voxel is only 50 % of the PRESS voxel. To ensure that the imaging gradients encode all coherences (including any unwanted coherences), the imaging gradients are positioned after the last RF pulse and magnetic field crusher gradients. Care should be taken to avoid rephasing of unwanted coherences by the phase-encoding gradient. Better defined slice profiles can be obtained when the localization sequences are executed with optimized frequency-selective RF pulses. Figure 6.16D and E shows the localized volumes of STEAM and PRESS executed with optimized  $90^\circ$  and  $180^\circ$  RF pulses (see Section 5.4). The slice profiles are near rectangular without any additional signal contamination. When the slice profile deviates from a rectangular profile, the actual localized volume differs from the nominal volume and correction factors must be applied. These correction factors can be obtained by numerical integration of the Bloch equations for the particular RF pulse used. Alternatively, the actual localized volume may be derived from images of the localized volume.

An even more stringent experiment to evaluate the localization accuracy can be performed by spectroscopic means. A suitable *in vitro* object may for example consist of a (spherical) phantom containing *in vivo* concentrations of metabolites ( $\sim 1\text{--}10\text{ mM}$ ) in water, which is placed in a second phantom containing lipids (e.g. olive oil). To approximate *in vivo* conditions, the metabolite solution may be doped with a paramagnetic relaxation agent. Localized NMR spectra of the inner phantom devoid of lipid resonances are indicative for good spatial localization (e.g. [36]). Similar *in vitro* objects may be developed for other nuclei (e.g.  $^{31}\text{P}$ ) or, for example, to measure the influence of motion on the localization accuracy. Comparable performance experiments *in vivo* will normally lead to the same conclusions (i.e. Figure 6.1), although stronger crusher gradients may be required to compensate for local  $B_0$  magnetic field inhomogeneity.

## 6.2.6 Effects of Imperfect RF Pulses

In the preceding section the PRESS sequence was described as a slice-selective  $90^\circ$  pulse, following by two  $180^\circ$  slice-selective refocusing pulses. The combination of frequency-selective RF pulses during magnetic field gradients leads, for uncoupled spins, to two deviations from a perfectly rectangular volume, namely a chemical shift dependent displacement as discussed in Section 6.2.2 and an imperfect slice profile (see Figure 6.16). For scalar-coupled spin systems both deviations will also occur, but the temporal and spatial effects will be more complex. The effect of imperfect RF pulses, and hence imperfect slices, on the scalar coupling evolution will be described here, while the chemical shift displacement effect for scalar-coupled spin systems will be discussed in Section 6.2.8.

Until now it has been assumed that the frequency-selective RF pulses in the presence of a magnetic field gradient generate perfectly rectangular slices, with full excitation or refocusing within the volume and no excitation or refocusing outside the volume. Clearly, this assumption can never be valid under realistic conditions, since a rectangular excitation profile can only be generated by an infinitely long RF pulse. Because a realistic RF pulse always has a finite pulse length, the excitation profile always includes a transition bandwidth in which the magnetization has a nutation angle distribution between  $0^\circ$  and  $90^\circ$  (or  $180^\circ$  for refocusing). Figure 6.17A and B shows two typical RF pulses used in localized NMR spectroscopy, namely a (truncated) Gaussian shaped pulse (Figure 6.17A) and a numerically optimized  $180^\circ$  pulse ([37], Figure 6.17B). Figure 6.17C and D shows the corresponding refocusing profiles. A refocused component of zero indicates no refocusing, while a value of one is indicative for complete refocusing (see also Section 5.7.3). Therefore, when the refocused component equals 0.5, the refocusing pulse effectively performs a  $90^\circ$  rotation.

Imperfect excitation profiles do not have a large effect on the performance of STEAM localization, as far as the detection of coupled spin systems is concerned. Clearly, for all localization techniques a near rectangular volume definition is desired, since this gives the highest localization accuracy and best volume definition. However, imperfect excitation profiles do not substantially modify the TE and TM dependent modulations for coupled spin systems as detailed in Section 6.2.3. This is because the TM magnetic field gradient (and to a lesser extent the TE magnetic field gradients) dephases all generated coherences besides the zero quantum and longitudinal coherences. The absolute (and relative) amounts of coherences may change as a function of the excitation profile, but the types of coherences leading to observable signal remain constant.

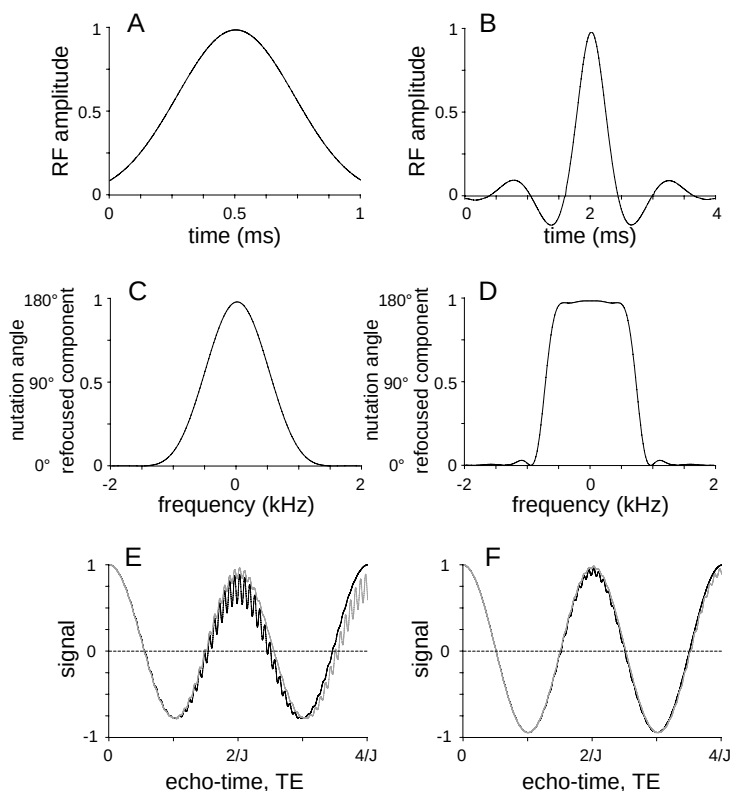


**Figure 6.17** The effect of imperfect refocusing pulses in PRESS localization on the detection of scalar-coupled two-spin systems. (A) Gaussian and (B) optimized sinc pulse profiles and (C and D) their corresponding frequency profiles expressed as a refocused component [Equation (5.44)]. The frequency profile of the optimized pulse gives a much better approximation of the ideal rectangular profile. (E, F) Integrated signal intensity for an AX spin system as a function of the echo-time TE for PRESS executed with (E) Gaussian and (F) optimized refocusing pulses ( $TE_1 = TE_2$ , black line). The high-frequency oscillations arise from polarization transfer effects between the two spins. For an asymmetric PRESS sequence ( $TE_2 = 4TE_1$ , gray line), the oscillations are spread over all echo times.

For PRESS localization the situation becomes more complicated. In the case of ideal  $90^\circ$  and  $180^\circ$  pulses, PRESS only generates single quantum coherences (SQC) and does not allow for polarization transfer between the coupled spins. However, in the case of realistic (nonideal) RF pulses, many more coherences can be generated. Fortunately, the majority of these coherences can be dephased by strategically placed magnetic field gradients (see Figures 6.13 and 6.14). For example, the first  $180^\circ$  pulse can generate multiple quantum coherences (MQCs) in those regions of the slice where the nutation angle is close to  $90^\circ$ . Although the double quantum coherences (DQCs) will be dephased by the subsequent magnetic field gradient, the zero quantum coherences (ZQCs) will survive and may be converted to observed magnetization by the final (nonideal)  $180^\circ$  pulse. If the magnetic

field gradient surrounding the  $180^\circ$  pulses would be identical, the pathway would certainly contribute to the observed signal. However, placing the magnetic field gradient along orthogonal directions dephases this pathway. Three additional coherence transfer pathways are generated by imperfect refocusing profiles that will survive the magnetic field gradient combination as shown in Figure 6.13. These pathways are single polarization transfer from the coupling partner to the spin of interest during each of the  $180^\circ$  pulses and double polarization transfer from the spin of interest to the coupling partner and back to the spin of interest. Because the coherences for both pathways remain in a single quantum state during the entire sequence, they can not be dephased by magnetic field gradients.

The derivation of an analytical expression to describe these phenomena [38–43] can be performed similar to that presented for the TE and TM modulations during STEAM (see also Exercise 6.11). Because only the SQCs need to be considered, the derivation is in fact substantially simplified and only the final result for an  $A_3X$  spin system (like lactate) will be given here. Consider the PRESS sequence shown in Figure 6.13 in which the  $90^\circ$  and  $180^\circ$  RF pulses have been replaced by RF pulses with nominal nutation angles of  $\alpha^\circ$  (excitation along x) and  $\beta^\circ$  and  $\gamma^\circ$  (refocusing along x or y). The observed signal at the beginning of acquisition can be described in terms of in-phase and anti-phase coherences of spin A according to:

$$SI(TE) = (k_1 A_y + k_2 A_x + k_3 2A_y X_z + k_4 2A_x X_z) \sin(\alpha) \quad (6.11)$$

where the coefficient  $k_1$  to  $k_4$  are given by [41, 42]:

$$\begin{aligned} k_1 = & \frac{P}{4} \sin\left(\frac{\pi JTE1}{2}\right) \sin\left(\frac{\pi JTE2}{2}\right) \cos\left(\frac{\pi JTE}{2}\right) \cos\left(\frac{\Delta\omega_{AX}TE}{2}\right) \\ & - \frac{A}{2} \cos^2\left(\frac{\pi JTE2}{2}\right) (1 - \cos(\gamma)) - \frac{A}{2} \sin^2\left(\frac{\pi JTE2}{2}\right) \cos(\gamma) (1 - \cos(\gamma)) \\ & + \frac{B}{2} \sin\left(\frac{\pi JTE2}{2}\right) \cos\left(\frac{\pi JTE2}{2}\right) (2\cos(\gamma) - 1 - \cos^2(\gamma)) \end{aligned} \quad (6.12)$$

$$k_2 = -\frac{P}{4} \sin\left(\frac{\pi JTE1}{2}\right) \sin\left(\frac{\pi JTE2}{2}\right) \cos\left(\frac{\pi JTE}{2}\right) \sin\left(\frac{\Delta\omega_{AX}TE}{2}\right) \quad (6.13)$$

$$k_3 = \frac{P}{4} \sin\left(\frac{\pi JTE1}{2}\right) \cos\left(\frac{\pi JTE2}{2}\right) \cos\left(\frac{\pi JTE}{2}\right) \sin\left(\frac{\Delta\omega_{AX}TE}{2}\right) \quad (6.14)$$

$$\begin{aligned} k_4 = & \frac{P}{4} \sin\left(\frac{\pi JTE1}{2}\right) \cos\left(\frac{\pi JTE2}{2}\right) \cos\left(\frac{\pi JTE}{2}\right) \cos\left(\frac{\Delta\omega_{AX}TE}{2}\right) \\ & + \frac{B}{2} \sin^2\left(\frac{\pi JTE2}{2}\right) (1 - \cos(\gamma)) + \frac{B}{2} \cos^2\left(\frac{\pi JTE2}{2}\right) \cos(\gamma) (1 - \cos(\gamma)) \\ & - \frac{A}{2} \sin\left(\frac{\pi JTE2}{2}\right) \cos\left(\frac{\pi JTE2}{2}\right) (2\cos(\gamma) - 1 - \cos^2(\gamma)) \end{aligned} \quad (6.15)$$

and

$$A = \frac{1}{2} \cos^2 \left( \frac{\pi JTE1}{2} \right) (1 - \cos(\beta)) + \frac{1}{2} \sin^2 \left( \frac{\pi JTE1}{2} \right) \cos(\beta) (1 - \cos(\beta)) \quad (6.16)$$

$$B = \frac{1}{2} \sin \left( \frac{\pi JTE1}{2} \right) \cos \left( \frac{\pi JTE1}{2} \right) (2 \cos(\beta) - 1 - \cos^2(\beta)) \quad (6.17)$$

$\Delta\omega_{AX}$  equals  $2\pi|\nu_A - \nu_X|$ , where  $\nu_A$  and  $\nu_X$  are the resonance frequencies (in Hz) of the A and X spins and  $P = \sin^2(\beta) \sin^2(\gamma)$ . The most striking feature from Equations (6.11)–(6.17) is that, as a result of the polarization transfer process, during the period in between the refocusing pulses the magnetization evolves in the transverse plane at a frequency given by the difference of the coupled spins  $\Delta\omega_{AX}$ . This gives rise to a high frequency modulation on top of the regular co-sinusoidal modulation. Figure 6.17E and F gives graphical representations of Equations (6.11)–(6.17) for the PRESS sequence executed with the refocusing pulses shown in Figure 6.17A and B, respectively. For the highly imperfect Gaussian refocusing pulses, a significant amount of polarization transfer occurs around  $TE = 2/J$  (for  $TE1 = TE2$ ) giving rise to overall signal loss and a high-frequency modulation. The frequency of this modulation increases with increasing magnetic field strength. The effect is most pronounced at  $TE = 2/J$ , because at this echo time the transverse magnetization is completely converted to antiphase coherence during the  $180^\circ$  RF pulses [at  $1/(2J)$  and  $3/(2J)$ ], thereby allowing maximum polarization transfer efficiency. Figure 6.17F shows the same simulations but now for the optimized refocusing pulse of Figure 6.17B. Because the nominal nutation angle over the voxel is much closer to the ideal  $180^\circ$ , the polarization transfer efficiency is significantly reduced and the observed modulation is close to the theoretical co-sinusoidal modulation. Note that when an asymmetrical PRESS sequence is used ( $TE2 = 4 TE1$ ), the high-frequency modulation is spread over all echo times.

The undesirable polarization transfer effects displayed in Figure 6.17E and F can be minimized by (1) using a short echo time or (2) using optimized refocusing pulses that produce a near rectangular frequency, and hence slice profile. The use of optimized RF pulses is recommended in general, since it allows for a better volume definition.

## 6.2.7 Localization by Adiabatic Selective Refocusing (LASER)

LASER is a single-scan 3D localization method executed with adiabatic excitation and refocusing RF pulses [44]. It is a modification of the previously described SADLOVE technique [45] and is based on the well-known principle of frequency-selective refocusing with pairs of AFP pulses as described in Section 5.7.4. The basic LASER sequence is depicted in Figure 6.18. In short, a single AFP pulse in the presence of a magnetic field gradient can achieve slice-selective refocusing. However, the frequency modulation of the pulse induces a nonlinear  $B_1$  and position dependent phase across the slice which will lead to severe signal cancellation. In Section 5.7.4 it was shown that a second, identical AFP pulse can refocus the nonlinear phase such that perfect refocusing can be achieved. Since frequency-selective adiabatic excitation pulses remain elusive, the entire sample is excited with a nonselective adiabatic excitation pulse after which three pairs of AFP pulses achieve 3D localization by selectively refocusing three orthogonal slices. The advantages of the LASER technique over STEAM and PRESS are twofold, namely (1) the method

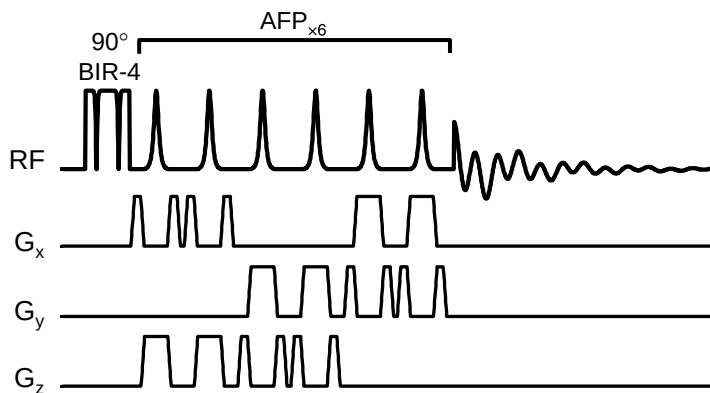


**Figure 6.18** Pulse sequence for 3D LASER localization. Excitation is achieved with BIR-4 or AHP 90° pulses, while refocusing is performed with three pairs of AFP pulses, each selecting an orthogonal spatial slice, thereby defining a 3D volume. Magnetic field crusher gradients surrounding the AFP pulses ensure removal of all unwanted coherences from outside the localized volume. The echo-time  $TE$  is defined from the end of the BIR-4 pulse to the beginning of acquisition.

is completely adiabatic and (2) by employing high bandwidth AFP pulses the localization can be extremely well-defined, both in terms of minimal chemical shift displacement, as well as sharpness of the localization edges. The localization can be further improved by using frequency offset corrected inversion (FOCI) pulses for refocusing [46].

At first sight LASER may not appear suitable for the detection of short- $T_2$  or scalar-coupled spin systems due to the requirement of six AFP refocusing pulses. However, on state-of-the-art MR systems, the echo-time can typically be reduced to less than 20 ms such that  $T_2$ -induced signal loss can be minimal. Scalar coupling evolution during a regular spin-echo can lead to significant signal loss for strongly coupled spin systems like glutamate and glutamine even for modest echo times (10–40 ms). Fortunately, LASER is not a regular spin-echo method, but is closer related to the CPMG multiple spin-echo pulse train [47,48]. It is well-known that evolution due to scalar coupling can be inhibited [49,50] when:

$$\sqrt{\Delta\omega_{AB}^2 + J_{AB}^2} TE_{\text{CPMG}} \ll 1 \quad (6.18)$$

where  $\Delta\omega_{AB}$  and  $J_{AB}$  are the frequency separation and scalar coupling constant between spins A and B, respectively, and  $TE_{\text{CPMG}}$  is the echo-time surrounding one 180° pulse, which equals  $TE/6$  for LASER as shown in Figure 6.18. Equation (6.18) can not be readily satisfied for weakly coupled spin systems like lactate, since the large frequency separation between lactate-H2 and H3 demands an unrealistically short echo-time. However, for strongly coupled spin systems like glutamate, even modest LASER echo-times up to 40 ms satisfy Equation (6.18) thereby leading to greatly enhanced signal detection. Figure 6.19 compares glutamate detection with LASER and a conventional spin-echo method at 4.0 T for increasing echo-times. While the minimum LASER echo-time on this particular

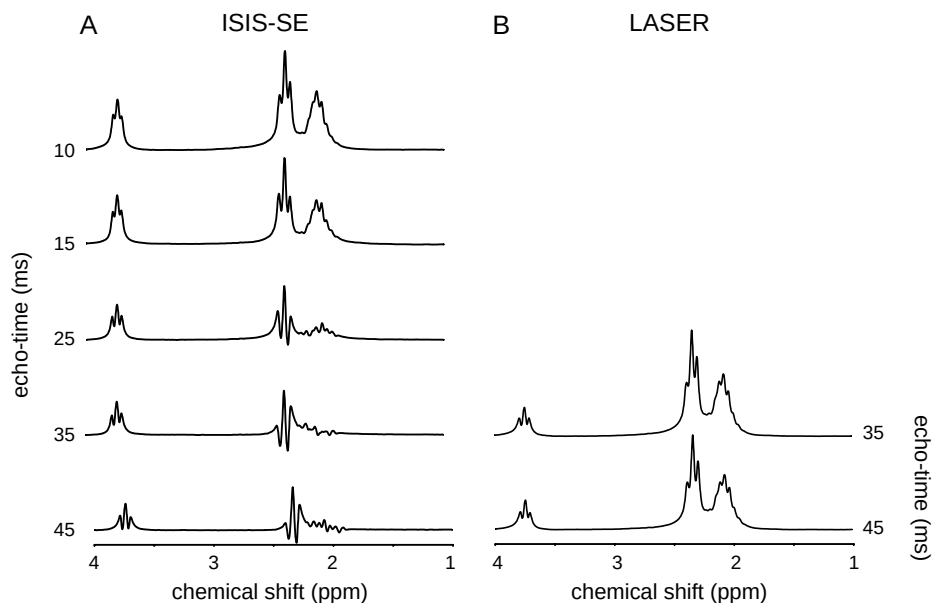


**Figure 6.19** Scalar coupling evolution of glutamate at 4.0 T as a function of the echo-time for (A) a regular spin-echo sequence preceded by 3D ISIS localization and (B) a 3D LASER sequence. While the minimum echo-time for LASER is much longer than for ISIS-SE, the scalar-coupling evolution for a strongly coupled spin system like the glutamate H3 and H4 protons is greatly reduced due to the CPMG character of the sequence. See text for more details.

system was limited to 35 ms (Figure 6.19B), the signal intensity for glutamate-H3 and -H4 corresponds to that obtained at TE = 10 ms during a regular spin-echo acquisition (Figure 6.19A). Increasing the LASER echo-time to 45 ms has minimal effects on the spectral appearance, while the spin-echo glutamate intensity has essentially reduced to zero. Therefore, despite the increased echo-time, LASER is an excellent method to detect strongly coupled spin systems like glutamate and glutamine.

## 6.2.8 Chemical Shift Displacement – Scalar-coupled Spins

In Section 6.2.2 it was described that all RF/gradient-based localization methods like ISIS, STEAM, PRESS or LASER exhibit a chemical shift displacement artifact. For homonuclear scalar-coupled spin systems additional complications can arise. Consider a homonuclear scalar-coupled  $A_nX$  ( $n = 1, 2, 3$ ) spin system. In the case of spin-1/2 nuclei, the NMR spectrum will consist of an A doublet of relative intensity  $n$  and a X ( $n+1$ ) multiplet of relative intensity one. For simplicity only the A doublet is considered in the following discussion, but similar arguments can be used to describe the X multiplet behavior. Figure 6.20 shows two dimensions of the voxel selected for spin A. The directions shown coincide with the directions refocused by the two  $180^\circ$  pulses of a PRESS method. Because of the chemical shift artifact, the voxel for the X spins is shifted (in all three dimensions) by the amount given in Equation (6.3). This has the consequence that not all X nuclei *within*

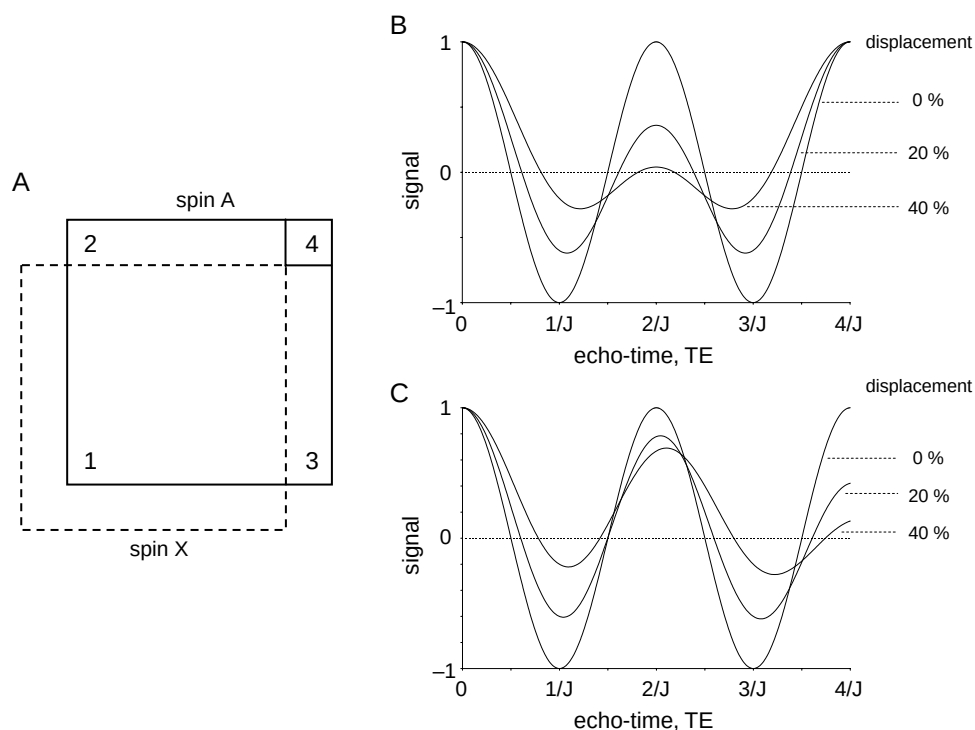


**Figure 6.20** The effects of chemical shift displacement during PRESS localization on the scalar coupling evolution of coupled spins. (A) Localized volume of spin A (solid line) and spin X (dotted line). The dimensions shown correspond with the slices selected by the two  $180^\circ$  pulses. The voxel displacement of spin X relative to spin A causes a selective refocusing of A in volume elements 2 and 3 during the first and second  $180^\circ$  pulse, respectively. In volume 4, spin A is selectively refocused during both  $180^\circ$  pulses, while in volume 1 A is nonselectively refocused, i.e. A and X are both refocused. (B) Scalar coupling evolution of the A spins in an  $A_nX$  ( $n = 1, 2$  or  $3$ ) spin system as a function of the echo-time TE ( $TE_1 = TE_2$ ). The curves represent voxel displacements of 0 (ideal), 20 and 40 % of the nominal volume. (C) Scalar coupling evolution for  $A_nX$  spin systems during an asymmetric PRESS sequence ( $TE_2 = 4TE_1$ ) with voxel displacements of 0, 20 and 40 % of the nominal volume.

the A voxel experience the two  $180^\circ$  pulses. Therefore, at certain spatial positions the A nuclei experience a selective spin-echo (when the X nuclei are not refocused), while at other positions they experience a nonselective spin-echo. This will in turn lead to a complicated modulation of the A resonance intensity, which will depend on the sequence timing and on the amount of spatial voxel displacement. To quantitatively assess the A modulations, consider the four subvoxels within the total A voxel. In all subvoxels the A spins experience both  $180^\circ$  pulses. In subvoxel 1, the X nuclei experience both  $180^\circ$  pulses. In subvoxels 2 and 3 the X spins only experience the first and second  $180^\circ$  pulse, respectively, while in subvoxel 4 the X spins do not experience any  $180^\circ$  pulse. The volumes of the subvoxels



are determined by the amount of voxel displacement and are given by:

$$\begin{aligned} V_1 &= \left(1 - \frac{\Delta\omega}{\Delta\omega_{\max}}\right)^2 V \\ V_2 &= V_3 = \left(1 - \frac{\Delta\omega}{\Delta\omega_{\max}}\right) \left(\frac{\Delta\omega}{\Delta\omega_{\max}}\right) V \end{aligned} \quad (6.19)$$

$$\begin{aligned} V_4 &= \left(\frac{\Delta\omega}{\Delta\omega_{\max}}\right)^2 V \\ V &= V_1 + V_2 + V_3 + V_4 \end{aligned} \quad (6.20)$$

When assuming complete in-phase coherence of the A spins immediately after excitation, the evolution of A coherence (by summing the individual subvoxels) is given by:

$$\begin{aligned} A_x \longrightarrow A_x [\rho_1 V_1 \cos(\pi JTE) + \rho_2 V_2 \cos(\pi JTE1) + \rho_3 V_3 \cos(\pi JTE2) + \rho_4 V_4] \\ + 2A_y X_z [\rho_1 V_1 \sin(\pi JTE) + \rho_2 V_2 \sin(\pi JTE1) + \rho_3 V_3 \sin(\pi JTE2)] \end{aligned} \quad (6.21)$$

$\rho_i$  ( $i = 1, 2, 3, 4$ ) is the spin density of subvoxel  $i$ . A similar equation can be deduced for antiphase coherence. It can be seen from Equation (6.21) that within subvoxel 1 the A spins evolve due to scalar coupling during the entire echo-time TE. Scalar evolution within subvoxels 2 and 3 is only effective during TE1 and TE2, respectively, while scalar evolution in subvoxel 4 is refocused completely. Figure 6.20B gives a graphical representation of Equation (6.21) for TE1 = TE2 = 0.5TE and chemical shift displacements of 0.2iV ( $i = 0, 1, 2$ ). It can be seen that when no chemical shift displacement occurs, the in-phase A coherence follows a co-sinusoidal modulation pattern, with a period of  $2/J$ . In the presence of chemical shift displacement, the modulation pattern substantially deviates from the ideal co-sinusoidal modulation. Especially for echo-times between  $1/J$  and  $3/J$  the signal intensity is substantially reduced (and even becomes zero) when compared to the ideal situation. Note that the signal acquired at TE =  $4/J$  is insensitive to the chemical shift displacement artifact, because at this echo time the magnetization is in-phase during the  $180^\circ$  refocusing pulses (at  $t = 1/J$  and  $3/J$ ). Similarly, the effect is largest at TE =  $2/J$ , because the magnetization has a pure anti-phase character during the  $180^\circ$  pulses [at  $t = 1/(2J)$  and  $3/(2J)$ ]. When an asymmetrical PRESS sequence is used (TE2 = 4TE1), the signal modulations get even more complicated (Figure 6.20C). The signal at  $4/J$  is no longer independent to the effect, because the coherences during the  $180^\circ$  pulses have a mixed in-phase and anti-phase character. Complete refocusing of the coherences now occurs at TE =  $10/J$ . From Figure 6.20 it is apparent that an enormous underestimation of the real concentration can result if the chemical shift displacement effect is not taken into account.

Several options exist to either minimize or eliminate the unwanted modulations, as pointed out by Yablonskiy *et al.* [51]. The most straightforward solution is to increase the RF bandwidth  $\Delta\omega_{\max}$  (by decreasing the pulse length), such that higher magnetic field gradients need to be employed to select the same spatial voxel, thereby minimizing  $\Delta\omega/\Delta\omega_{\max}$  in Equation (6.19). Unfortunately this option has only limited applicability

because the required RF power increases with decreasing pulse length. Employing magnetic field gradient modulation as described for VERSE (Section 5.9) and FOCI (Section 6.2.2) pulses, can increase the effective bandwidth without increasing the required RF peak power. Other options to minimize the modulation problem include the use of short echo-times ( $TE \ll 1/J$ ), use of specific sequence timing (e.g.  $TE = 4/J$ ,  $TE_1 = TE_2 = 2/J$ ), cycling the sign of the magnetic field gradients and employing sequences with additional RF pulses which refocus unwanted modulations. However, independent of the compensation method employed, it is most important that the experimentalist is aware of the problem. For example, when measuring the transverse relaxation time constant of a coupled spin system with a localization sequence like PRESS, it is not recommended to measure the signal intensity by increasing the PRESS echo-time, because this will lead to a complicated time-dependent modulation of the signal. Instead, one should separate the localization and variable echo-time parts, by employing a (spectrally selective) spin-echo following the localization sequence.

In summary, significant polarization transfer effects may be observed during PRESS localization of scalar-coupled spin systems. The origin of these effects can be found in the imperfect refocusing profile of the  $180^\circ$  pulses. The effects can be minimized (but never 100 % solved) by optimizing the refocusing profile of the  $180^\circ$  pulses to resemble a perfect rectangular profile. Frequency differences between spins will lead to a chemical shift displacement which, for scalar-coupled spin systems, will lead to a TE-dependent deviation from the regular scalar coupling evolution. Clearly, the voxel misregistration and polarization transfer effects can not be separated under practical conditions. Therefore, the observed modulation function will be a convolution of these two effects. A quantitative evaluation of the two effects combined is given by Marshall and Wild [41,42].

### 6.3 Water Suppression

Water is the most abundant compound in mammalian tissue and as a result the proton NMR spectrum of almost all tissue is dominated by a resonance at circa 4.7 ppm originating from the two protons of water (e.g. Figure 6.21A). In some tissues, like muscle and breast, the second most abundant compounds are a wide variety of lipids which give rise to multiple resonances in the 1–2 ppm range. The concentration of metabolites is often  $>10\,000$  lower than that of water. Modern 16-bit analog-to-digital converters (ADCs, see Chapter 10) are able to adequately digitize the low metabolite resonances in the presence of a large water resonance without degrading the metabolite SNR (Figure 6.21B). However, the presence of a large water resonance leads to baseline distortions and spurious signals due to vibration-induced signal modulation (Figure 6.21B) which in turn make the detection of metabolites unreliable [52–54]. Suppression of the water signal eliminates baseline distortions and spurious signals, leading to a reliable and consistent detection of metabolite spectra (Figure 6.21C).

The suppression of a particular resonance in a NMR spectrum requires a difference in a property between the molecule of interest and the compound that is interfering with detection (i.e. water). This property need not be a magnetic one, but it must be reflected in a NMR observable parameter. Thus besides differences in chemical shift, scalar coupling and  $T_1$  or  $T_2$  relaxation, properties like diffusion and exchange can also be exploited. Although

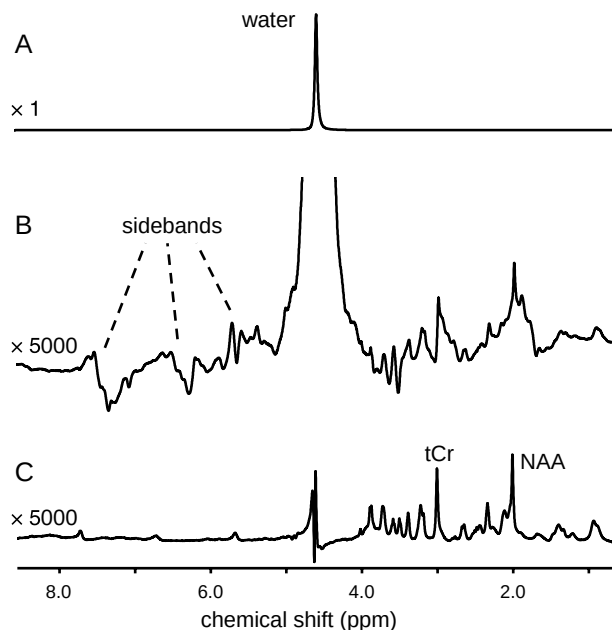


**Figure 6.21** Demonstration of the necessity of water suppression in vivo. (A) A 3D localized  $^1\text{H}$  NMR spectrum acquired from rat brain is dominated by the singlet resonance of water. (B) While the dynamic range of modern AD converters is sufficient to detect small metabolite signals in the presence of a large water signal, small (typically less than 1 %) vibration-induced sidebands of the water obscure the metabolite resonances. (C) Removal of the water resonance (and consequently any associated sidebands) results in an artifact-free  $^1\text{H}$  NMR spectrum that allows reliable detection and quantification of metabolites.

there is not a universal technique there are some criteria by which the existing water suppression methods can be evaluated. These criteria include: (1) degree of suppression; (2) insensitivity to RF imperfections (inhomogeneity); (3) ease of phasing the spectra; (4) insensitivity to relaxation effects; (5) perturbation of other resonances; and (6) detection of resonances near or at the water resonance frequency.

The existing water suppression techniques can be divided into four groups, namely: (1) methods that employ frequency-selective excitation and/or refocusing; or (2) utilize differences in relaxation parameters; (3) spectral editing methods like polarization transfer; and (4) other methods, including software-based water suppression. These four groups will be discussed in detail in the next sections.

### 6.3.1 Binomial and Related Pulse Sequences

One of the most obvious choices for water suppression is to utilize the difference in chemical shift (resonance frequency) between water and the other resonances. These methods are most widely used and in general they satisfy most of the mentioned criteria, giving suppression factors of more than 10 000. However, the criteria [6] can never be fulfilled,

i.e. since there is no chemical shift difference, resonances at or near the water resonance will also be suppressed.

Among the first water suppression methods to be used in FT NMR spectroscopy are the sequences of short high-amplitude RF pulses interleaved with delays to achieve frequency-selective excitation [55–61]. The sequences are designed in such a manner that metabolite resonances are rotated to the transverse plane for detection, while the water magnetization is returned to the longitudinal axis at the end of the sequence, making it unobservable. Most of these frequency-selective hard pulse sequences were derived within the small nutation angle approximation, such that the NMR response can be described as a linear system. Within this approximation the frequency-domain excitation profile equals the Fourier transformation of the time-domain pulse. Despite this assumption, the performance of the pulses is exceptional even for large nutation angles.

Short, high-amplitude (i.e. ‘hard’) RF pulses can be seen as time-domain delta ( $\delta$ ) functions. Since the Fourier transform of a sinusoidal function is a  $\delta$  function, a series of hard RF pulses can be used to generate frequency-domain sinusoids. Consider the frequency-domain function  $S_n(\omega)$ , given by:

$$S_n(\omega) = \sin^n \left( \frac{\omega\tau}{2} \right) \quad (6.22)$$

where  $n$  is a positive integer. The inverse Fourier transformation of  $S_n(\omega)$  is given by:

$$S_n(t) = \sum_{k=0}^n (-1)^k \binom{n}{k} \delta \left( t + \left( k - \frac{n}{2} \right) \tau \right) \quad (6.23)$$

where

$$\binom{n}{k} = \frac{n!}{k!(n-k)!} \quad (6.24)$$

The time-domain signal consists of  $(n+1)$  equally spaced delta functions (i.e. hard pulses) separated by delays  $\tau$ . The delta functions have alternating signs and amplitudes given by the binomial coefficients  $n$  and  $k$ . Equation (6.23) describes a series of ‘binomial pulses’ which have historically been very popular for water suppression [56, 58, 60, 61]. For instance, the pulse sequence corresponding to  $n = 1$  is denoted 11. This pulse sequence corresponds to two equal-amplitude hard pulses separated by a delay  $\tau$ . The second pulse is  $180^\circ$  phase shifted relative to the first pulse, as denoted by the bar. This pulse produces a frequency-domain excitation profile given by  $\sin(\omega\tau/2)$ . The  $n = 3$  pulse sequence is denoted 1331 and consists of four pulses with relative magnitudes of 1:3:3:1. The second and fourth pulses are  $180^\circ$  phase shifted relative to the first and third pulses. All pulses are separated by a delay  $\tau$ . This binomial pulse sequence produces a frequency-domain excitation profile given by  $\sin^3(\omega\tau/2)$ . It should be realized that Equation (6.23) is only valid for the case of time-domain functions of which the amplitudes are given by a binomial distribution. For a practical NMR experiment, the delta functions need to be approximated by hard pulses of finite duration, where the nutation angles rather than the amplitudes of the individual pulses are binomially distributed. Figure 6.22 shows some typical binomial pulse sequences.

When using binomial pulse sequences for water suppression, the carrier frequency of the pulse sequence is set on the water resonance frequency. As a consequence the water will

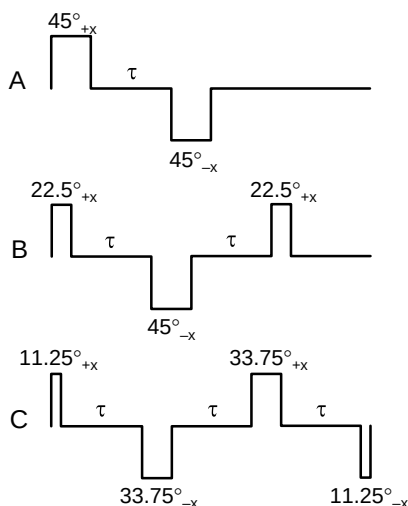


**Figure 6.22** Binomial water suppression sequences according to Equation (6.23) for (A)  $n = 1$ , (B)  $n = 2$  and (C)  $n = 3$ . On-resonance the nutation angle adds up to  $0^\circ$  (i.e. no excitation), while  $1/2\tau$  Hz off-resonance the net nutation angle is  $90^\circ$  (i.e. full excitation).

not be excited onto the transverse plane. Figure 6.23 shows simulations based on the Bloch equations for the first three binomial pulses. It can be seen that the on-resonance excitation null widens as  $n$  increases (resulting in a better water suppression), but that the off-resonance excitation profile becomes smaller with increasing  $n$  (leading to unwanted suppression of metabolite resonances). Thus, the best choice of  $n$  represents a compromise between optimal water suppression and detection uniformity for metabolite resonances. However, the choice may also be governed by the phase of the resulting transverse magnetization. Figure 6.23B shows that with increasing number of pulses the (linear) phase across the spectrum also increases. However, this linear phase can easily be corrected by first-order (linear) phasing of the spectrum. The slight deviation from exact linear phase across the spectrum can be minimized by reducing the overall nutation angle of the binomial pulse.

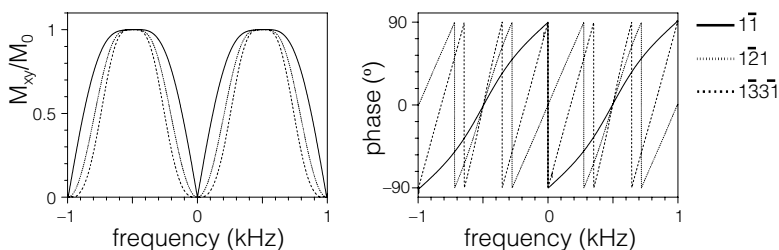


**Figure 6.23** Excitation profiles for the binomial RF pulses shown in Figure 6.22. Higher order binomial pulses exhibit a wider suppression region, but have a smaller excitation bandwidth and a larger first-order phase rotation across the spectrum.

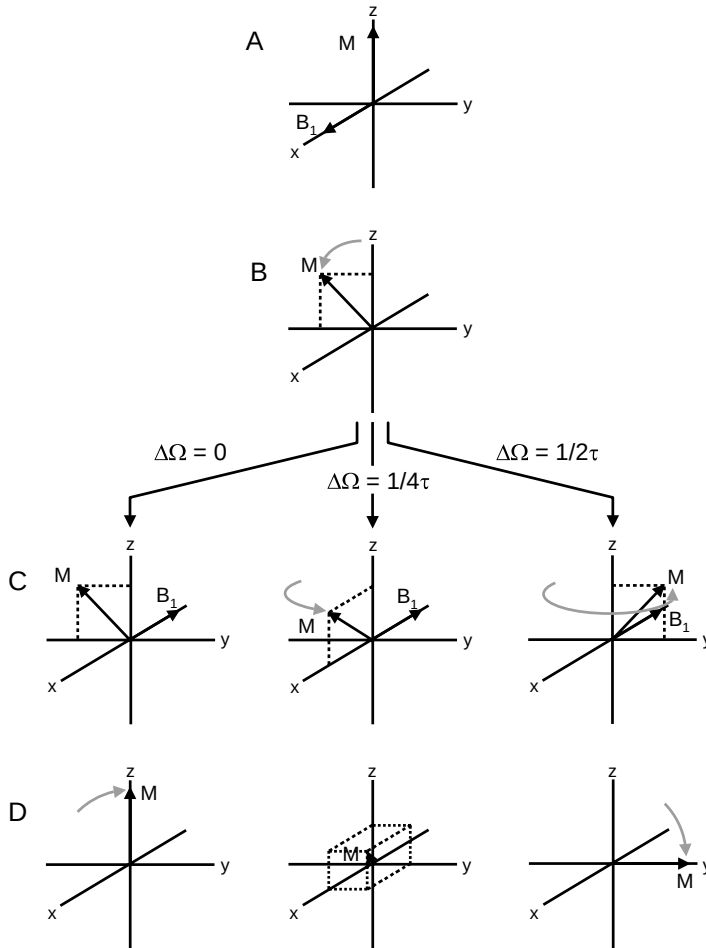


**Figure 6.24** Rotations during the  $45^\circ(+x) - \tau - 45^\circ(-x)$  binomial pulse sequence, for anticlockwise rotations. (A) Prior to the first pulse, the magnetization is assumed to be in thermal equilibrium. (B) Situation after  $45^\circ$  excitation. (C) During the delay  $\tau$ , spins at different frequencies  $\Delta\Omega$  rotate in the transverse plane (i.e. acquire a phase shift  $\phi$ ) according to  $\phi = 2\pi \Delta\Omega \tau$ . (D) On-resonance magnetization vectors (left) are rotated back to the  $+z$  axis, while off-resonance magnetization vectors at  $\Delta\Omega = 1/2\tau$  (right) are being excited. Vectors at intermediate frequencies (middle) are being partially excited.

The mechanism underlying water suppression with binomial pulses can easily be understood with the use of classical vector diagrams. Figure 6.24 shows the rotations of the magnetization vector  $\mathbf{M}$  during a  $1\bar{1}$  pulse sequence. Normally, water is on-resonance ( $\Delta\Omega = 0$ ) and the metabolite resonance frequencies are such that excitation occurs (complete excitation for  $\Delta\Omega = 1/2\tau$ ). The initial  $45^\circ(+x)$  pulse rotates all magnetization towards the transverse plane. During the following delay, the water magnetization remains stationary

in the rotating frame, while the metabolite magnetization rotates by  $180^\circ$ . The last  $45^\circ(-x)$  pulse rotates the metabolite magnetization completely in the transverse plane, and simultaneously rotates the water magnetization back to the  $+z$  axis. Magnetization with intermediate resonance frequencies has an intermediate response, i.e. partial excitation and partial return to the longitudinal axis. All binomial pulses can easily be described in a classical manner. Note that the total nutation angle on-resonance always adds to zero, i.e. on-resonance a 1331 pulse sequence equals  $(11.25^\circ - 33.75^\circ + 33.75^\circ - 11.25^\circ) = 0^\circ$ , while off-resonance full excitation occurs since  $(11.25^\circ + 33.75^\circ + 33.75^\circ + 11.25^\circ) = 90^\circ$ . Therefore, as long as the correct ratios between the pulses are used, the on-resonance water suppression is completely  $B_1$ -insensitive, since the total nutation angle always adds to zero. Further note, that the 'odd' binomial sequences [e.g. 11 ( $n = 1$ ) or 1331 ( $n = 3$ )] are generally preferred over the 'even' binomial sequences [e.g. 121 ( $n = 2$ ) or 14641 ( $n = 4$ )], because 'odd' binomial sequences exhibit an on-resonance null even when the binomial ratios are incorrectly calibrated [i.e.  $11.75^\circ - 50^\circ + 50^\circ - 11.75^\circ = 0$  ( $n = 3$ ), while  $22.5^\circ - 60^\circ + 22.5^\circ \neq 0$  ( $n = 2$ )].

As an alternative to the generation of sinusoidal frequency-domain profiles, cosine profiles can be used. These pulses are identical to the already described binomial pulse sequence, except that all pulses have the same phase (e.g. 1331 instead of 1331). Cosine binomial pulses generate maximum excitation on-resonance, while the off-resonance null is located at  $\pm 1/2\tau$  Hz. When used for water suppression, the carrier frequency of a cosine binomial sequence should be placed on the metabolites of interest, while the delay  $\tau$  is adjusted to give a null at the water Larmor frequency. Cosine binomial sequences do not have any particular advantage over sine binomial sequences. In some special cases they could be used when changes in carrier frequency are undesirable.

Another useful pulse sequence for water suppression which only utilizes hard RF pulses and delays is the so-called jump-return (JR) sequence [57, 59]. This sequence consists of  $90^\circ(+x) - \tau - 90^\circ(-x)$ , in which  $\tau = 1/4\Delta\Omega$  for complete excitation  $\Delta\Omega$  Hz off-resonance.  $\tau$  is therefore only half of the intrapulse delays during binomial pulses. Figure 6.25A shows the transverse magnetization and phase profiles of a  $90^\circ$  JR pulse. The main advantage of the JR sequence over the binomial 11 pulse is that the phase is virtually constant across the excitation profile (except from a phase inversion on-resonance). Another advantage of the JR sequence is that it is readily converted to an adiabatic version, known as a solvent suppression adiabatic pulse (SSAP [62]). Figure 6.25B shows an experimental verification of the excitation profile of a SSAP JR pulse.

As was mentioned earlier, the excitation profiles for binomial and JR pulses are only described by sinusoidal functions when the RF pulses approximate  $\delta$  (delta) functions. The typical  $B_1$  fields encountered in *in vivo* MRS ( $\gamma B_{1\max}/2\pi = 10$  kHz and typically much smaller), make the  $90^\circ$  pulse length of the same order as the intrapulse delay  $\tau$ , such that the  $\delta$  function approximation breaks down. Figure 6.26 shows simulations based on numerical integration of the Bloch equations for JR pulses of different pulse lengths with  $\tau = 500$   $\mu$ s. The  $90^\circ$  pulselengths were 25, 50, 100 and 200  $\mu$ s (corresponding to  $\gamma B_1/2\pi = 10, 5, 2.5$  and  $1.25$  kHz, respectively). It follows that the excitation maximum shifts to lower frequencies as the pulse lengths become longer. Furthermore, the phase across the excitation profile becomes nonlinear with longer  $90^\circ$  pulses. If not accounted for, this can have significant effects on signal quantification. The degradation of the excitation profiles arises from the fact that the magnetization experiences a larger, offset-dependent phase

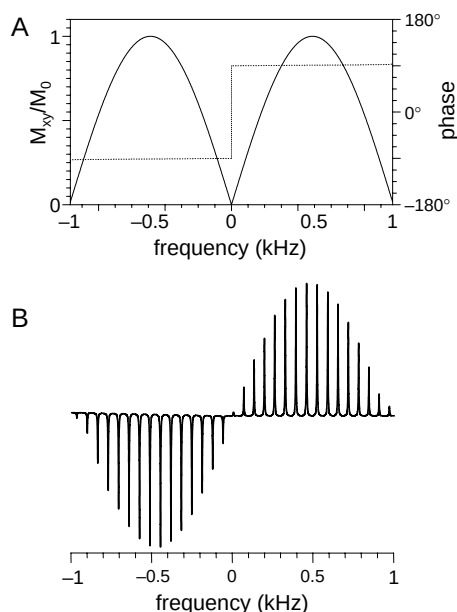


**Figure 6.25** Excitation profile of a JR  $[90^\circ(+x) - \tau - 90^\circ(-x)]$  RF pulse. The excitation profile is theoretically given by  $\sin(2\pi\Delta\Omega t)$ , such that magnetization  $1/4\tau$  Hz off-resonance is completely excited. Note that, apart from the on-resonance phase inversion, the phase is constant across the excitation profile. The sinusoidal excitation profile and constant phase can easily be verified using vector diagrams as shown in Figure 6.24. (B) Experimental verification of an adiabatic JR pulse (SSAP) on a water-filled spherical phantom. The SSAP pulse consists of a  $0^\circ$  BIR-4 pulse with an intrapulse delay  $\tau$  between the first and the second segment.

shift during longer RF pulses (see Chapter 5). As was derived in Section 5.2, a finite square RF pulse of length  $T$  can be approximated to first order by an ideal, infinitely short pulse (i.e. a delta function) followed by a free precession period of  $(2T/\pi)$ . Therefore, the phase shift generated by the RF pulses can be removed by recalculating the intrapulse delay as  $(\tau - 2(2T/\pi))$ . For example, the third JR pulse sequence of Figure 6.26, i.e.  $90^\circ_x(100 \mu s) - \tau(500 \mu s) - 90^\circ_{-x}(100 \mu s)$ , will be calculated as  $90^\circ_x(100 \mu s) - \tau(373 \mu s) - 90^\circ_{-x}(100 \mu s)$ . Figure 6.26C and D shows the results for the compensated JR sequences. The excitation maximum of all JR sequences occurs at the theoretical predicted maximum of  $\pm 500$  Hz. Note that the phase across the excitation profiles does not significantly improve, because the phase is more sensitive to the off-resonance amplitude modulation of longer RF pulses.

The nulling of the ‘odd’ binomial RF pulses and of the JR sequence is completely  $B_1$  insensitive, because the sum of nutation angles on-resonance is always zero. Off-resonance, a different situation arises since the sum of nutation angles is no longer constant (e.g.  $90^\circ$ ). When an inhomogeneous coil, such as a surface coil, is used for pulse transmission this will lead to signal loss of the metabolite resonances, even though the water suppression can be excellent. Because binomial and JR pulse sequences are composed of square RF pulses,



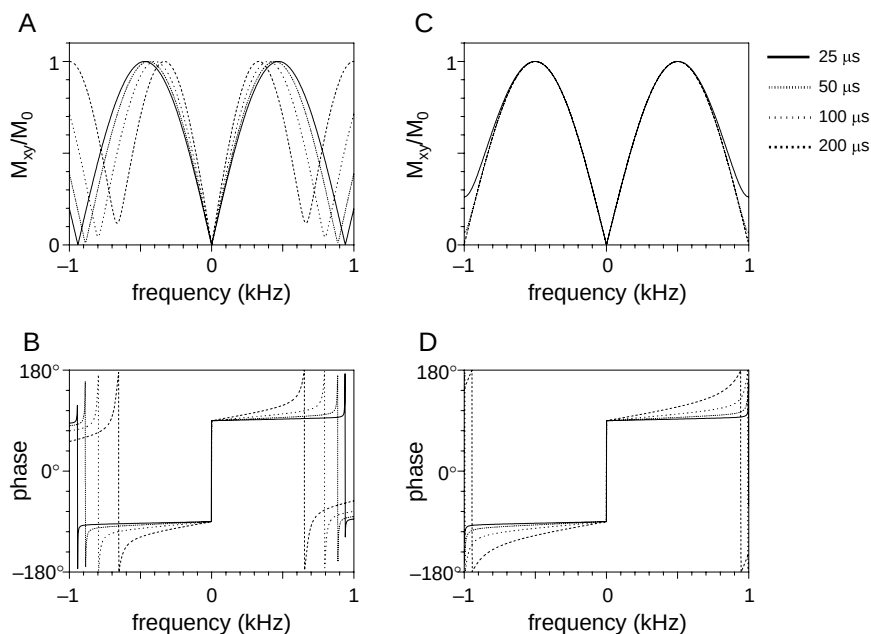


**Figure 6.26** (A and B) Excitation profiles for a JR pulse of different pulse lengths, e.g.  $90^\circ$  ( $25\ \mu\text{s}$ )— $\tau$  ( $500\ \mu\text{s}$ )— $90^\circ$  ( $25\ \mu\text{s}$ ), without compensating for phase shifts during the pulses. (A)  $M_{xy}$  and (B) phase. Note that the excitation maximum shifts and the phase rotation becomes nonlinear for longer pulse lengths. (C and D) Excitation profiles when the intrapulse delay  $\tau$  is corrected for phase rotations during the pulses according to  $(\tau - 2(2T/\pi))$ , e.g.  $90^\circ$  ( $25\ \mu\text{s}$ )— $\tau$  ( $468\ \mu\text{s}$ )— $90^\circ$  ( $25\ \mu\text{s}$ ). The frequency position of the excitation maximum is now independent of the pulse length, but the nonlinear phase across the spectrum remains.

they can easily be transformed to their adiabatic analog by substitution of individual square RF pulses by BIR-4 pulses, thereby providing complete  $B_1$  insensitivity on-resonance, but also off-resonance.

The JR pulse has very successfully been converted to an adiabatic RF pulse under the acronym SSAP (solvent suppression adiabatic pulse) [62]. Instead of individually substituting the  $90^\circ$  RF pulses of a JR sequence, a single  $0^\circ$  BIR-4 pulse suffices. Introducing a delay  $\tau$  between the first and second (or alternatively between the third and fourth segment of BIR-4) completes the conversion. Note that because the individual adiabatic segments are inherently refocused, the delay  $\tau$  does not have to be corrected for the duration of the segments.

Despite the great success of water suppression with binomial pulses in high-resolution NMR, the performance of these methods when used with typical *in vivo* localization methods can be surprisingly poor. This can often be explained by the presence of time-varying  $B_0$  magnetic fields due to residual (uncompensated) eddy currents (see Section 10.4.1). The performance of binomial pulse sequences depends critically on the relative phases between the individual RF pulses and should typically be  $0^\circ$  or  $180^\circ$ . However, when time-varying  $B_0$  magnetic fields from a preceding magnetic field gradient persist

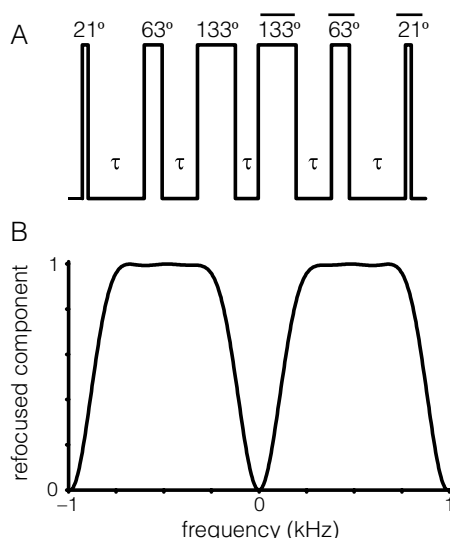


**Figure 6.27** (A) WATERGATE pulse sequence and (B) corresponding refocusing frequency profile. The intrapulse delay was adjusted to give maximum refocusing at  $\pm 500$  Hz off-resonance. The bars above the nutation angles in (A) indicate that the last three pulses have a  $180^\circ$  phase change relative to the first three pulses.

during the pulse, the transverse magnetization during the intra-pulse delay  $\tau$  experiences an additional phase shift which will lead to a shift in the excitation profile and therefore to incomplete water suppression. The original sequence performance can easily be restored by calibrating the phase of the individual RF pulses in order to counteract the additional phase acquired by the transverse magnetization.

The performance of semi-selective RF pulses, like binomial and JR pulses, can be substantially improved through numerical optimization of the pulse durations, pulse phases and intra-pulse delays [63]. A popular, numerically optimized pulse is the so-called 3-9-19 or WATERGATE sequence, described by Sklenar *et al.* [64]. As can be seen from Figure 6.27, WATERGATE generates a significantly improved frequency profile, with a sufficiently wide frequency ‘null’ for water suppression and a relatively uniform response across the majority of the spectral range.

It should be realized that all binomial and related methods generate a wide range of nutation angles depending on the exact frequency position in the spectrum. As such it is to be expected that binomial pulses can affect scalar coupling evolution through the generation of MQCs and polarization transfer effects ([65], see also Exercise 6.10).

### 6.3.2 Frequency Selective Excitation

The implementation of binomial RF pulses as discussed in Section 6.3.1. allows for simple, but robust water suppression. The primary drawback of binomial methods is that their frequency response is periodic and not highly selective. For these reasons binomial sequences

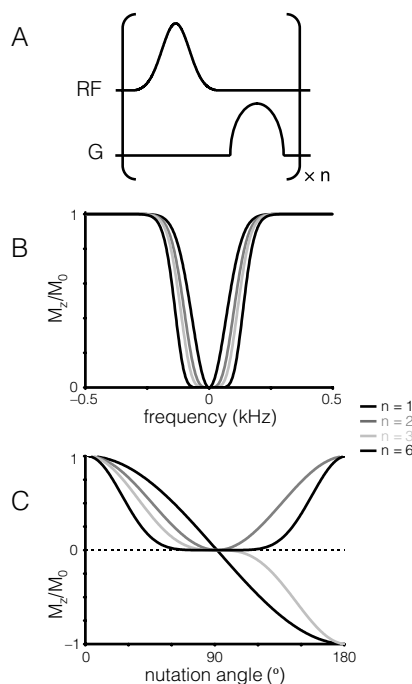


**Figure 6.28** (A) Generalized pulse sequence element for water suppression by frequency selective excitation. After the water has been excited by the frequency selective RF pulse (e.g. Gaussian), the following magnetic field ‘crusher’ gradient dephases the water coherences. The sensitivity to (B)  $B_0$  and (C)  $B_1$  magnetic field inhomogeneity can be improved by repeating the element shown in (A)  $n$  times. More water suppression elements lead to (B) a broader frequency range over which suppression is achieved and (C) a greater tolerance to variations in the nutation angle of the excitation pulse.

are often referred to as semi-selective RF pulses. Frequency-selective, shaped RF pulses were discussed in Chapter 5 and their use in combination with magnetic field gradients has been demonstrated for slice selection in MRI and volume selection in MRS. The frequency selectivity of shaped RF pulses is readily utilized in water suppression through the pulse sequence element shown in Figure 6.28A. In essence, the selective RF pulse excites the water onto the transverse plane after which all coherences are dephased by the following  $B_0$  magnetic field gradient. This sequence was originally described by Haase *et al.* [66] and was named CHES (chemical shift selective) water suppression. A similar sequence was described by Doddrell and coworkers [67] given the name SUBMERGE (suppression by mistimed echo and repetitive gradient episodes). A major advantage of CHES is that it can precede any pulse sequence, since it leaves the metabolite resonances unperturbed. To avoid water signal recovery by  $T_1$  relaxation, the pulse length and the delay between CHES and the excitation of metabolites should be as short as possible. Most often Gaussian shaped RF pulses are used for frequency selective excitation because of their favorable bandwidth–pulse length product and the well-defined frequency profile. The suppression

efficiency of CHESS clearly depends on the ability of the RF pulse to generate transverse magnetization for all water spins in the ROI. Therefore,  $B_0$  and  $B_1$  magnetic field homogeneity are crucial for adequate water suppression. A single CHESS element should suffice when  $B_0$  and  $B_1$  magnetic field inhomogeneity is negligible. In practice however, the CHESS element is repeated two to six times to improve the water suppression (Figure 6.28B and C). Repeating the basic CHESS element of Figure 6.28A will have two effects that independently improve the water suppression. Firstly, repeating the CHESS element will broaden and flatten the frequency range around the water resonance (Figure 6.28B), so that the suppression becomes less sensitive towards frequency offsets caused by  $B_0$  magnetic field inhomogeneity. Secondly, the additional elements reduce the sensitivity towards  $B_1$  magnetic field inhomogeneity (Figure 6.28C). As can be seen from Figure 6.28C, a single CHESS element only achieves perfect suppression when the nutation angle equals  $90^\circ$  (ignoring  $T_1$  relaxation). For any other nutation angle there will be residual magnetization along the longitudinal axis, which will lead to incomplete water suppression. In the absence of  $T_1$  relaxation it is trivial to show that the residual longitudinal magnetization  $M_z(\theta)$  after  $n$  CHESS elements is a function of the nutation angle  $\theta$  according to:

$$M_z(\theta) = M_0(\cos \theta)^n \quad (6.25)$$

Therefore after six repeated CHESS elements, the residual magnetization along the  $z$  axis is less than 0.035 % for nutation angles,  $75^\circ \leq \theta \leq 105^\circ$ , providing sufficient water suppression with a tolerance to  $B_1$  magnetic field inhomogeneity of 30 % around the nominal nutation angle of  $90^\circ$ . Exercise 6.4 deals with the extension of Equation (6.25) to account for  $T_1$  relaxation in between the CHESS elements. It should be realized that even though the on-resonance water suppression has a high tolerance towards  $B_1$  magnetic field inhomogeneity, the off-resonance frequency profile does depend on the exact  $B_1$  amplitude. This should be taken into account when studying metabolites close to the water resonance.

CHESS cycles have been combined with STEAM localization under the name DRYSTEAM [68]. In the view of water suppression, the STEAM sequence is more favorable over PRESS, since one or more CHESS cycles can be incorporated in the TM period. For instance, Figure 6.29 shows a 3,3-DRYSTEAM sequence, in which three CHESS cycles have been incorporated prior to the localization and three CHESS cycles in the TM period. This combination of six CHESS elements and STEAM localization can give suppression factors of  $>15\,000$  even for very short echo times ( $TE < 20$  ms) and surface coil transmission [68].

Some further improvements in water suppression can be made by individual adjustments of the nutation angles in the repeated CHESS cycles (see also Section 6.4), but far more important is the removal of unwanted echoes that can arise from a series of repeated RF and gradient pulses. For example,  $n$  pulses give rise to  $3^{n-1}$  coherence transfer pathways. Therefore, strategical placement of  $B_0$  magnetic field gradient crushers is essential for optimal water suppression. Theoretical and practical considerations of CHESS water suppression have been presented by Moonen and van Zijl [68]. Using the formalism of coherence pathways they demonstrated that water is suppressed optimal when linear combinations of the applied  $B_0$  magnetic field gradients during the repeated CHESS cycles do not lead to complete refocusing of transverse magnetization (i.e. the net applied gradient should be nonzero for all coherences at any point following the first excitation pulse). For three

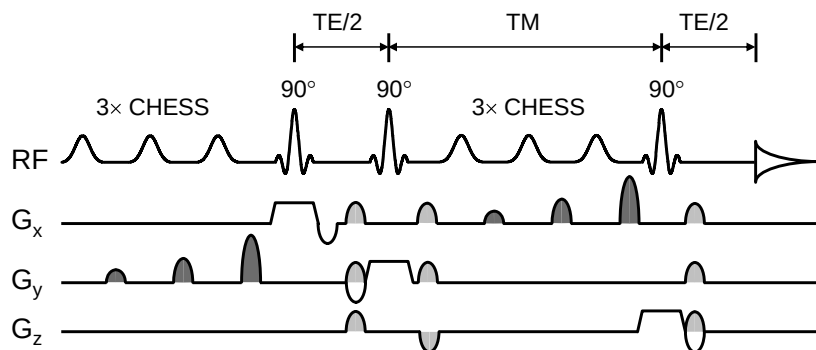


**Figure 6.29** Combination of STEAM localization and CHES water suppression (3,3 – DRYSTEAM). The dark shaded magnetic field gradients (in a 1:2:4 ratio) are used for dephasing of water coherences. Light shaded and nonshaded magnetic field gradients are used for crushing and localization, respectively. Incomplete water suppression prior to the STEAM localization sequence can be improved by a number of CHES elements during the TM period that will give  $T_1$ -independent water suppression. While the six CHES elements should give excellent water suppression (see also Figure 6.28), the actual amount of residual water is largely determined by the ability to avoid refocusing of water coherences by the large number of magnetic field gradients. Using the amplitude ratios and orientations shown, gives a suppression factor  $>15\,000$ .

CHES elements this condition is achieved by applying the magnetic field gradients in a 1:2:4 (or 4:2:1) ratio. In a 3, 3–DRYSTEAM sequence, the direction of the magnetic field gradients prior to localization and during the TM period should be different to avoid unwanted echoes.

When the selective RF pulse shown in Figure 6.28A is a long (in the order of  $T_1$ ), low power, constant amplitude irradiation field, the sequence element corresponds to (pre)saturation [69]. During RF presaturation the field is continuously applied at the water frequency in order to reach a (hypothetical) situation of equal spin populations (i.e. completely dephase the water magnetization in a plane perpendicular to the excitation axis). In practice, complete saturation can not be obtained, since saturation is competing with  $T_1$  relaxation. The best results are obtained when irradiation is maintained for  $2\text{--}3T_{1\text{water}}$ . In practice, the saturation pulse length is chosen, after which the pulse power is optimized with respect to optimal water suppression (for a given repetition time TR). Presaturation is a popular water suppression technique in high-resolution liquid-state NMR and it has received some attention in early *in vivo* NMR studies. This was mainly governed by the ease of implementation and reasonable suppression factor ( $\sim 1000$ ) of presaturation. However, the disadvantages of presaturation have dramatically reduced its applications for *in vivo* MRS. Excessive heat deposition is a consideration, but presaturation may also obscure actual metabolite concentrations by off-resonance magnetization transfer between NMR invisible ('bound' or immobile) metabolites and NMR visible, mobile metabolites. Further, presaturation does in general not allow (or at least complicates) the observation of exchangeable protons. Finally, presaturation requires sufficient  $B_0$  magnetic

homogeneity, although this is a requirement for all chemical shift based water suppression techniques.

The absence of a frequency selective adiabatic excitation pulse has prevented a direct adiabatic analog of CHESS. However, de Graaf and Nicolay [70] realized that a frequency selective inversion, as achieved by for example a hyperbolic secant AFP pulse, equals a frequency selective excitation at the two frequencies where the longitudinal magnetization becomes zero following selective inversion. Since the inversion bandwidth of AFP pulses is constant over a large  $B_1$  range, the points of excitation are essentially adiabatic and can be used in a CHESS-type water suppression sequence, dubbed SWAMP (selective water suppression with adiabatic-modulated pulses) [70]. Using four or six AFP pulses interleaved with magnetic field crusher gradients typically reduces the residual water well below the metabolite resonances, without subject-dependent power adjustments. The insensitivity of SWAMP towards variations in  $T_1$  relaxation can be further improved by adjustment of frequency offsets and inter-pulse delays [71].

### 6.3.3 Frequency Selective Refocusing

As an alternative to suppressing the water resonance prior to excitation, suppression can be achieved during a subsequent spin-echo period. The main advantage of water suppression following excitation is that the suppression efficiency is not degraded by  $T_1$  relaxation. The only drawback of frequency selective refocusing over excitation methods is that the minimum echo-time is prolonged due to the requirement for frequency selective  $180^\circ$  pulses. While this prevents short-TE MRS, it does not impede experiments at longer echo-times, like spectral editing (see Chapter 8). Three spin-echo-based methods are WATERGATE [72], excitation sculpting [73] and MEGA [74] of which the basic MEGA sequence is shown in Figure 6.30. Note that even though the WATERGATE sequences mentioned here and in Section 6.3.1 share the same acronym, they are distinctly different methods. In essence all three methods rely on the fact that the water is selectively dephased, while the metabolites of interest are rephased during the spin-echo period. For instance, consider the MEGA sequence of Figure 6.30. All resonances are excited by a nonselective  $90^\circ$  pulse. In the transverse plane, the spins experience two orthogonal magnetic field gradients  $G_1$  and  $G_2$ . The essential ‘trick’ of all three water suppression techniques is, that the water resonance experiences an even number of refocusing pulses during two equal magnetic field gradients, while the metabolites are refocused by only one  $180^\circ$  pulse. This will result in a dephasing of the water by the orthogonal magnetic field gradients, while the metabolites can be observed. A detailed theoretical treatment of these techniques using the product operator formalism is given elsewhere [73, 74]. From these calculations it followed that residual, unsuppressed signal of excitation sculpting and MEGA is proportional to  $\cos^4(\theta/2)$ , where  $\theta$  is the nutation angle of the selective refocusing pulses (Figure 6.30B). Both excitation sculpting and MEGA can be transformed to adiabatic sequences, thereby providing complete  $B_1$ -insensitive water suppression. This is achieved by replacing the selective refocusing pulses with AFP pulses and the nonselective pulses with BIR-4 pulses. Figure 6.30C shows an *in vivo* evaluation of the  $B_1$  insensitivity of MEGA water suppression. Variation of the RF amplitude by a factor of  $>10$  has negligible effect on the spectral appearance.

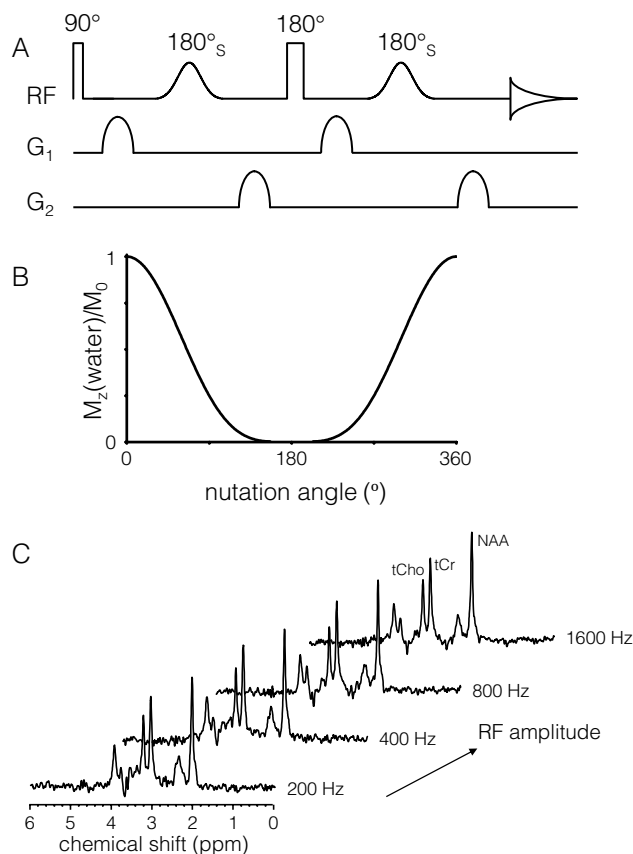


**Figure 6.30** (A) RF and gradient combinations as used for MEGA water suppression. (B) Residual water signal as a function of nutation angle for conventional frequency selective RF pulses. (C) <sup>1</sup>H NMR spectra obtained from rat brain in vivo at 4.7 T (TR/TE = 3000/144 ms, 125  $\mu$ l, numbers of experiments = 64) with MEGA water suppression executed with adiabatic AFP pulses. The water suppression remains excellent despite an eightfold variation in the RF amplitude.

### 6.3.4 Relaxation Based Methods

Techniques exploiting chemical shift differences are currently most often used for *in vivo* water suppression. However, for some specific applications, longitudinal and/or transverse relaxation may be used to discriminate between water and other resonances. When the water and metabolite relaxation times are sufficiently different, it is even possible to suppress the water signal and observe metabolites in the close proximity to the water resonance. Most relaxation based methods make use of differences in longitudinal  $T_1$  relaxation. WEFT (water eliminated Fourier transform) is among the oldest  $T_1$  based water suppression methods [75–77]. The pulse sequence of WEFT is identical to an inversion recovery sequence used for  $T_1$  relaxation time measurements. It consists of a nonselective 180°

pulse followed by an adjustable delay  $t$  and a magnetic field gradient to remove transverse components during  $t$ . After the delay  $t$ , the magnetization is excited by a nonselective  $90^\circ$  pulse and acquired. The delay  $t$  is chosen so that the water magnetization has recovered to its null point [i.e.  $t = T_{1,\text{water}} \ln(2)$ , see Figure 1.21], whereas the metabolites have partially recovered. In order to ensure near-complete recovery of the magnetization, the repetition time TR between successive excitations needs to be  $4\text{--}5T_{1,\text{water}}$ . Unfortunately, this would lead to excessive measurements time, since the *in vivo*  $T_1$  relaxation time of water can be 1.5–2.0 s (see Tables 3.1 and 3.2). It is possible to operate in a steady-state mode, such that  $\text{TR} < 4\text{--}5T_1$ , making the simple expression  $t_{\text{null}} = T_{1,\text{water}} \ln(2)$ , slightly more complicated and TR dependent. However, the exact dependency needs not to be known if the delay  $t$  is optimized empirically.

A comparable method is the so-called driven equilibrium Fourier transform (DEFT) technique [78–80] given by the sequence  $90^\circ\text{--}t\text{--}180^\circ\text{--}t\text{--}90^\circ$ . The magnetization is excited by the first pulse. Longitudinal magnetization that has recovered during the  $t$  period is inverted by the  $180^\circ$  pulse. The water resonance can be nulled just prior to the last  $90^\circ$  pulse by proper choice of  $t$ . The metabolites are supposed to have relaxed back during  $2t$  and will be excited by the last  $90^\circ$  pulse. The advantage of DEFT over WEFT is that the choice of  $t$  is not so critical, since it is only necessary to remove water that relaxed back during the first  $t$  delay. Furthermore, phase cycling during DEFT may improve suppression.

The main disadvantages of WEFT and DEFT are rather obvious. Since the difference in *in vivo*  $T_1$  relaxation time between water and metabolites is only small, good water suppression is always accompanied by a severe reduction in sensitivity of the metabolite resonances since the metabolites are incompletely recovered during the delay  $t$ . Further, *in vivo*  $T_1$  relaxation times of water are very heterogeneous (see Tables 3.1 and 3.2). This results in incomplete water suppression, because the delay  $t$  can only be optimized for a single  $T_1$  relaxation time. Finally,  $T_1$  based water suppression makes quantification of metabolite resonances cumbersome.

As mentioned in Section 6.3.2, the performance of all water suppression techniques that rely on selective removal of the water resonance prior to signal excitation are degraded by (variations in)  $T_1$  relaxation. Several methods have been reported [21, 81] in which the  $T_1$  and  $B_1$  sensitivity of CHESS-based water suppression is decreased by optimizing the nutation angles of subsequent CHESS elements and/or optimizing the inter-pulse delay. One of these methods, variable pulse powers and optimized relaxation delays (VAPOR, [29]) combines  $T_1$ -based water suppression and optimized frequency-selective perturbations to provide excellent water suppression with a large insensitivity towards  $T_1$  and  $B_1$  inhomogeneity. The VAPOR method is depicted in Figure 6.31A. It consists of seven frequency selective RF pulses interspersed with optimized  $T_1$  recovery delays. For a nutation angle,  $\alpha$ , of  $90^\circ$  the longitudinal magnetization prior to nonselective excitation ends up very close to zero despite the presence of  $T_1$  relaxation. And even when the nutation angle is not calibrated corrected, the magnetization ends up close to zero due to the optimized pulse and delay combination (Figure 6.31B). The VAPOR sequence is also relatively insensitive to variations in  $T_1$  relaxation, as shown in Figure 6.31C. Figure 6.31D shows the performance of a 7-pulse CHESS sequence for comparison. While excellent water suppression has been achieved in a number of studies [29, 81], the long inversion times and repeated water perturbations significantly decrease the amount of water-exchangeable protons [29].



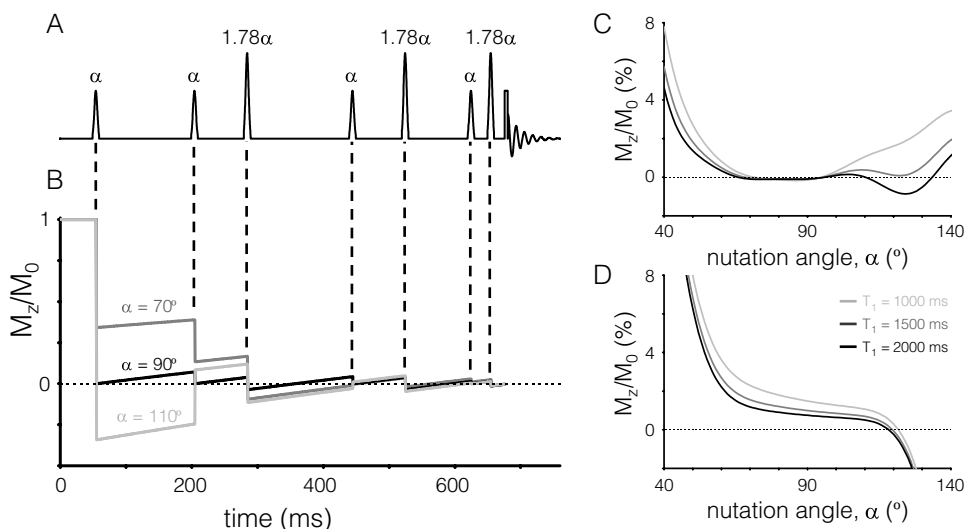


**Figure 6.31** Principle of VAPOR water suppression. (A) The specific RF pulse and delay combination used in VAPOR leads to a water suppression largely independent of the nutation angle  $\alpha$ . (B)  $M_z$  trajectory for  $\alpha = 70^\circ, 90^\circ$  and  $110^\circ$  are shown ( $T_1 = 1500$  ms) and all end up close to zero at the end of the sequence. Residual longitudinal magnetization as a function of nutation angle following (C) VAPOR and (D) a 7-element CHESS sequence (intrapulse delay = 20 ms) for  $T_1 = 1000, 1500$  and  $2000$  ms.

### 6.3.5 Non-water-suppressed NMR Spectroscopy

The majority of  $^1\text{H}$  MRS studies have focused on selectively suppressing the water resonance to unambiguously observe the metabolite resonances. However, detection of the water resonance can be very useful for (1) internal concentration referencing, (2)  $B_0$  eddy current and vibration correction (see Chapter 10), (3) automated phasing and (4) magnetic field drift monitoring and correction.

While arguments concerning limited ADC dynamic range are not longer valid with modern hardware, the primary reason against non-water-suppressed  $^1\text{H}$  MRS is the interference of strong spectral sidebands (Figure 6.21) arising from the water due to mechanical vibrations [52–54, 82]. Several reports have appeared addressing this problem using 2D approaches [82] and their 1D variants [83], sideband symmetry arguments and very long echo-times during which the sidebands have decayed [52]. However, none of these reports is generally applicable under all experimental conditions. A modification of the method described by Dreher and Leibfritz [84] is depicted in Figure 6.32. It involves the acquisition of two separate scans in which either the upfield ( $-1.6$  ppm to  $4.4$  ppm) metabolite resonances (Figure 6.32A) or the downfield ( $5.0$  ppm to  $11.0$  ppm) metabolite resonances (Figure 6.32B) are inverted. The large (unsuppressed) water resonance, as well as the water-related sidebands are not inverted in either scan. The difference between the two scans therefore results in a water-subtracted (or suppressed) metabolite spectrum without

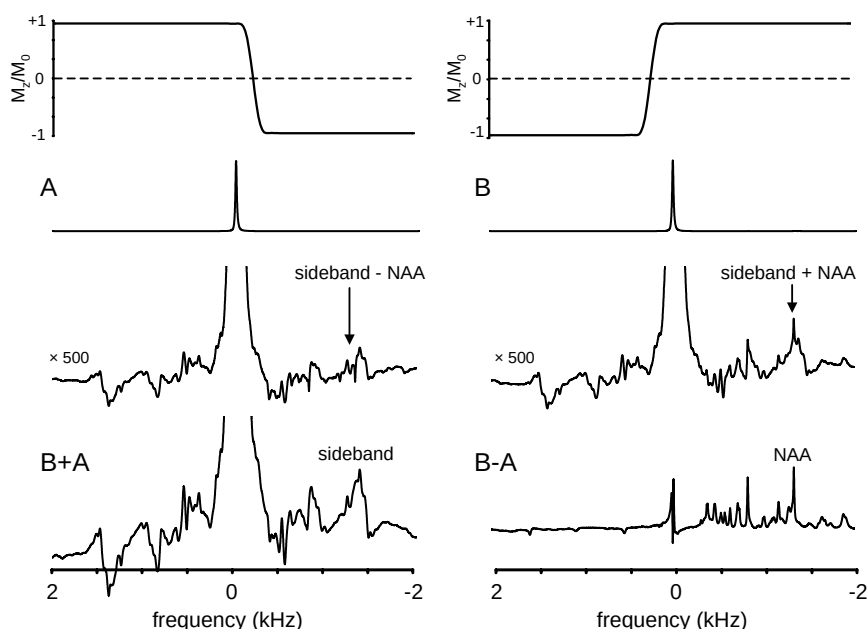


**Figure 6.32** Principle of non-water-suppressed MRS using a two-scan approach for post-acquisition water removal. (A) In the first scan, the upfield region of the  $^1\text{H}$  NMR spectrum is selectively inverted while leaving the water resonance unperturbed. (B) In the second scan, the downfield region of the  $^1\text{H}$  NMR spectrum is selectively inverted, again without perturbing the water resonance. Since the water resonance and associated sidebands are identical in both scans they are suppressed in the difference spectrum ( $B-A$ ), thereby revealing the metabolite resonances. Since the water resonance is not suppressed during acquisition, signal from water-exchangeable protons in the downfield region of the spectrum are greatly enhanced (see also Figure 2.1).

any interfering water-related sidebands. The sum of the two acquisitions gives the water and water-related sidebands and can be used for  $B_0$  eddy current and vibration correction, phase correction or internal concentration referencing. Unlike the methods described in the previous sections, the method shown in Figure 6.32 only minimally perturbs the water resonance. As a result, water-exchangeable protons are detected at greatly increased sensitivity, as can be seen in the example spectrum of Figure 2.1.

## 6.4 Exercises

**6.1** Consider the PRESS localization method with the following parameters:

TR = 2500 ms, TE = 274 ms (TE1 = TE2 = 137 ms), 2 ms linear SLR90 ( $R = 6.08$ ) excitation, 4 ms optimized sinc ( $R = 5.75$ ) refocusing,  $B_0 = 4$  T, voxel size =  $3 \times 3 \times 3$  cm.

- A** Calculate the percentage spatial overlap of voxel locations for the NAA methyl (2.01 ppm), creatine methyl (3.03 ppm) and choline methyl (3.22 ppm) resonances. Assume perfectly rectangular slice profiles.
- B** Given the limitations of the RF and gradient power supplies ( $B_{1\max} = 2500$  Hz,  $G_{\max} = 20$  mT m<sup>-1</sup>), calculate the percentage voxel overlap and pulse lengths that give the highest amount of spatial voxel overlap.
- C** In the case of lactate (assume two-spin system with  $J = 7.3$  Hz) calculate the amount of observable lactate as percentage of the amount that would have been observed in the absence of a chemical shift artifact.
- D** Repeat the calculation for  $TE = 548$  ms.
- 6.2** Consider a CHESS-2 sequence prior to a nonselective excitation executed with two 10 ms Gaussian pulses followed by 20 ms crusher gradients. Assume the first pulse is calibrated to give a  $90^\circ$  excitation.
- A** In the absence of  $B_1$  magnetic field inhomogeneity calculate the optimal nutation angle of the second pulse that gives the best suppression of brain gray matter water at 4 T ( $T_1 = 1250$  ms). Neglect  $T_1$  relaxation during the RF pulses and assume a long repetition time ( $TR > 5 T_1$ ).
- B** Calculate the relative water intensity ( $M_z/M_0$ ) for the optimized sequence calculated under (A) when the  $B_1$  field is off by  $-10\%$  and  $+10\%$  due to a miscalibration or  $B_1$  inhomogeneity.
- C** For a voxel composed of 60 % gray matter (50 M water,  $T_1 = 1250$  ms) and 40 % white matter (48 M water,  $T_1 = 1050$  ms) calculate the water resonance intensity relative to the creatine methyl resonance (10 mM,  $T_1 = 1400$  ms) for the optimized sequence calculated under (A).
- D** Repeat the calculation under (C) for a miscalibration of the  $B_1$  field by  $-10\%$  and  $+10\%$ .
- 6.3** **A** Under the assumption that sufficient inhibition of scalar coupling evolution occurs when  $(\Delta\omega^2 + J^2)^{1/2} TE_{\text{CPMG}} = 0.2$  calculate the maximum  $TE_{\text{CPMG}}$  for citrate at 1.5 T.
- B** Repeat the calculation for a magnetic field strength of 7.0 T.
- C** Repeat the calculation for lactate at 7.0 T.
- 6.4** **A** Derive an expression for the residual longitudinal magnetization  $M_z$  following  $n$  CHESS elements during the  $TR$  period of a PRESS sequence in the presence of  $T_1$  relaxation. Assume CHESS elements of length  $\Delta t$  and  $TR \gg T_1$ .
- B** Derive an expression for the residual longitudinal magnetization  $M_z$  following  $n_1$  and  $n_2$  CHESS elements during the  $TR$  and  $TM$  periods of a STEAM sequence in the presence of  $T_1$  relaxation. Assume CHESS elements of lengths  $\Delta t$ ,  $TR \gg T_1$  and  $TM$  equals the length of  $n_2$  CHESS elements.
- C** Calculate the residual signals following the PRESS sequence for CHESS ( $n = 4$ ) nutation angles of  $70^\circ$ ,  $90^\circ$  and  $110^\circ$  in the absence of  $T_1$  relaxation. Repeat the calculation for a  $T_1$  relaxation time of 1500 ms and 25 ms CHESS elements. Assume  $TR \gg T_1$ .
- D** Calculate the residual signals following the STEAM sequence for CHESS ( $n_1 = 2$ ,  $n_2 = 2$ ) nutation angles of  $70^\circ$ ,  $90^\circ$  and  $110^\circ$  in the presence of  $T_1$  relaxation ( $T_1 = 1500$  ms, 25 ms CHESS elements,  $TM = 50$  ms). Assume  $TR \gg T_1$ .

**6.5** The excitation profile for a Gaussian-shaped RF pulse is given by:

$$M_{xy}(\delta) = M_0 e^{-2(\delta-4.7)^2}$$

where  $\delta$  is the chemical shift in ppm.

- A** In the absence of  $T_1$  relaxation, calculate the signal intensity for the total creatine resonance at 3.92 ppm when a single pulse is used for CHESS water suppression followed by nonselective excitation.
- B** Repeat the calculation when the CHESS element is repeated six times prior to nonselective excitation in order to improve the water suppression.
- C** Summarize the conclusions of the calculation performed under (A) and (B) and explain how finite metabolite  $T_1$  relaxation would affect the results.
- 6.6 A** During a comparison between STEAM and LASER localization it is noticed that the localized STEAM spectra display a tenfold lower SNR. Name at least three possible reasons for the large difference in SNR and suggest means to eliminate them.
- B** During the development of a novel localization method, the localized volume is visualized by incorporating frequency- and phase-encoding gradient into the sequence. The image shows strong ripples across and outside the localized volume, indicating an inadequate localization performance. However, a spectroscopic experiment on a two-compartment water-lipid phantom yields a  $^1\text{H}$  NMR spectrum from the water compartment devoid of any lipid contamination. Explain these apparently contradicting results and suggest experimental modifications to test the answer.
- C** The  $T_2$  relaxation time for glycine in an *in vitro* sample doped with  $\text{MnCl}_2$  is determined as  $150 \pm 8$  ms. The glycine linewidths as measured from  $^1\text{H}$  NMR spectra acquired from  $5 \times 5 \times 5$  mm = 125  $\mu\text{l}$ , 64  $\mu\text{l}$ , 27  $\mu\text{l}$ , 8  $\mu\text{l}$  and  $1 \times 1 \times 1$  mm = 1  $\mu\text{l}$  volumes are given by 5.0, 3.8, 2.5, 2.6 and 3.6 Hz, respectively. Give an explanation for the variation in spectral linewidth for the different volume sizes.
- 6.7 A** For a 2D ISIS sequence verify that all signal outside the VOI is canceled at the end of the four required scans.
- B** Discuss if the presence of short  $T_1$  relaxation times would compromise the localization quality.
- 6.8 A** For a regular spin-echo sequence show that a simple two-step phase cycle on the  $180^\circ$  pulse (+x, -y) in conjunction with a receiver phase (+x, -x) is sufficient to eliminate imperfections introduced by the refocusing pulse (i.e. when the nutation angle deviates from  $180^\circ$ ). Assume a perfect  $90^\circ$  nutation angle for the excitation pulse.
- B** Show that the two-step phase-cycle under (A) is insufficient when the excitation pulse has a nutation angle different from  $90^\circ$ . Suggest an extended phase cycle for the  $180^\circ$  pulse that will be valid for any nutation angle of the excitation pulse.
- C** Determine the number of phase cycle steps required for a PRESS sequence.
- D** Show that the placement of two equal magnetic field ‘crusher’ gradients on either side of the  $180^\circ$  pulse is equivalent to the extended phase cycle derived under (B).

- 6.9 A** For a stimulated-echo sequence (without magnetic field gradients) with  $TE = 20$  ms and  $TM = 200$  ms calculate the number of signals that can be expected following the final RF pulse.
- B** With the insertion of equal-area magnetic field crusher gradients in the two  $TE/2$  and one  $TM$  period, calculate the number of signals that can be expected following the final  $TE$  crusher gradient.
- C** Recalculate the answer when the  $TM$  crusher gradient is made twice the area of a single  $TE$  crusher gradient.
- D** Discuss the spatial origin of the detected signal obtained with a STEAM sequence (see Figure 6.12) for the  $TE/TM$  crusher gradient combinations discussed under (B) and (C).
- 6.10** All five intra-pulse delays required during a WATERGATE sequence executed with ideal, infinitely short RF pulses are equal (see also Figure 6.27).
- A** Calculate the intra-pulse delay to achieve refocusing at  $\pm 250$  Hz off-resonance.
- B** Calculate the required pulse lengths when the maximum available  $B_1$  magnetic field strength equals 2.0 kHz.
- C** Recalculate the intra-pulse delays obtained under (A) to account for chemical shift evolution during the RF pulses.
- D** Derive an expression for the evolution of a scalar-coupled two-spin system  $AX$  resonating at frequencies  $\omega_A$  and  $\omega_X$  during a spin-echo sequence with echo time  $TE$  and a  $JR$  refocusing pulse with null and maximum refocusing frequencies of  $\omega_{\text{null}}$  and  $\omega_{\text{max}}$ , respectively. Assume two equal  $TE$  crusher gradients surrounding the jump-return pulse and ideal RF pulses. Exclude the  $JR$  pulse from the echo-time.
- 6.11 A** Derive Equation (6.9) for an  $AX$  two-spin system under the assumption of ideal RF pulses and complete dephasing by the  $TE$  and  $TM$  magnetic field ‘crusher’ gradients.
- B** For lactate at 9.4 T ( $^3J_{HH} = 6.9$  Hz) calculate the shortest  $TM$  delays which give the minimum and maximum amounts of detectable signal for  $TE = 1/3 J_{HH}$ . Consider lactate an  $A_3X$  spin system.

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# 7

## Spectroscopic Imaging and Multivolume Localization

### 7.1 Introduction

The localization techniques discussed in the previous chapter allow the detection of signal from a single volume. The advantages of this approach are that (1) the volume is typically well-defined with minimal contamination (e.g. extracranial lipids in brain MRS), (2) the magnetic field homogeneity across the volume can be readily optimized, leading to (3) improved water suppression and spectral resolution. The main disadvantage of single voxel localization methods is that no signal is acquired from large parts of the object, thereby potentially missing important areas of interest.

Multivoxel localization or magnetic resonance spectroscopic imaging (MRSI) allows the detection of localized NMR spectra from a multidimensional array of locations. While technically more challenging, due to (1) significant magnetic field inhomogeneity across the entire object, (2) spectral degradation due to intervoxel contamination, (3) long data acquisition times and (4) processing of large multidimensional datasets, MRSI can detect metabolic profiles from multiple spatial positions, thereby offering an unbiased characterization of the entire object under investigation. This chapter will review the principles of multivoxel localization techniques and in particular of spectroscopic imaging.

### 7.2 Principles of Spectroscopic Imaging

The previous edition of this monograph contained a short section on rotating frame spectroscopic imaging which achieves spatial localization based on  $B_1$  magnetic fields. However, with the exception of a limited number of specialized applications, rotating frame spectroscopic imaging has essentially been replaced with  $B_0$ -based spectroscopic imaging, which

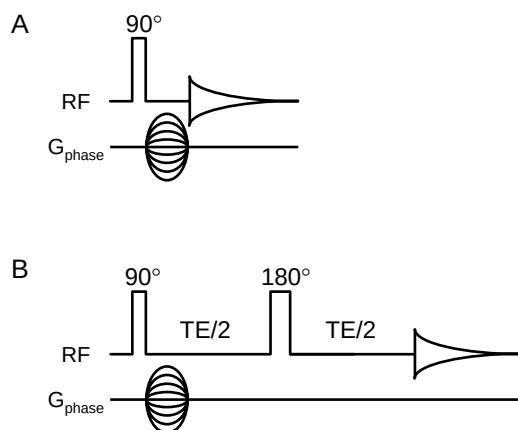


**Figure 7.1** Basic pulse sequences for 1D MRSI. (A) Pulse-acquire and (B) spin echo pulse sequences. NMR spectra acquired with sequence (A) require a linear phase correction in order to compensate for the delayed start of acquisition.

will be the topic for the rest of this chapter. The interested reader is referred to the literature [1–4] for more details on rotating frame spectroscopic imaging.

The principles of MRSI or spectroscopic imaging (SI) [5, 6] are very similar to phase encoding in magnetic resonance imaging (MRI). The basic pulse sequences (Figure 7.1) are also very similar to gradient and spin echo MRI sequences. Extending the MRSI experiment with an additional frequency axis, i.e. the chemical shift dispersion, is the main difference. After the first *in vitro* [5, 6] and *in vivo* [7, 8] demonstrations, MRSI has been developed to a localization technique which is now routinely used in clinical research.

Before proceeding with a formal, quantitative k-space description of MRSI, a qualitative and intuitive explanation of MRSI will be provided. Consider the basic MRSI sequence of Figure 7.1B. Following excitation, a 180° refocusing pulse in the middle of the spin echo period refocuses all phase evolution due to chemical shift and magnetic field inhomogeneity. During a MRSI experiment, a magnetic field gradient pulse  $G_{\text{phase}}$  (where phase can be any of the orthogonal directions x, y and z or combinations thereof for oblique MRSI) is applied whose amplitude is incremented in subsequent acquisitions in analogy with phase encoding in MRI. During application of the gradient pulse (e.g.  $G_x$ ), the precession frequencies of the spins are modified according to:

$$\omega(x) = \gamma x G_x \quad (7.1)$$

where x is the spatial position relative to the gradient isocenter (which normally coincides with the magnetic isocenter). For a constant-amplitude gradient pulse of duration t, Equation (7.1) is equivalent to a spatially dependent phase shift  $\phi$  given by:

$$\phi(x) = \gamma x G_x t \quad (7.2)$$

Since this gradient is only executed during one of the  $TE/2$  periods, the phase shift given by Equation (7.2) will not be refocused and will therefore encode the acquired FID or

echo with spatial information. A graphical explanation is given in Figure 7.2. Consider a theoretical experiment with point samples containing NAA (2.0 ppm), glutamate (2.4 and 3.8 ppm), creatine (3.0 ppm and 3.9 ppm) and choline (3.2 ppm). The spatial distribution of these metabolites along the  $x$  direction can be obtained by applying a phase-encoding gradient  $G_x$  as shown in Figure 7.1. For each gradient increment, a phase-encoded FID is acquired and stored (Figure 7.2A). The spatially acquired phase can be visualized more readily in the spectral domain obtained following Fourier transformation (Figure 7.2B). In the experiment where the amplitude of the phase-encoding gradient is zero, all resonances appear in-phase, as they would in a normal spin echo experiment. When the phase-encoding gradient is nonzero, the resonances acquire a phase shift dependent on their spatial position. In subsequent experiments, the real  $R(\omega, x)$  and imaginary  $I(\omega, x)$  spectra are sinusoidally modulated according to:

$$\begin{aligned} R(\omega, x) &= A(\omega) \cos \phi(x) - D(\omega) \sin \phi(x) \\ I(\omega, x) &= A(\omega) \sin \phi(x) + D(\omega) \cos \phi(x) \end{aligned} \quad (7.3)$$

where  $A(\omega)$  and  $D(\omega)$  represent the absorption and dispersion components of the metabolite resonances of interest, respectively, and  $\phi(x)$  is given by Equation (7.2). From the spectra in Figure 7.2B it can be seen that the creatine resonances do not modulate as a function of the phase-encoding gradient amplitude  $G_x$ . Using Equations (7.2) and (7.3) this can only be explained when  $\phi(x) = 0$  for all  $G_x$ , so that  $R(\omega, x) = A(\omega)$ . The phase can only be zero for all gradient amplitudes when creatine resides in the magnet isocenter ( $x = 0$ ). For the other resonances the situation is more complex. However, comparing the NAA and choline resonances, indicates that the NAA and choline samples must reside on opposite sides of the magnet isocenter (in the  $x$  direction), since the phase shift is in opposite directions for these resonances. Furthermore, since the choline resonance acquires more phase than the NAA resonance, it can also be deduced that the choline sample must reside further away from the isocenter than the NAA sample. The principle demonstrated in Figure 7.2 is that the spatial position  $x$ , which is proportional to frequency  $\omega(x)$  [Equation (7.1)], is encoded as phase  $\phi(x)$  in the second dimension. For point samples (with infinitely small dimensions) the  $(G_x, \phi(x))$  curves reveal the spatial frequency  $\omega(x)$  which, through Equation (7.1), is directly related to the spatial position  $x$ . However, realistic samples do not consist of point samples and may have identical resonances at multiple spatial positions. As has been demonstrated throughout Chapters 1 and 4, Fourier transformation is the standard technique to separate multiple frequencies. Therefore, a Fourier transformation with respect to the applied phase-encoding gradient will reveal the spatial sample distribution (Figure 7.2C).

Using the  $k$ -space formalism as introduced in Chapter 4, the relation between the acquired time-domain signal and the displayed frequency-domain spectra can be described quantitatively. The total acquired signal  $S(t)$  is the sum of signal from elementary volume elements  $s(x, t)dx$  from each point  $x$  in the sample:

$$S(t) = \int_{-\infty}^{+\infty} s(x, t) dx \quad (7.4)$$

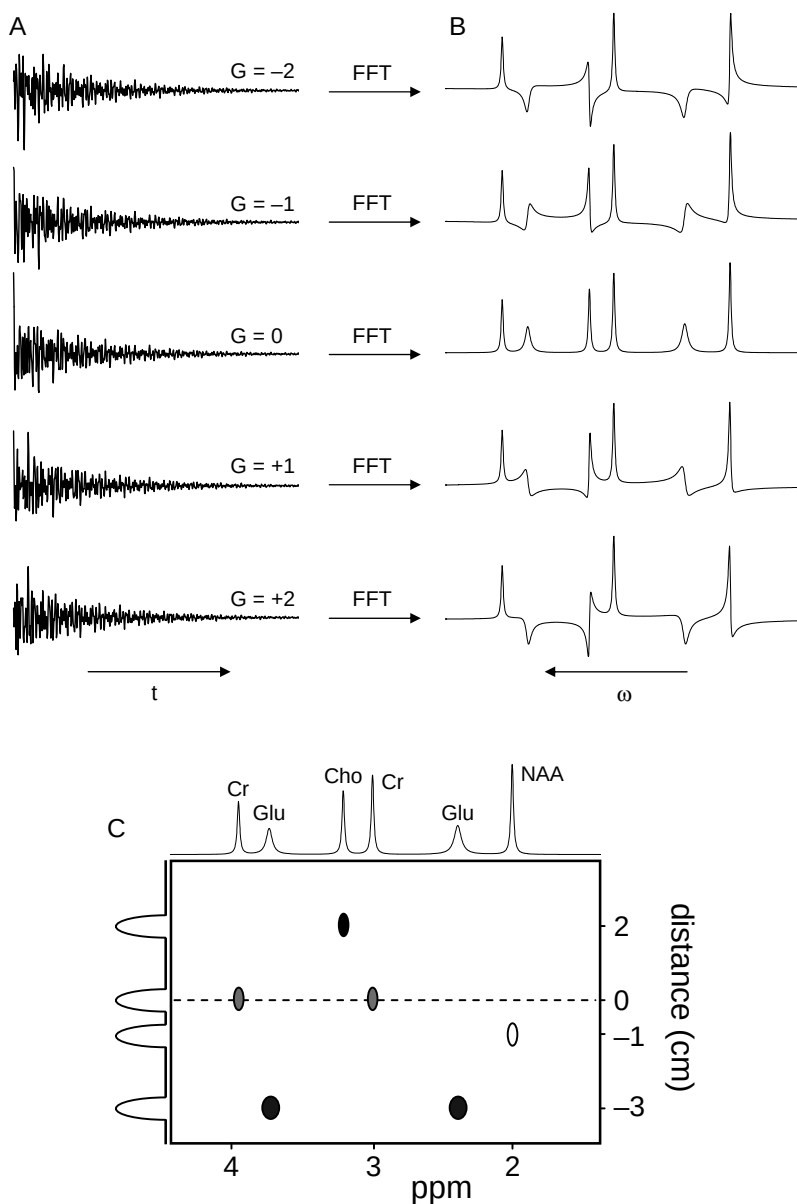


**Figure 7.2** Principle of MRSI. (A) FID signals acquired in the presence of different phase-encoding gradients. In the absence of a phase-encoding gradient ( $G = 0$ ), the FID is not spatially encoded and following a fast Fourier transformation (FFT) (B) reveals the spectral composition of the sample under investigation (showing resonances from creatine, glutamate, choline and NAA). In the presence of a phase-encoding gradient ( $G \neq 0$ ) different compounds acquire different phases depending on their spatial location. (C) Fourier transformation with respect to the phase-encoding gradient reveals the 1D spatial distribution of all the metabolites. See text for more details.

The total spectrum of the sample  $F(\omega)$  is obtained by Fourier transformation of  $S(t)$  and equals the sum of spectra from elementary volume elements  $f(x, \omega)dx$ :

$$F(\omega) = \int_{-\infty}^{+\infty} S(t)e^{-i\omega t}dt = \int_{-\infty}^{+\infty} f(x, \omega)dx \quad (7.5)$$

Until this point there is no difference between Equation (7.5) and the Fourier transformation given by Equation (1.52). However, as was already intuitively demonstrated in Figure 7.2, the spatial distribution of  $F(\omega)$ , i.e.  $f(x, \omega)$  can be obtained by application of a phase-encoding gradient which induces a phase shift on each elementary volume element according to:

$$f'(x, \omega) = f(x, \omega)e^{i\gamma x G_x t} \quad (7.6)$$

such that the entire spectrum can be written as:

$$F(G_x, \omega) = \int_{-\infty}^{+\infty} f(x, \omega)e^{i\gamma x G_x t}dx \quad (7.7)$$

The introduction of the k-space formalism,  $k_x = \gamma G_x t$ , converts Equation (7.7) to:

$$F(k_x, \omega) = \int_{-\infty}^{+\infty} f(x, \omega)e^{ik_x x}dx \quad (7.8)$$

Clearly, the phase-modulated spectra of the entire sample  $F(k_x, \omega)$  represents the inverse Fourier transformation of the spectra  $f(x, \omega)$  from the individual volume elements. Therefore,  $f(x, \omega)$  can easily be obtained by Fourier transformation of  $F(k_x, \omega)$ :

$$f(x, \omega) = \int_{-\infty}^{+\infty} F(k_x, \omega)e^{-ik_x x}dk_x \quad (7.9)$$

This calculation can easily be extended to three spatial dimensions by applying three orthogonal gradients independently in subsequent experiments. The volume elements are then calculated according to:

$$f(x, y, z, \omega) = \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} F(k_x, k_y, k_z, \omega)e^{-i(k_x x + k_y y + k_z z)}dk_x dk_y dk_z \quad (7.10)$$

The first spectral Fourier transformation of a complete 4D SI dataset  $s(k_x, k_y, k_z, t)$  yields the spectra  $F(k_x, k_y, k_z, \omega)$  after which a 3D spatial fourier transformation according to Equation (7.10) gives the spectra  $f(x, y, z, \omega)$  from the spatial positions  $x, y$  and  $z$ .

While Equation (7.10) suggests that the signal is continuously sampled in k-space over an infinitely long period, in reality the signal is only acquired over a finite period at discrete k-space positions. The spatially resolved spectra are obtained through a discrete Fourier transformation. Following the acquisition of  $N$  k-space samples, most discrete Fourier transform algorithms provide as output  $N$  spatial positions located between  $-N/2$  and

$N/2 - 1$ . As a result, the zero frequency point is not centered in the ‘spectrum’, but appears at a position  $-0.5$ . This is sometimes referred to as a half-pixel shift and should be taken into account when accurate localization or co-registration between high-resolution MRI and low-resolution MRSI data is required.

The gradient amplitude increments determine the digitization rate in the spatial frequency or  $k$ -space domain. By analogy with the acquisition of a 1D spectrum, this digitization is governed by the Nyquist sampling criterion, i.e. the maximal phase shift difference between two gradient increments over the entire field of view (FOV) equals  $2\pi$ :

$$2\pi = \gamma \text{FOV} \Delta G t, \quad \text{or} \quad \text{FOV} = \frac{1}{\gamma \Delta G t} = \frac{1}{\Delta k} \quad (7.11)$$

where  $\gamma = \gamma/2\pi$  and  $\Delta k$  is expressed in  $\text{cm}^{-1}$ . The nominal voxel size  $\Delta V$  is directly related to the FOV and the number of phase-encoding increments  $N_p$  and is given by:

$$\Delta V = \frac{\text{FOV}}{N_p} \quad (7.12)$$

In practice the minimum voxel size is, besides the FOV, determined by the allowable measurement time and sensitivity. For conventional MRSI, the encoding of  $N_1 \times N_2 \times N_3$  volume elements (voxels) requires  $N_1 \times N_2 \times N_3$  acquisitions.

### 7.3 Spatial Resolution in MRSI

The nominal voxel size in a MRSI experiment is simply given by Equation (7.12), i.e. the entire FOV divided by the number of phase-encoding gradient increments. However, the actual voxel size can substantially deviate from this nominal value due to the characteristics of the Fourier transformation [9–14].

A time-domain signal  $s(k_x)$  measured over an infinitely long period of time will produce a single frequency upon Fourier transformation. However, under realistic conditions  $s(k_x)$  is sampled only over a finite time, described by the sampling function  $F_{\text{sample}}(k_x)$ . The Fourier transformation must therefore be made over the product of  $s(k_x)$  and  $F_{\text{sample}}(k_x)$ , leading to a convolution of  $s(x)$  and  $F_{\text{sample}}(x)$  in the spatial domain (see also Appendix A3). When  $F_{\text{sample}}(k_x)$  is given by a constant grid of  $N_p$  samples (i.e. phase-encoding steps), the convolution is given by:

$$\text{PSF} = \text{FFT}[F_{\text{sample}}(k_x)] = \Delta k_x \frac{\sin(\pi N_p \Delta k_x x)}{\pi N_p \Delta k_x x} \quad (7.13)$$

This is the case for MRS, MRI, MRSI and in general all techniques requiring Fourier transformation. The Fourier transformation of the sampling points (or sampling grid when more dimensions are involved) is often referred to as the point spread function (PSF). Although the PSF always influences the Fourier transformed data, it has not been mentioned earlier, because the PSF was not a dominating factor. Provided that the signal has decayed to zero at the end of the acquisition period, the PSF of a time-domain free induction decay (FID) is much narrower than the typical line widths which are dominated by  $T_2^*$  relaxation. The PSF only becomes apparent when the acquisition time is too short, leading to a truncation artifact in the NMR spectrum (e.g. see Figure 1.10).

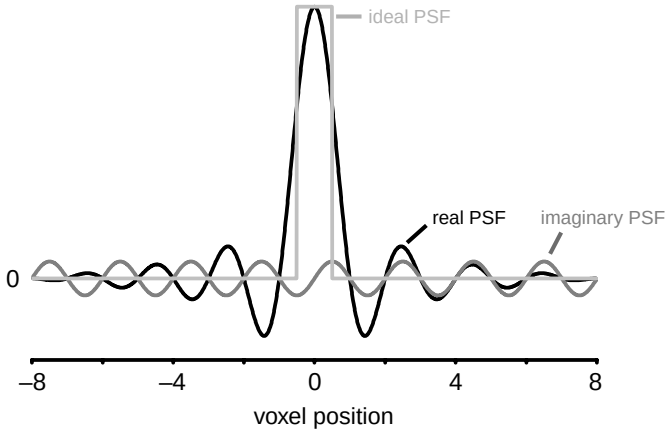


**Figure 7.3** PSF of a single voxel in a 1D SI experiment with 32 phase-encoding increments. The ideal PSF is indicated by the rectangular gray box. Clearly, the experimental PSF significantly deviates from the ideal profile.

However, with MRSI the effects become more pronounced because of (1) the limited number of phase-encoding increments (and consequently the limited number of k-space samples) and (2) the spatial time-domain data are not influenced by parameters as  $T_2^*$  (since  $T_2^*$  effects are refocused in a spin echo sequence or are identical for all phase-encoding increments in a pulse-acquire sequence). Figure 7.3 shows the PSF of a k-space region sampled by 32 points. Three noticeable features can be extracted from Figure 7.3. Firstly, while the nominal resolution in MRSI is given by the FOV divided by the number of phase-encoding increments  $N_p$ , i.e. Equation (7.12), the actual resolution as defined as the full width at half maximum (FWHM) of the PSF is  $1.21(\text{FOV}/N_p)$ . In other words, the actual voxel size is 21 % larger than the nominal voxel size. Secondly, due to residual phase dispersal, the PSF is not uniform across the nominal voxel size leading to the observation that only 87.3 % of the observed signal originates from the desired (or intended) spatial location [15]. Thirdly, the remaining 12.7 % of the signal is spread to adjacent voxels, a phenomenon referred to as signal leakage or bleeding. Similarly, voxels at other positions contaminate the voxel displayed in Figure 7.3. The exact contribution of the PSF to remote voxels depends on the object under investigation and the relative positions to the points of the spatial grid. In the best case (particularly for larger objects) the signal contamination may partially cancel out due to the positive and negative sinc lobes. Note that for a 2D MRSI grid, the real voxels measured at the FWHM are 18.5 % larger than the nominal voxel size. 76.2 % of the signal originates from the theoretical square voxel position.

The PSF can be artificially improved by applying apodization functions in analogy to those used for MRS [9,16]. However, in the case of MRSI the apodization functions needs to be applied in the spatial frequency or k-space domain. Since MRSI data are normally acquired with the spatial frequencies centered around the origin of k-space, the apodization function needs to be symmetrical with respect to the origin. Figure 7.4 shows the effects of some commonly used apodization functions on the PSF. Figure 7.4A shows the PSF



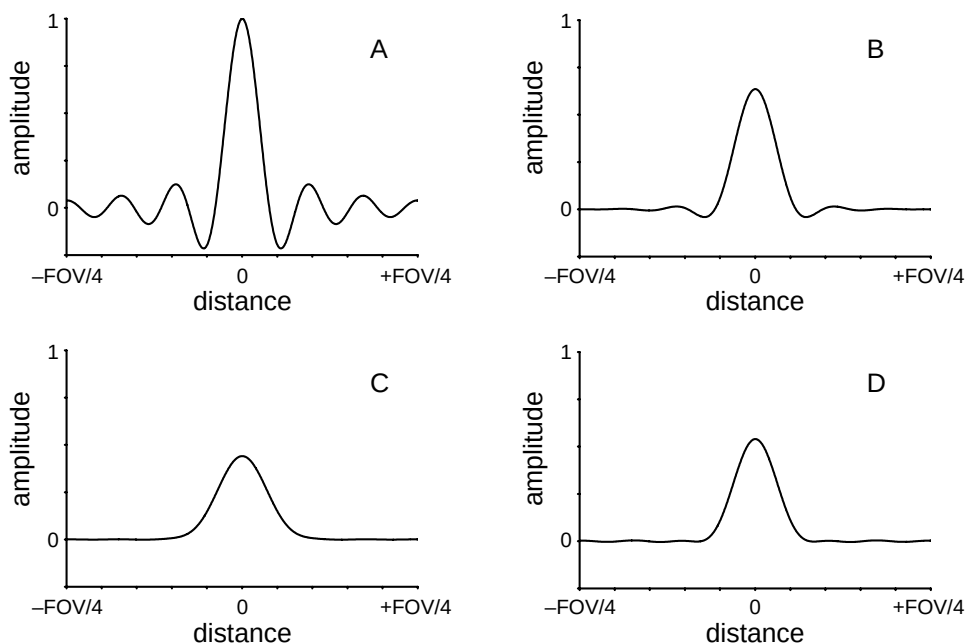


**Figure 7.4** Effect of spatial apodization on the PSF. (A) No apodization and apodization according to (B) a cosine function [Equation (7.14)], (C) a Gaussian function [Equation (7.15)] and (D) a Hamming function [Equation (7.16)].

without any apodization. Figure 7.4B shows the PSF after the application of a cosine apodization function according to:

$$W(k) = \cos\left(\frac{\pi k}{2k_{\max}}\right) \quad \text{for } -k_{\max} \leq k \leq k_{\max} \quad (7.14)$$

where  $k_{\max}$  is the maximum sampled position in  $k$ -space. The ripples are significantly reduced at the expense of a slight increase in the width (and integrated area) of the main lobe, i.e. the actual, localized volume increases. The ripples can be reduced further by using apodization which is described by a Gaussian function (Figure 7.4C)

$$W(k) = e^{-4(k/k_{\max})^2} \quad \text{for } -k_{\max} \leq k \leq k_{\max} \quad (7.15)$$

The reduction of ripples is again accompanied by an increase in the FWHM, leading to a decreased spatial resolution. Theoretically, the optimal filter in terms of maximal ripple reduction versus minimal FWHM is given by the Dolph–Chebyshev windowing function [16]. However, this function is rarely used as it has a dependence on the number of sampling points and is relatively cumbersome to calculate. An analytical function that closely approximates the performance of the Dolph–Chebyshev filter is given by the

Hamming function (Figure 7.4D, [16]):

$$W(k) = 0.54 + 0.46 \cos\left(\frac{\pi k}{k_{\max}}\right) \quad \text{for } -k_{\max} \leq k \leq k_{\max} \quad (7.16)$$

Note that the FWHM of the 1D PSFs without and with cosine, Gaussian and Hamming filtering are 21, 69, 119 and 85 % broader than the ideal or nominal volume.

The application of post-acquisition spatial frequency apodization functions is not optimal in terms of time efficiency and consequently sensitivity [12]. This is because all k-space samples are acquired with the same number of averages, after which post-acquisition apodization reduces the signal of the high-frequency k-space coordinates. In Section 7.4.1 methods are described that can achieve k-space apodization during acquisition.

## 7.4 Temporal Resolution in MRSI

The main drawback of MRSI is the large number of required acquisitions and hence the long acquisition times. For a 3D phase-encoded MRSI experiment, the measurement time can be calculated as:

$$T_{\text{measurement}} = N_A N_x N_y N_z TR \quad (7.17)$$

where  $N_n$  ( $n = x, y, z$ ) are the number of phase-encoding increments,  $N_A$  the number of averages and  $TR$  the repetition time. For a medium resolution dataset ( $N_x = N_y = N_z = 16$ ),  $N_A = 1$  and a repetition time that is such that  $T_1$ -weighting is not a major concern (e.g. 2000 ms at 1.5 T), the measurement time will be 136 min, well outside acceptable MR examination times for human subjects. Even a moderately high resolution 2D dataset ( $N_x = N_y = 32$ ) acquired in 34 min will challenge subject compliance limits. For most applications it is therefore crucial to increase the temporal resolution of MRSI. Here all techniques that achieve an increase in temporal resolution will be divided into three groups, namely (1) conventional methods, requiring minimal additional pre- or post-processing, (2) methods based on fast MRI sequences that typically require reordering and sometimes regridding of k-space and (3) methods that use prior knowledge to minimize and optimize k-space sampling.

### 7.4.1 Conventional Methods

*Circular k-Space Sampling.* As was demonstrated in Chapter 4, the low spatial frequency coordinates of k-space generally contribute to the bulk of the observed signal with minimal information about the object shape and boundaries. The high spatial frequency coordinates of k-space hold information about the detailed features of an object, like boundaries, but contribute minimally to the bulk signal. Because of sensitivity restrictions, MRSI is inherently a low-to-medium spatial resolution technique and as such the decision can be made to emphasize the low k-space coordinates over the higher ones in order to favor sensitivity over detail and resolution. Circular 2D or spherical 3D k-space sampling [17,18] achieves this goal by simply not acquiring the high k-space coordinates (Figure 7.5A). This reduced k-space sampling leads to a circa 21.5 % and 47.6 % reduction in measurement time for 2D and 3D MRSI, respectively. The non-acquired k-space coordinates are typically replaced by zeros, such that standard Fourier transformation can be used to process the

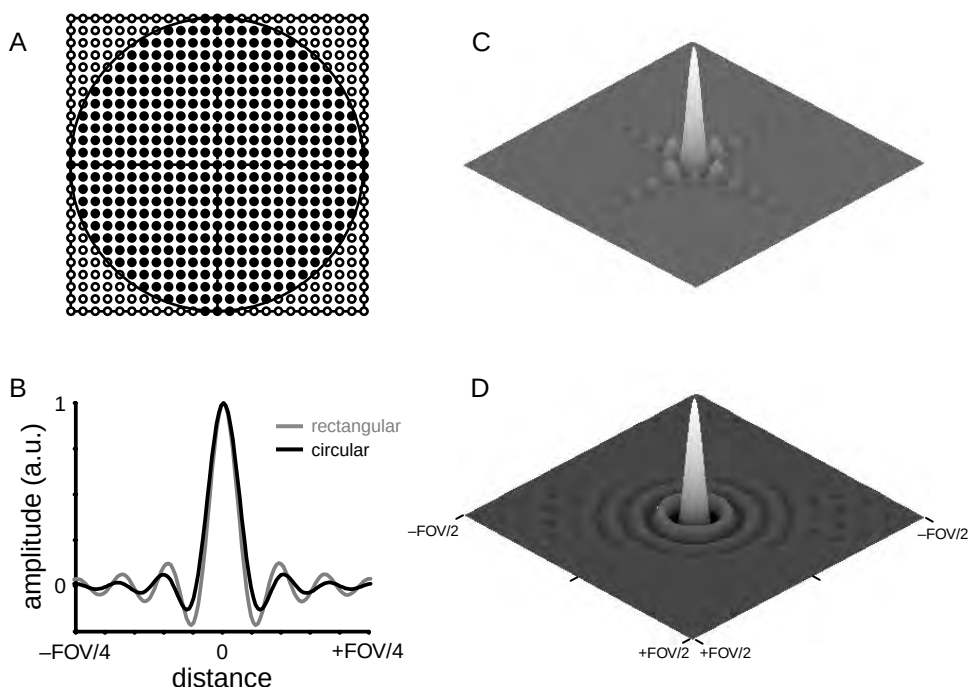


**Figure 7.5** (A) Circular  $k$ -space sampling over a  $25 \times 25$  grid. During regular  $k$ -space sampling all  $k$ -space positions are acquired, whereas circular  $k$ -space sampling only acquires the black  $k$ -space positions. (B) 1D and (C, D) 2D PSFs for (B, C) regular and (B, D) circular  $k$ -space sampling.

MRSI data. However, ignoring the higher  $k$ -space coordinates will have an effect on the PSF of the experiment. Figure 7.5B compares the 2D PSF of a regular, rectangular sampled  $k$ -space with that of a circularly sampled  $k$ -space, as shown in Figure 7.5A. As expected, the main PSF lobe of the circularly sampled  $k$ -space grid is wider than the PSF lobe of the standard, rectangular  $k$ -space grid, leading to an effective volume that is circa 55 % larger than the nominal volume (and circa 29 % larger when compared with the volume obtained with standard sampling). In addition to the wider main lobe, the contamination to other voxels is significantly reduced by circular  $k$ -space sampling.

**$k$ -Space Apodization During Acquisition.** In Section 7.3 it was demonstrated that post-acquisition  $k$ -space apodization can improve the PSF by suppressing voxel bleeding at the expense of a broader main lobe. However, performing this operation post-acquisition is not optimal in terms of sensitivity per unit time, since the high  $k$ -space coordinates are suppressed by the apodization [12]. A better approach would be to spend less time acquiring the high  $k$ -space coordinates and more time on acquiring the low  $k$ -space coordinates. This can be achieved by (1) performing fewer signal averages or (2) introducing more  $T_1$ -weighting at high  $k$ -space coordinates. The first option is obviously only applicable when signal averaging is required, which typically limits it to  $^{31}\text{P}$  MRSI. The sensitivity of  $^1\text{H}$

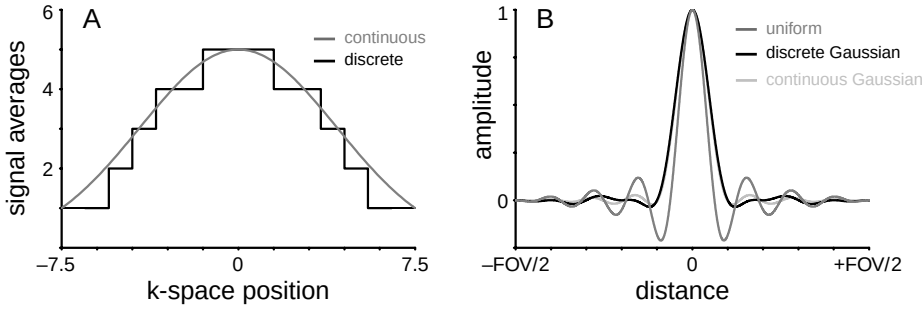


**Figure 7.6** (A) Discrete averaging schedule for  $k$ -space weighting during signal acquisition approximating a continuous Gaussian function for 16 phase-encoding increments. (B) PSFs for uniform, discrete and continuous Gaussian  $k$ -space weighting.

MRSI is typically sufficient in a single average. Figure 7.6 shows the principle of  $k$ -space weighting during signal acquisition for a 1D MRSI experiment. Instead of acquiring all 16  $k$ -space coordinates with the same number of averages ( $NA = 3$ ), the number of averages can be varied between  $NA = 1$  (outer edges of  $k$ -space) and  $NA = 5$  (middle of  $k$ -space) to approximate a Gaussian apodization function according to Equation (7.15) in which the factor 4 has been replaced by  $\ln(5)$ . Figure 7.6B shows the greatly improved PSF for the weighted  $k$ -space acquisition, with only minor differences due to the stepwise approximation of a continuous Gaussian function.

In the particular example shown in Figure 7.6, the weighted acquisition did not result in increased temporal resolution since the total number of averages remained constant. Instead the sensitivity increased, since more time was spent acquiring the low  $k$ -space coordinates. When a large number of averages are required, the temporal resolution can be increased by keeping low  $k$ -space sampling constant, but decreasing the high  $k$ -space sampling. However, a relatively smooth weighting function can only be obtained when using a large number of averages, a situation not commonly encountered *in vivo* due to time constraints.

An alternative to  $k$ -space weighting by variable signal averaging was proposed by Kuhn *et al.* [19]. Different  $k$ -space coordinates can be weighted differently by employing variable repetition time acquisitions. The amount of signal at position  $k$ , relative to  $k = 0$  essentially determines the weighting (or apodization) function according to:

$$W(k) = \frac{M_{xy}(TR, k)}{M_{xy}(TR, k=0)} = \frac{1 - e^{-TR(k)/T_1}}{1 - e^{-TR(k=0)/T_1}} \quad (7.18)$$

where the far right side of Equation (7.18) is valid for  $90^\circ$  excitations. The desired apodization function  $W(k)$  can, for example, be given by Equations (7.14)–(7.16). In combination with Equation (7.18) an expression for  $TR$  as a function of  $k$  can be obtained. Figure 7.7 shows the  $k$ -position dependence of  $TR$  using the Hamming apodization function [Equation (7.16)]. Therefore, in order to modulate the transverse magnetization according to the Hamming apodization function, the  $TR$  must be varied from 3000 ms in the middle to below 1000 ms at the edges of  $k$ -space. In the 1D example, the  $TR$ -weighted sampling leads

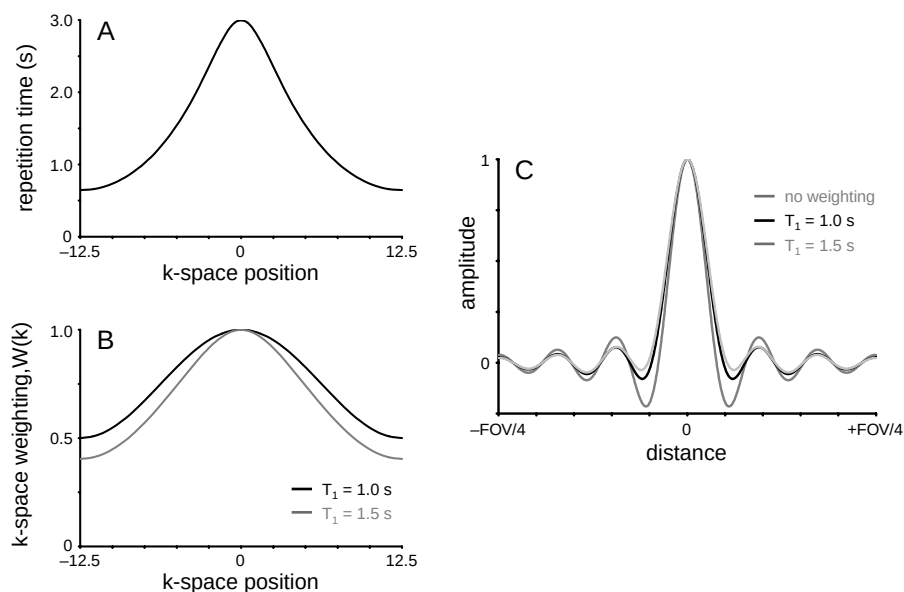


**Figure 7.7** *k*-space apodization through variation of the repetition time. (A) Repetition times calculated with Equation (7.18) for 26 *k*-space positions to (B) achieve a modified Hamming apodization function,  $0.75 \pm 0.25 \cos(\pi k/k_{\max})$  with  $-k_{\max} \leq k \leq +k_{\max}$ , for  $T_1 = 1000$  ms. (C) PSFs for *k*-space sampling without apodization and with TR-driven Hamming apodization for  $T_1 = 1000$  ms and 1500 ms.

to a 35 % reduction in measurement time relative to constant TR sampling (TR = 3000 ms). For 2D and 3D acquisitions the reduction in measurement time will be even greater since the number of low *k*-space coordinates (with a long TR) becomes smaller relative to the number of high *k*-space coordinates (with a short TR). In the original manuscript of Kuhn *et al.* [19], a 55 % reduction in measurement time was achieved for a 2D MRSI acquisition.

A potential concern of TR-weighted *k*-space sampling is that the point spread function becomes dependent on the  $T_1$  relaxation time constant. Figure 7.7B compares the PSF of the apodization shown in Figure 7.7A for  $T_1 = 1000$  and 1500 ms. There are only minor differences, which typically do not impact signal quantification.

**Zoom MRSI.** The temporal resolution of a MRSI experiment is directly proportional to the number of phase-encoding increments, which for a given spatial resolution, is directly proportional to the field of view (FOV). The FOV in turn is determined by the boundaries of the object under investigation. Therefore, if the FOV can be reduced by restricting the observed signal to a smaller region, the number of phase-encoding increments and hence measurement time can be reduced while maintaining the same nominal voxel size. Numerous methods are available to remove unwanted signal from the object under investigation in order to reduce the FOV. Typically outer volume suppression (OVS) or single shot localization methods like PRESS or STEAM are used for this purpose. As these techniques are often also used for lipid suppression, they are discussed in detail in Section 7.5.

## 7.4.2 Methods Based on Fast MRI Sequences

So far the reduction in measurement times has been achieved through reduced or modified k-space sampling. However, the limits are fairly quickly encountered since there simply needs to be a minimum amount of data to adequately characterize the object. Rather than aiming for reduced k-space sampling, many of the fast MRI sequences focus on more efficient k-space sampling. This leads in the most extreme case to single scan echo planar or spiral imaging in which the entire k-space is sampled in a single acquisition. The principles of fast MRI are often equally applicable to MRSI, which has resulted in MRSI methods based on EPI [20–23], RARE [24], spiral [25] and steady-state [26, 27] sequences. MRSI methods based on EPI will be discussed in the next section.

*Proton Echo Planar Spectroscopic Imaging (PEPSI).* The main difference with conventional MRSI sequences is that the fast MRSI sequences, just as the MRI methods, employ (shaped) magnetic field gradients during signal acquisition [20–23, 28]. Figure 7.8 shows a fast MRSI sequence based on EPI, known under the commonly used acronym PEPSI. The main difference with a conventional EPI method (see Chapter 4) is that the phase-encoding blips in between the oscillating readout gradient lobes are missing. Therefore, rather than being encoded with information about the second spatial dimension, the echoes are encoded with chemical shift information. As time progresses, phase evolution due to chemical shifts increases. This can be expressed by a spectroscopic frequency k-space parameter  $k_\omega = \omega t$ . Figure 7.9A shows the corresponding trajectory in k-space. From the previous arguments and Figure 7.9A it is now immediately obvious that when conventional MRSI samples a

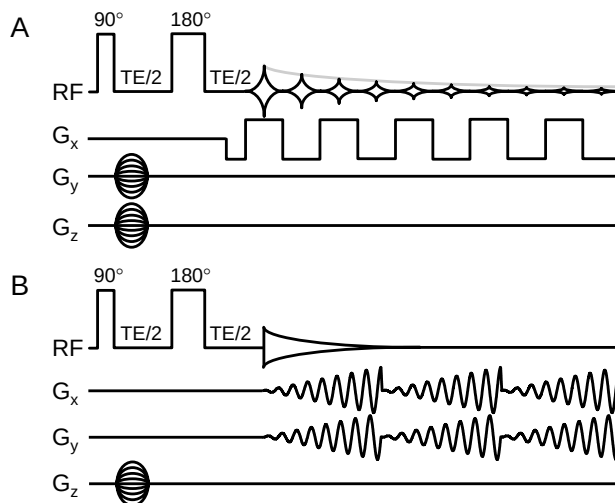


**Figure 7.8** Pulse sequences for ultrafast MRSI. (A) 3D spin-echo EPI with conventional phase-encoding in two dimensions and (B) spiral MRSI with conventional phase-encoding in one dimension. Both sequences are readily modified for 2D multislice acquisitions by replacing one phase-encoding dimension with multislice selection.

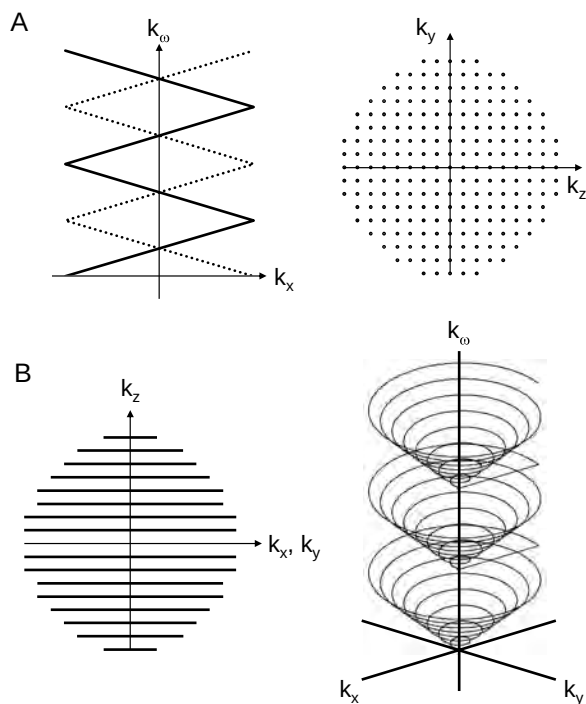


**Figure 7.9** *k*-space sampling trajectories for the sequences in Figure 7.8. (A) The frequency-encoding ‘readout’ magnetic field gradient samples the  $(k_x, k_\omega)$  plane (i.e. one spatial and the spectral dimension). To increase the spectral resolution, the  $(k_x, k_\omega)$  plane may be oversampled (dotted line and nonindicated lines of higher frequency). The other two spatial dimensions ( $y, z$ ) are sampled by regular phase-encoding. (B) With spiral-based MRSI, the entire  $(k_x, k_y)$  plane is sampled, while the  $k_\omega$  direction is automatically sampled as time progresses. The third spatial dimension  $z$  is again sampled by regular phase-encoding.

1D  $k_\omega$  line for each  $k_x$  point (and each excitation), the PEPSI method samples an entire 2D  $(k_\omega, k_x)$  plane for each excitation. Therefore, PEPSI can in principle be  $N_x$  times as fast as conventional MRSI.

However, in analogy to conventional EPI, the  $k$ -space data requires pre-processing before the final MRSI dataset can be obtained. Firstly, every other  $k$ -space line needs to be reversed. Reversal of the readout gradient in the presence of magnetic field inhomogeneity and asymmetries in gradient switching introduce periodicities in  $k$ -space that lead to aliasing artifacts. While this can be corrected, the problem is completely eliminated by not using data acquired during the negative lobes, of course at the expense of a two-fold reduction in the spectral bandwidth.

PEPSI at higher magnetic fields becomes more challenging because of the increased spectral bandwidth. Suppose that on a 9.4 T magnet PEPSI is performed on a  $32 \times 32$  spatial grid. The required spectral bandwidth is circa 10 ppm or 4.0 kHz, making the dwell time 250  $\mu$ s. From Figure 7.8A it can be seen that the spectral dwell time in a PEPSI experiment is essentially equal to the gradient plateau of one readout gradient lobe

(ignoring gradient rise times). Therefore, the 32  $k_x$  points need to be acquired in 250  $\mu\text{s}$ , making the ‘spatial’ dwell time 7.8  $\mu\text{s}$  and the readout bandwidth 128 kHz. When the data is acquired over a 1.6 cm FOV, the required readout gradient strength is 188 mT  $\text{m}^{-1}$ . While this is a reasonable gradient strength on most modern animal MR systems, it may be a limitation on older systems. If the gradient strength and hence the spectral bandwidth can not be achieved, the spectral bandwidth can be increased by interleaving two scans as shown in Figure 7.9A. This is referred to as spectral-spatial oversampling [28] and increases the measurement duration by the number of interleaves.

Even though PEPSI can provide greatly reduced measurement times, a point of concern with PEPSI and any high-speed MRSI sequence is sensitivity. This is because the signal is typically acquired using large (20–200 kHz) receiver bandwidths. Ignoring intrinsic parameters as relaxation and experimental imperfections such as residual gradient effects, it can be shown by simple arguments that the S/N per unit of time is the same for conventional and high-speed MRSI sequences. Continuing with the example of the previous paragraph, suppose that a conventional 1D MRSI dataset, acquired with 32 phase-encoding increments (one average) and a spectral bandwidth of 4000 Hz, has a (S/N) of  $(\text{S/N})_{\text{conv}}$ . The same MRSI dataset can be acquired with a high-speed PEPSI sequence with a 128 kHz receiver bandwidth. Since the noise is proportional to the root of the bandwidth, this results in  $(\text{S/N})_{\text{fast}} = (1/\sqrt{32})(\text{S/N})_{\text{conv}}$ . In the time needed to acquire the conventional MRSI dataset, one can repeat the fast PEPSI sequence 32 times, such that  $(\text{S/N})_{\text{fast}} = (\text{S/N})_{\text{conv}}$  in the same measurement time. This result may, of course, be influenced by specific pulse sequence characteristics, including relaxation, RF and/or gradient imperfections and magnetic field inhomogeneity. However, Pohmann *et al.* [29] have performed a rigorous comparison between a wide range of MRSI methods, including PEPSI and other multi-echo approaches, and they concluded that conventional MRSI is and remains the gold standard for sensitivity per unit time.

Therefore, in conclusion, high-speed MRSI sequences do not improve the attainable S/N, they merely allow a flexible trade-off between sensitivity and measurement time. Conventional MRSI does not have this degree of freedom, since the number of phase-encoding increments determines the minimum achievable measurement time. When sufficient sensitivity is obtained with fewer acquisitions, high-speed MRSI may be used to increase the time resolution of the experiments as may be useful in functional spectroscopic studies [30, 31]. Furthermore, high-speed MRSI methods allow the acquisition of an additional frequency axis, as encountered for example when 2D NMR is combined with 2D MRSI [32–34]. Figure 7.10 shows an example of a complete 3D SI dataset of human brain acquired in only 20 min.

**Parallel MRSI.** A relatively novel and potentially interesting method to increase the temporal resolution of MRSI data acquisition employs the principles of parallel MRI [35–37]. Parallel MRSI [38, 39] is identical to parallel MRI in all but two important ways. Firstly, parallel MRSI has an additional spectral dimension, but since this dimension is acquired without undersampling it requires no further consideration. Secondly, an implicit assumption in the original SENSE description [Equation (4.32)] does in general not hold for spectroscopic SENSE. For a two-coil array with 1D encoding, Equation (4.32) assumes that the signal in the aliased images is a linear sum of the signals in the unaliased, full FOV image:

$$\begin{aligned} I_{\text{coil},1}(x_1) &= C_1(x_1)I_{\text{sample}}(x_1) + C_1(x_2)I_{\text{sample}}(x_2) \\ I_{\text{coil},2}(x_1) &= C_2(x_1)I_{\text{sample}}(x_1) + C_2(x_2)I_{\text{sample}}(x_2) \end{aligned} \quad (7.19)$$



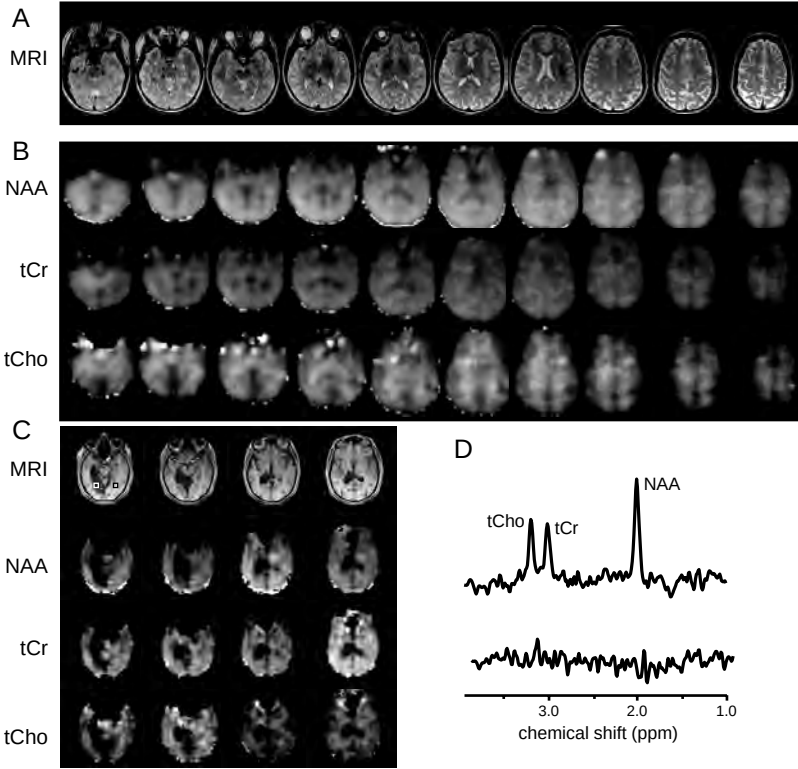


**Figure 7.10** (A) MRI and (B) MRSI data of a normal subject at 3 T acquired with multislice 2D MRSI based on echo-planar data acquisition. Data were acquired with a phased-array receiver at a nominal spatial resolution of  $4.4 \times 4.4 \times 7.0$  mm ( $\approx 0.14$  ml) in 20 min ( $TE = 70$  ms). (C) MRI and echo-planar MRSI data acquired on a stroke patient. (D) Representative  $^1\text{H}$  NMR spectra from the positions indicated in (C). The abundant  $^1\text{H}$ -containing metabolites NAA, tCr and tCho can be detected with excellent sensitivity (Courtesy of A. A. Maudsley.)

For MRI applications Equation (7.19) is valid, as a pixel can be well approximated by a Dirac function. However, MRSI acquisitions are inherently low-to-medium resolution experiments and as a consequence the PSF (or ‘shape of a pixel’) is a sinc-like function only remotely resembling a Dirac function. As a result, a pixel in the aliased dataset is not only a sum of the corresponding unaliased signals, but through the PSF also contains contributions from many other spatial positions. In this case the expressions for  $C_n(x_i)I_{\text{sample}}(x_i)$  in Equation (7.19) need to be convolved with the PSF of the experiment according to:

$$\int_{-\infty}^{+\infty} C_n(x')I_{\text{sample}}(x')\text{PSF}(x_i - x')dx' \quad (7.20)$$

When the original SENSE reconstruction algorithm of Equation (4.34) is applied to MRSI data, artifacts in terms of lipid contamination and incorrect metabolite ratios can

be expected [40]. While Equation (7.20) in combination with knowledge of the PSF can eliminate all artifacts, the calculation of Equation (7.20) is computationally intensive, such that several alternatives have been proposed to approximate Equation (7.20) [40].

While SENSE spectroscopic imaging has been demonstrated with a several-fold improvement in temporal resolution, the maximum acquisition time reduction is ultimately limited by the S/N of the low concentration metabolites. SENSE MRSI, as all other high-speed MRSI methods, does not improve the sensitivity per unit of time, it merely allows a flexible trade-off between temporal resolution, spatial resolution and sensitivity.

### 7.4.3 Methods Based on Prior Knowledge

One of the strengths of MRSI is that no assumptions are made about the object under investigation. However, this often results in the acquisition of voxels containing no or unwanted signals, for example from outside the object. In addition, many voxels can arise from a homogenous compartment, like the cerebral cortex, for which it would have been more advantageous to obtain a single high-sensitivity spectrum.

In many cases, some prior knowledge of the object, like spatial position, orientation and compartments, is available which can be used to increase the efficiency of k-space sampling. The SLIM [41] and SLOOP [42] techniques use anatomical prior knowledge to restrict k-space sampling, thereby greatly increasing the temporal resolution. Theoretically,  $N$  compartments of arbitrary shape can be accurately described by  $N$  k-space samples, potentially leading to greatly increased temporal resolution.

Suppose that the brain is divided into  $N$  ROIs and that the MRSI data are acquired with  $M$  different phase encoding increments (where  $M \geq N$ ). The amount of signal  $P_m(t)$  acquired during phase-encoding step  $m$  can then be expressed as:

$$P_m(t) = \sum_{n=1}^N c_{mn} S_n(t) \quad \text{with} \quad c_{mn} = \int_{\text{compartment } n} e^{i\mathbf{k}_m \mathbf{r}} d^3\mathbf{r} \quad (7.21)$$

where  $S_n(t)$  represents the signal from compartment  $n$  and  $c_{mn}$  is a complex weighting factor expressing the acquired phase of all positions within compartment  $n$  during phase-encoding increment  $m$ , characterized by k-space vector  $\mathbf{k}_m$ . Equation (7.21) can be written in matrix form as:

$$\mathbf{P}_M(t) = \mathbf{C} \mathbf{S}_N(t) \quad (7.22)$$

where  $\mathbf{P}_M(t)$  designates the vector of  $M$  acquired phase-encoded signals and  $\mathbf{S}_N(t)$  a vector of the  $N$  signals from each of the compartments in the absence of phase encoding.  $\mathbf{C}$  is a complex  $M \times N$  matrix with the elements  $c_{mn}$ . The desired compartmentalized spectra  $\mathbf{S}_N(t)$  can be found by factoring  $\mathbf{C}$  with a singular-value decomposition according to:

$$\mathbf{C} = \mathbf{U} \mathbf{S} \mathbf{V}^\dagger \quad (7.23)$$

with  $\mathbf{U}$  and  $\mathbf{V}$  being unitary matrices of dimensions  $M \times M$  and  $N \times N$ , respectively. The operator  $\dagger$  indicates complex-conjugate transpose.  $\mathbf{S}$  is a  $M \times N$  matrix containing the  $N$  singular values of  $\mathbf{C}$ . A unique solution to Equation (7.22) for the time-domain signals in the  $N$  compartments is then given by:

$$\mathbf{S}_N(t) = \mathbf{V} \mathbf{S}^* \mathbf{U}^\dagger \mathbf{P}_M(t) \quad (7.24)$$

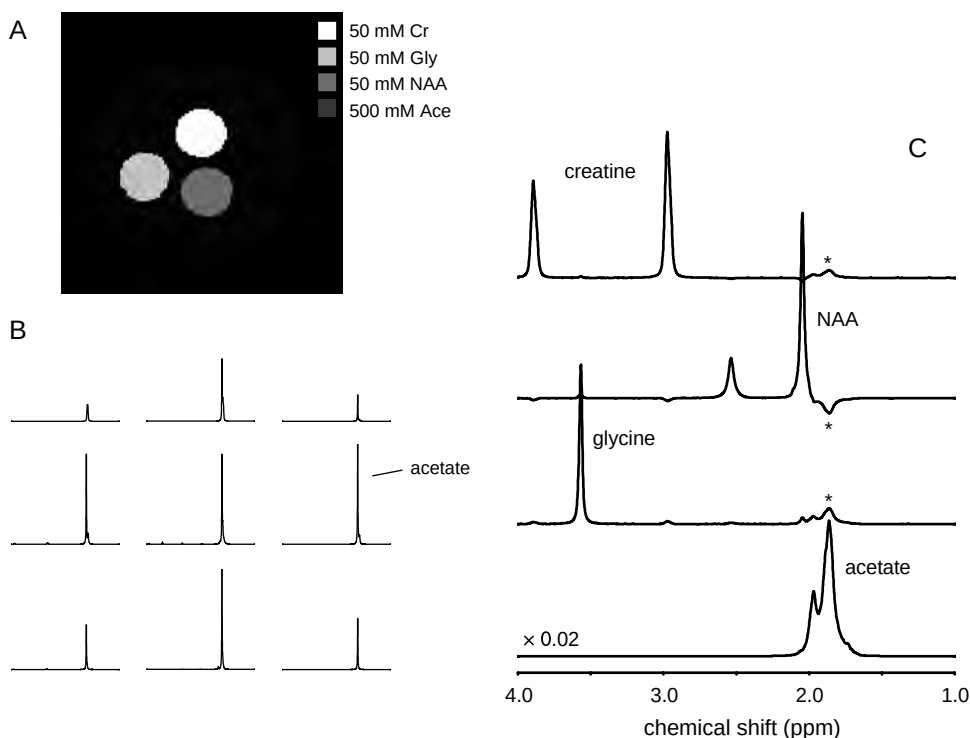


**Figure 7.11** Improved spatial localization through the use of anatomical prior knowledge. (A) Four compartment phantom, containing different metabolites in each compartment. (B)  $3 \times 3$   $^1\text{H}$  MRSI dataset, reconstructed by 2D FFT processing. The low spatial resolution in combination with extensive PSF-related signal bleeding leads to the observation that every pixel is heavily dominated by the concentrated acetate resonance. (C) By utilizing prior knowledge on the spatial localizations of the compartments as shown in (A), the SLIM algorithm can reconstruct compartmentalized  $^1\text{H}$  NMR spectra from a  $3 \times 3$  k-space with greatly reduced contamination (\*) from other compartments.

where  $\mathbf{S}^*$  is a  $N \times M$  matrix containing the reciprocal eigenvalues on the diagonal. Temporal Fourier transformation of the reconstructed  $S_n(t)$  finally provides all compartmentalized spectra. Equation (7.24) shows the principle underlying the SLIM method: the localized spectra of the individual compartments are obtained as a linear combination of the acquired phase-encoded signals, weighted by the complex coefficients  $c_{mn}$  of the matrix  $\mathbf{C}$ .

Figure 7.11 shows an example of the incorporation of prior knowledge to increase the temporal resolution of MRSI data acquisition using the SLIM algorithm. Figure 7.11A shows an anatomical image of a four-compartment phantom containing 50 mM creatine, glycine and NAA in the smaller tubes and 500 mM acetate in the larger tube. In order to obtain compartmentalized NMR spectra from the four compartments with negligible amounts of signal leakage from other compartments, a roughly  $32 \times 32$  MRSI dataset would be required. However, under the assumption of homogenous compartments, the SLIM algorithm would only require four phase-encoded FIDs (e.g. a  $2 \times 2$  matrix).

Figure 7.11C shows the compartmentalized NMR spectra calculated from an overdetermined  $3 \times 3$  MRSI dataset, while Figure 7.11B shows the  $3 \times 3$  FFT-based spectroscopic image. Clearly by imposing anatomical prior knowledge the SLIM algorithm allows the calculation of accurate compartmentalized NMR spectra with only a small number of phase-encoding increments. The small amount of acetate contamination must be attributed to inhomogeneity between the compartments. The inter-compartment signal contamination can be further reduced with extended k-space sampling and the incorporation of additional prior knowledge, for instance on  $B_1$  magnetic field inhomogeneity.

## 7.5 Lipid Suppression

The PSF as described in Section 7.3 leads to a less than ideal voxel definition which gives rise to intervoxel contamination or bleeding. Fortunately, the contribution of all tissue types to a given voxel can be quantitatively calculated by using the PSF in combination with segmented anatomical MRI data (see Section 7.6). This allows the measurement of metabolism in pure tissue types despite significant voxel contamination [43, 44].

However, when the small metabolite signals are detected in the presence of large signals, like extracranial lipids during MRSI of the brain, the intervoxel contamination can dominate the appearance of the spectra. Figure 7.12A shows a  $^1\text{H}$  NMR spectrum from a single slice through the human brain acquired at 4 T without any lipid suppression. Even though the extracranial lipids are residing outside the brain, the finite intensity of the PSF at positions located well inside the brain is sufficient to cause significant contamination from intense lipid resonances, thereby complicating the quantification of the underlying metabolite resonances. In most cases the solution to this problem is found by removing the lipid signals prior to or during acquisition as will be described next for the most commonly employed methods.

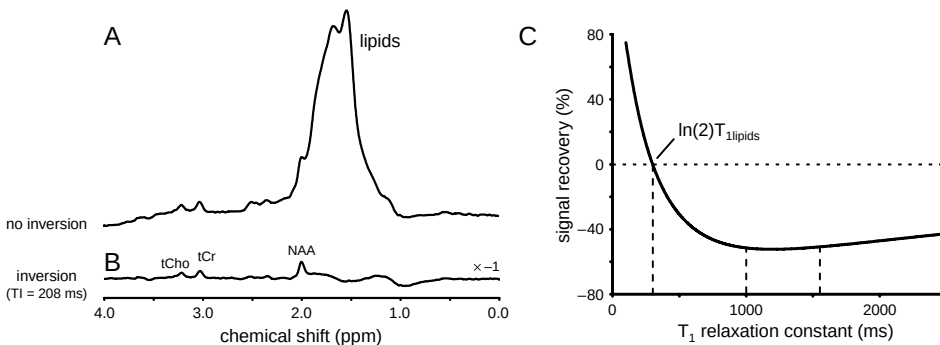


**Figure 7.12** (A)  $^1\text{H}$  NMR spectrum ( $TR/TE = 2000/50$  ms) acquired without lipid suppression from a single slice through the human brain at 4 T. (B) The lipid resonances can be significantly reduced through the use of an IR method in which the inversion time is adjusted to minimize signal with a  $T_1$  of 300 ms. Metabolites with a longer  $T_1$  can be detected at circa 50 % of their maximum intensity. (C) Signal recovery curve as a function of  $T_1$  relaxation for an IR sequence with  $TR/TI = 2000/208$  ms.

### 7.5.1 Relaxation Based Methods

Methods based on the differences in relaxation between lipids and metabolites are among the oldest techniques to achieve lipid suppression. The methods are typically straightforward to implement and can give reasonable lipid suppression. However, there are two related methodological considerations that need to be taken into account, namely signal loss of the metabolites of interest and the attainable degree of lipid suppression.

Transverse  $T_2$  relaxation is typically not used for lipid suppression, since the difference between metabolite and lipid  $T_2$  relaxation is too small. At the echo-time required to achieve sufficient lipid suppression, the metabolites would also have greatly decayed due to  $T_2$  relaxation. This would lead to a low metabolite S/N, as well as a heavily  $T_2$ -weighted spectrum which makes signal quantification more complicated.

The difference in longitudinal  $T_1$  relaxation between metabolites and lipids is typically larger. The field-dependent metabolite  $T_1$ s as summarized in Table 3.3 are well above 1000 ms, whereas lipid resonances are characterized by  $T_1$ s in the range of 250–350 ms [45,46]. This provides an opportunity for selective lipid suppression through the use of an inversion recovery (IR) sequence. By choosing the inversion delay such that the longitudinal lipid magnetization is zero, the lipids are effectively not excited. The metabolites are still along the  $-z$  axis due to the much longer  $T_1$  relaxation time constants and will, upon excitation be detected as negative resonances. Figure 7.12C shows a signal recovery curve for a typical situation in which the inversion delay is optimized to null compounds with a  $T_1$  of 300 ms [e.g. inversion delay =  $T_1 \ln(2) = 208$  ms] and TR = 2000 ms. It follows that while lipids are suppressed, the metabolites ( $T_1 = 1000$ –1600 ms at 4.0 T [47]) are observed at circa 50 % of the thermal equilibrium magnetization. Note that the  $T_1$  weightings introduced by the short TR and the inversion pulse partially cancel each other, leading to similar metabolite intensities which in turn simplify metabolite quantification. Increasing the TR to  $5T_{1\text{max}}$ , as often recommended to minimize  $T_1$  effects in metabolite quantification, would actually result in a strong  $T_1$ -dependent metabolite signal recovery. An obvious drawback of a single IR method is that perfect suppression can only be achieved for a single  $T_1$  relaxation constant. It has been shown that extracranial lipids exhibit a range of  $T_1$ s [46] thus making the suppression incomplete. Improved suppression can be obtained with a double IR method in which two inversion pulses and two delays can be optimized to achieve suppression over a wider range of  $T_1$  relaxation constants. However, the improved lipid suppression comes at the price of reduced metabolite signal recovery. Figure 7.12B shows a typical result of single IR-based lipid suppression on a single slice in the human brain at 4.0 T. While the lipid suppression is not perfect, it is sufficient to reduce the intervoxel bleeding in MRSI to negligible levels.  $T_1$ -relaxation-based lipid suppression can in principle be used in multi-slice acquisitions, by employing slice-selective inversion pulses. In order to minimize inter-slice interference and enhance the lipid suppression it is recommended to leave slice gaps in between the slices and make the inversion slice thickness slightly wider than the excitation/refocusing slice thickness.

### 7.5.2 Outer Volume Suppression and Volume Pre-localization

Currently the most commonly employed methods for lipid suppression, namely outer volume suppression (OVS) and volume pre-localization, utilize the difference in spatial origins of lipids and metabolites.

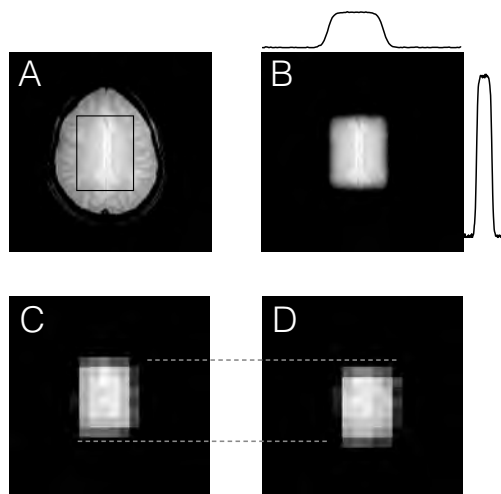


**Figure 7.13** (A) Desired and (B) actual shape and position of a localized volume obtained with the PRESS method when executed with optimized excitation and refocusing pulses (see Chapter 5). (C, D) Metabolic maps for (C) total creatine and (D) NAA as calculated by numerical integration of the corresponding spectral regions in a  $20 \times 20$  MRSI dataset. While lipid-related artifacts are absent, two other artifacts related to the volume selection can be observed, namely (1) both tCr and NAA metabolic maps have reduced signal intensity around all edges and (2) the NAA map is spatially shifted relative to the tCr map due to a chemical shift displacement artifact.

Volume pre-localization methods use single shot localization techniques like STEAM and PRESS as described in Chapter 6 to select a relatively large rectangular volume inside the brain (Figure 7.13). Since the extracranial lipids are not excited/refocused they do not contribute to the detected signal. All considerations discussed for STEAM and PRESS are also valid for volume pre-localization in MRSI. However, two effects become especially pronounced during volume pre-localization, namely imperfect slice profiles and the chemical shift displacement.

A well-behaved slice profile is characterized by uniform in-slice signal, no signal outside the slice and a relatively narrow transition zone (ideally less than 10 % of the total slice). However, over a wide slice as used in MRSI volume pre-localization, a 10 % transition zone can cover one or several MRSI voxels thereby leading to artificially reduced metabolite levels. One should therefore always use RF pulses with the narrowest transition zones (see also Chapter 5). However, since slice transition zones are unavoidable it is imperative that the slice profiles are measured or calculated and used to correct the MRSI data.

The chemical shift displacement has been extensively discussed in Chapter 6. Since the slices during MRSI volume pre-localization are much wider than in regular single volume localization, the absolute displacement is much larger and can span one or several spectroscopic volumes. Unlike single volume NMR spectroscopy, the chemical shift displacement during volume pre-localization becomes spatially resolved by the phase-encoding gradients which leads to distorted metabolite ratios even in a spatially homogeneous sample. For example, suppose that the absolute chemical shift displacement between creatine and NAA

is 1.0 cm and that the nominal MRSI voxel dimension is also 1.0 cm. Depending on the sign of the slice selection gradient, the outer most voxels on either side of the selected volume will not contain any creatine or NAA, as that compound is not excited/refocused at that particular location (see Exercise 7.1 for more details). As was already mentioned in Section 6.2.2, the only way to minimize the chemical shift displacement artifact is to increase the RF bandwidth. For MRSI volume pre-localization it is therefore always recommended using RF pulses with the largest bandwidth and smallest transition width, giving the limitations imposed by RF power deposition and magnetic field gradient amplitudes. However, chemical shift displacement and imperfect slice profile artifacts are always present and should be quantitatively assessed for data processing, as well as publications. Figure 7.13 gives a practical example of the considerations involved with volume pre-localization in MRSI applications.

While volume pre-localization gives excellent lipid suppression and is easy to use and implement, it also has a number of disadvantages. Firstly, using the basic STEAM or PRESS methods, the pre-localized volume must be rectangular in shape. The human (or animal) brain is not rectangular in any slice orientation, such that volume pre-localization necessarily destroys signal from cerebral tissues on the edge of the brain. This can be problematic when abnormalities are present at the brain periphery, such as encountered in stroke or tumors. Furthermore, whole-brain multi-slice acquisitions are complicated by through-slice interference of the volume pre-localization method. This is especially troublesome when the in-plane volume size and position must change to encompass the changing brain size and position across multiple slices. As a result, volume pre-localization is most commonly used over a limited number of slices without changes in size or position of the localized volume.

OVS works essentially opposite to the strategy employed by volume pre-localization. Rather than avoiding the spatial selection of lipids, OVS excites narrow slices centered around the brain in lipid-rich regions (Figure 7.14). Following slice-selective excitation,

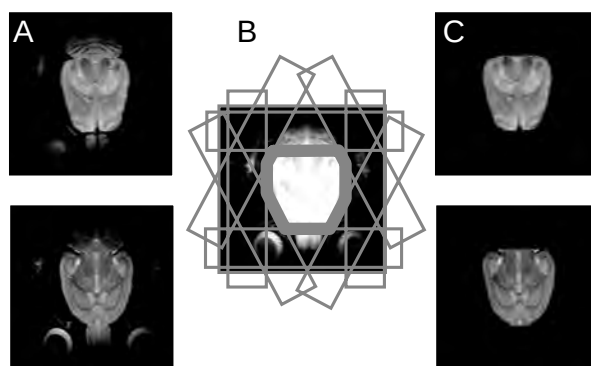


**Figure 7.14** OVS on eight oblique slices. The OVS performance can be evaluated spectroscopically or by incorporating imaging gradients and visualizing the obtainable localization. (A) Images of adjacent rat brain slices in vivo acquired without OVS. The slices are encoded with transverse Hadamard localization, as detailed in Section 7.7. (B) Position and orientation of the eight oblique OVS slices. (C) Images obtained with OVS. All extracranial signal from muscle, lipids and eyes have been obliterated without affecting signal within the brain.

the transverse magnetization is dephased ('spoiled') by a subsequent magnetic field crusher gradient. Following the OVS modules, the brain water and metabolites signals can be excited without lipid contamination. OVS is a general method to selectively remove unwanted signal. On most clinical MR systems, a minimum of eight OVS slices can be arbitrarily placed around the brain, thereby closely approximating the roughly elliptical shape of the brain as shown in Figure 7.14. OVS is readily used in multi-slice acquisitions, since the through-slice angle of the OVS slices can be adjusted to minimize slice-to-slice interference [48]. Furthermore, since OVS selects relatively narrow slices, artifacts originating from imperfect slice profiles and chemical shift displacements are typically negligible.

However, the complications for OVS arise from  $B_1$  magnetic field inhomogeneity and finite  $T_1$  relaxation times. Perfect lipid suppression requires that no lipid signals reside along the longitudinal axis when the global excitation pulse is executed. This means that the OVS pulses must achieve an exact  $90^\circ$  rotation to excite all the lipids towards the transverse plane. Furthermore, lipid signal should not significantly recover by  $T_1$  relaxation prior to global excitation. Neither requirement is true under realistic conditions. Inhomogeneity in the  $B_1$  magnetic field leads to a range of nutation angles, whereas the short lipid  $T_1$  relaxation leads to recovery of longitudinal lipid magnetization. As has been demonstrated for CHESS water suppression in Section 6.3.2, the sensitivity towards  $B_1$  magnetic field inhomogeneity can be greatly reduced by repeating the OVS modules. Figure 7.15C demonstrates the improved performance of repeated OVS modules. It should be noted that in the presence of strong  $B_1$  magnetic field inhomogeneity the choice of OVS excitation pulse becomes crucial to the performance of the suppression (Figure 7.15D and E). Figure 7.15D shows simulations of the frequency profile for a sinc pulse for a wide range of  $B_1$  magnetic fields ( $B_1 = 0.5\text{--}6.0$  kHz). It follows that while the slice profile is reasonable for lower  $B_1$  amplitudes, it becomes unsuitable for OVS at higher  $B_1$  amplitudes. The problem can be avoided by using small nutation angles, while repeating the OVS modules many ( $>8$ ) times. This forms the basis of projection presaturation, a versatile and robust OVS method [49–52]. Another solution can be found by using adiabatic full passage (AFP) pulses for excitation. In Section 5.7.2 AFP pulses were described as frequency selective inversion pulses that achieve inversion independent of the applied  $B_1$  field *above* a minimum  $B_1$  amplitude. At  $B_1$  amplitudes below the minimum threshold to satisfy the adiabatic condition, AFP pulses achieve partial excitation instead of inversion while retaining a highly selective frequency profile (Figure 7.15E). Insensitivity towards  $B_1$  magnetic field inhomogeneity can be regained by repeating the AFP-based OVS module several times [53]. AFP-based signal suppression has been shown to give excellent performance, especially when the amplitude of subsequent pulses is modulated to increase the insensitivity towards  $B_1$  magnetic field inhomogeneity [53].

### 7.5.3 Methods Utilizing Spatial Prior Knowledge

A completely different group of techniques designed for lipid suppression in MRSI is aimed at reducing intervoxel bleeding by measuring the lipid resonances over an extended k-space span. The lipid contamination in MRSI voxels located well within the brain can be attributed to the unfavorable PSF due to limited k-space sampling, as discussed in Section 7.3. Increasing the k-space sampling span does indeed greatly improve the PSF and thereby reduces the intervoxel contamination. Unfortunately, increasing the k-space sampling also



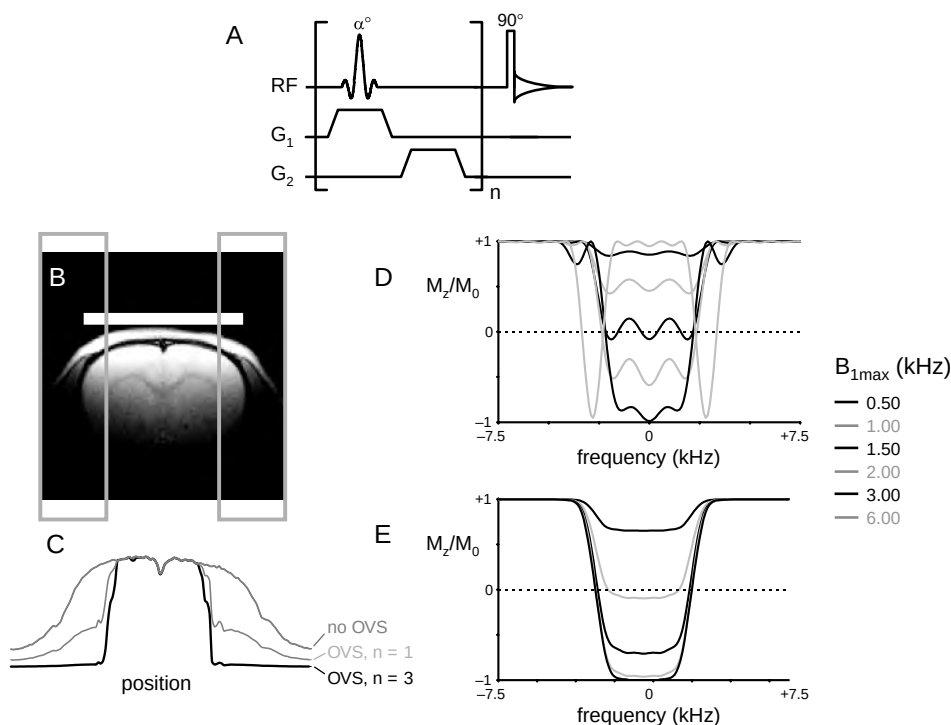


**Figure 7.15** (A) General OVS element.  $G_1$  and  $G_2$  represent the OVS slice selection and crusher gradients, respectively. (B) Desired OVS slice positions as indicated on a 2D MR image and (C) achieved OVS using one or three OVS elements per slice as indicated on a 1D readout profile along the horizontal direction. RF transmission and reception were achieved with a 14 mm diameter surface coil as indicated in (B). (D, E) Frequency profiles for (D) 1 ms sinc and (E) 2 ms AFP (sech/tanh modulation,  $R = 10$ ) pulses for a range of  $B_1$  magnetic field amplitudes.

leads to a reduced S/N for the metabolites. The strong lipid signals on the other hand are readily detected, even with extended k-space sampling. This difference forms the basis for a number of post-processing methods aimed at lipid removal [54, 55]. Figure 7.16A shows a human brain mask surrounded by a layer of lipids. Following the acquisition of a medium-resolution ( $16 \times 16$ ) MRSI dataset, the spatial locations inside the brain are dominated by lipid signals (Figure 7.16B). Extending the k-space sampling to a high-resolution ( $48 \times 48$ ) MRSI dataset, eliminates the lipid contamination inside the brain at the expense of a greatly reduced S/N (Figure 7.16C). Note that higher k-space coordinates are always sampled in a single scan, while the lower k-space coordinates may be acquired with multiple averages. The crucial step for most methods in to define a ‘lipid image’ from the high-resolution MRSI dataset and setting all pixels outside the lipid mask to zero (Figure 7.16D). Inverse Fourier transformation of the lipid image results in a k-space in which the higher k-space coordinates have a greatly reduced noise level (Figure 7.16E). Replacing the inner  $16 \times 16$  k-space coordinates of the enhanced ‘lipid’ k-space with the originally acquired inner  $16 \times 16$  k-space coordinates restores the medium-resolution metabolite MRSI, while maintaining the advantages of extended k-space sampling for the

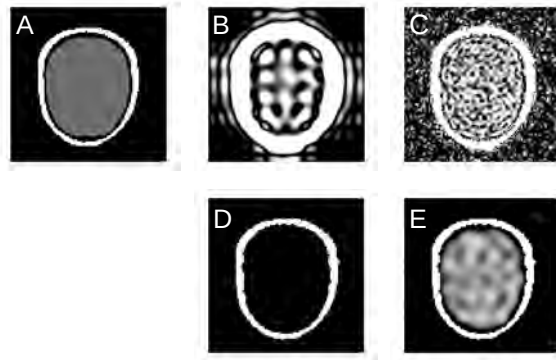


**Figure 7.16** (A) Binary mask of a human head showing brain tissue (gray) and lipid (white) regions. Metabolic maps obtained from (B)  $16 \times 16$  and (C)  $48 \times 48$  MRSI datasets by integration of the 1.8–2.2 ppm spectral region. (D) Lipid mask created by thresholding the metabolic map shown in (C). Combining the high-resolution, ‘noiseless’ lipid data from (D) with the inner  $16 \times 16$  k-space coordinates from (C) results in a high-sensitivity, low-resolution metabolite MRSI dataset without contamination from extracranial lipid signals (E).

lipid resonances. The main consideration for methods utilizing extended k-space sampling is that the acquisition needs to be extended to include the additional k-space samples. However, with MRSI methods based on fast k-space sampling, this consideration is of little concern.

More recently the SLIM algorithm (see Section 7.4.3) has been utilized to remove lipid contamination in MRSI applications on the human muscle [56].

## 7.6 Spectroscopic Imaging Processing and Display

Multidimensional SI datasets normally contain a vast amount of spatial and spectral information. This information can only be optimally evaluated when the data are processed correctly and displayed in a proper manner. The processing and display largely depend on the objective of the study and the nature of the acquired data. Here, a number of important considerations concerning the processing and display of SI datasets are discussed [57, 58].

Consider a  $n$  dimensional ( $n = 1, 2$  or  $3$ ) MRSI dataset, consisting of  $n$  spatial axes and one frequency axis. Prior to the spatial Fourier transformation the data can undergo apodization as described in Section 7.3. When no apodization is employed, the nominal voxel volume is simply given by the FOV divided by the number of phase-encoding increments. After apodization the real voxel size will be increased and can be obtained by (numerical) integration of the PSF (e.g. see Figure 7.3). To allow comparison between MRSI datasets, it is informative to calculate and report the real voxel volumes.

The k-space data could be zero-filled to improve the final appearance of the processed MRSI dataset (either in spectral or image form). It should be noted that zero-filling does not affect the PSF, such that the real resolution of the final image remains unchanged. Zero-filling merely allows the calculation of NMR spectra at intermediate spatial locations, but

does not enhance the information content. Following the spatial Fourier transformation, a  $n$  dimensional MRSI dataset of spatially resolved FIDs (or echoes) is obtained. These time domain signals can undergo the normal processing, like apodization and zero-filling, as described in Chapter 1. The final spectral Fourier transformation results in the spatially resolved NMR spectra.

Even though a 2D MRSI dataset typically holds information on hundreds of voxels, the spatial resolution is most often insufficient to ignore partial volume effects. This problem can be partially reduced by increasing the number of phase-encoding increments at the expense of an increased experimental measurement time. As an alternative the acquired MRSI dataset may be recalculated at intermediate spatial positions, such that the positions of the voxels coincide more closely to anatomical features. This can be achieved by the so-called shift theorem. Fourier transformation of a 1D MRSI dataset  $f(k_x, t)$  gives the spatially resolved FIDs or echoes  $F(x, t)$ . The spatially resolved FIDs can also be calculated at intermediate positions  $x + \Delta x$ , by multiplying the original MRSI data, prior to Fourier transformation, by  $e^{-i\Delta x k_x}$ , i.e.:

$$F(x + \Delta x, t) = \text{FT}(f(k_x, t)e^{-i\Delta x k_x}) \quad (7.25)$$

The shift theorem of Equation (7.25) is readily extended to three spatial dimensions. In fact, one of the advantages of acquiring a complete 4D (i.e. 3D spatial and 1D spectral encoded) MRSI dataset is that the metabolic images can be recalculated in all dimensions to match anatomical and/or pathological features.

Spectroscopic imaging datasets, exhibiting features of both MRI and MRS, can be displayed in several different ways which either focus on the spectroscopic or the spatially resolved information. One of the most straightforward methods of display is to extract and display a number of important spectra from the dataset (Figure 7.17A). This allows a rigorous evaluation of the spectral quality and allows the detection of small spectral changes. However, this method of display largely ignores the spatial information of the data and, if one is only interested in these particular voxels, it should be considered if the acquisition of a number of single voxel localized spectra (possibly by multivoxel localization, see Section 7.7) is more time efficient.

Alternatively, one can display spectra from all voxels in a 2D (or 3D) matrix, for example overlaying an anatomical image (Figure 7.17B). This allows the evaluation of (differences between) spectra in all voxels. However, this type of representation may be too complex in a clinical setting where it is desirable to evaluate spectral changes in voxels at a first glance. For this purpose, a so-called metabolic map may be generated of one or more metabolite resonances. This can be accomplished by integrating or fitting the resonance of interest in each voxel and mapping the signal intensity over the entire MRSI dataset (e.g. see Figure 7.19). The main problem encountered in calculating metabolic maps is the presence of  $B_0$  magnetic field inhomogeneity. Figure 7.18 shows the localized  $^1\text{H}$  NMR spectra from a 1D MRSI experiment of rat brain at 4.7 T. Due to  $B_0$  magnetic field inhomogeneity, the resonances are shifted from voxel to voxel. Clearly, improved shimming using higher order shim coils will decrease the spatial variation in frequencies, but will never completely eliminate the problem. Automatic integration between two predetermined frequencies will result in an underestimation of the metabolite signal intensity in voxels where the resonance lines are shifted with respect to the integration area. In principle, this could be solved by integrating each voxel individually, but this would lead to an unacceptable processing time.

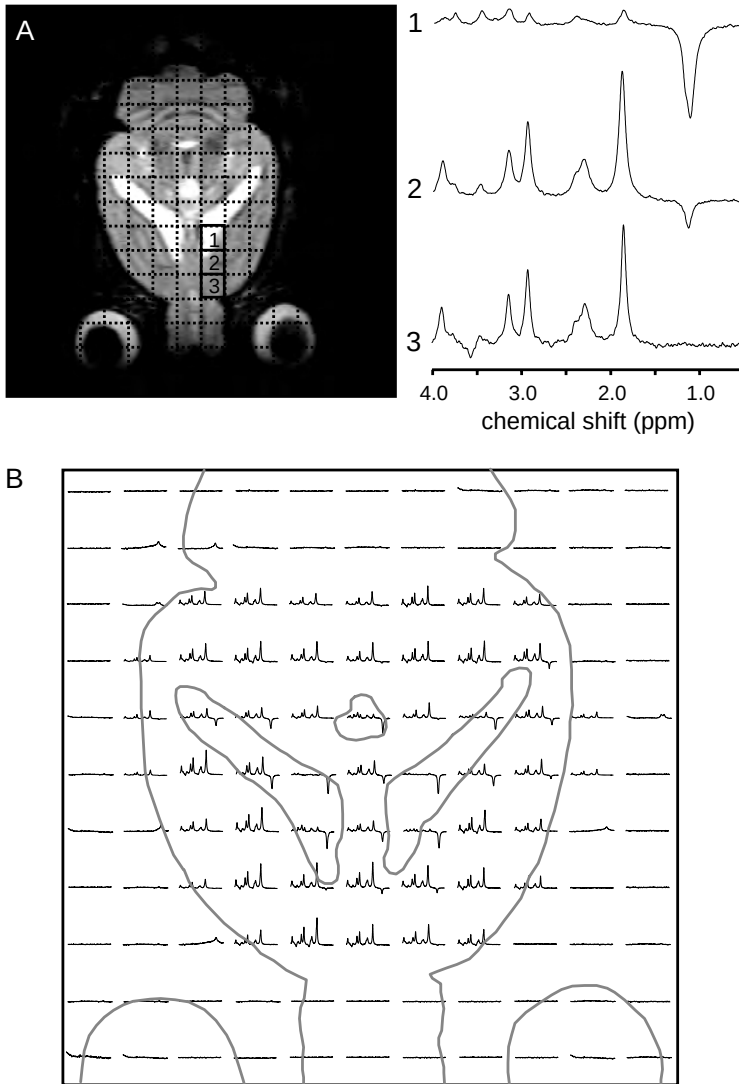


**Figure 7.17** Spectroscopic representations of a MRSI dataset. (A) Extraction of individual  $^1\text{H}$  NMR spectra from a  $16 \times 16$  MRSI dataset of a kaolin-induced hydrocephalic rat in vivo (surface coil transmission and reception,  $16 \mu\text{l}$  nominal voxel size). This representation allows a close inspection of the spectral quality, but ignores large parts of the MRSI dataset. (B) 2D representation of the inner 121  $^1\text{H}$  NMR spectra in an  $11 \times 11$  matrix. In this representation all spectral and spatial information is present and visible. However, it is difficult to get a good overview for individual resonances and observe subtle intensity changes. (Courtesy of K. P. J. Braun.)

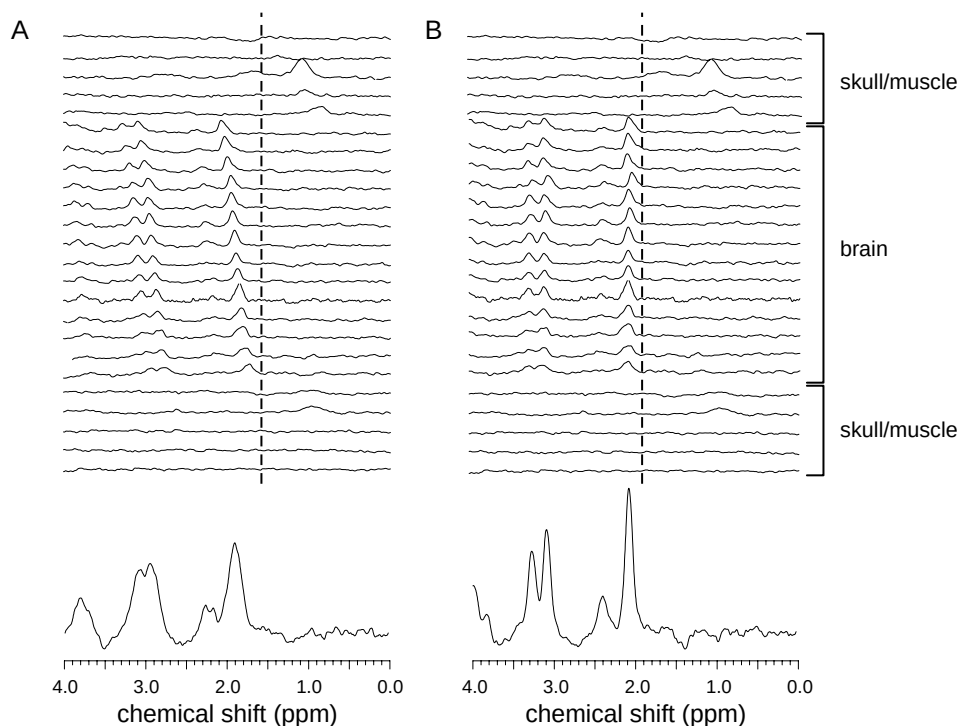


**Figure 7.18** Stacked plot of  $^1\text{H}$  NMR spectra obtained from a 1D MRSI experiment (32 phase encoding increments,  $TE = 144$  ms) of rat brain in vivo. Outside the skull some lipid resonances can be observed (top and bottom five spectra). (A) Note the  $B_0$  magnetic field inhomogeneity across the brain in the original MRSI dataset, as can also be judged from the broad resonances in the summation of the inner 10 spectra (bottom). (B) MRSI dataset after referencing the NAA resonance. All resonances within the brain are better aligned, resulting in narrower peaks in the summation spectrum. The MRSI dataset in (B) is better suited for automatic processing, since frequency shifts have been minimized.

Another method is to employ time- or frequency-domain fitting routines which can account for variations in metabolite resonance frequency. However, frequency alignment based on a strong signal (residual water, NAA) or a  $B_0$  magnetic field map is often part of a pre-processing procedure (Figure 7.18B). The advantage of using unsuppressed water is that besides frequency alignment all spectra can automatically be phased and possibly corrected for residual  $B_0$  eddy current effects (see Section 9.3). An additional advantage of a separate MRSI dataset of unsuppressed water is that it can function as an internal concentration reference (see also Chapter 9). Following frequency alignment, integration or spectral fitting will give the correct signal intensities such that a correct metabolic map can be generated, as shown in Figure 7.19. In order to aid in the evaluation of the correspondence between the separate metabolic maps and the anatomical image, the metabolic map can be overlaid on an anatomical image as a contour plot.

A final step in the acquisition, processing and display of MRSI data is the calculation of absolute metabolite concentrations (in mM or  $\mu\text{mol g}^{-1}$  of tissue). Chapter 9 summarizes

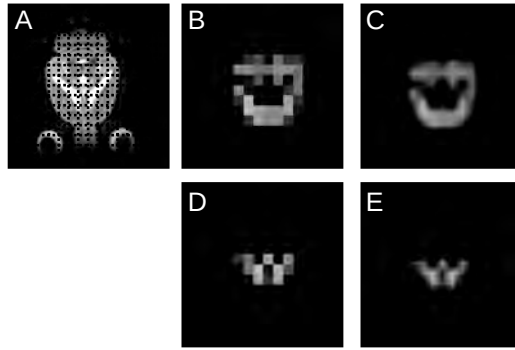


**Figure 7.19** Image representations of a MRSI dataset. (A) Spin-echo image of rat brain in vivo with kaolin-induced hydrocephalus overlaid with a  $16 \times 16$  grid. Metabolic maps of N-acetyl aspartate and lactate are shown in (B, C) and (D, E), respectively. The maps in (B) and (D) represent the real spatial resolution of  $16 \times 16$  voxels. The maps in (C) and (E) are generated after two times zero-filling prior to Fourier transformation to  $64 \times 64$  voxels. Although zero-filling results in a smoothing of the spatial appearance, the actual spatial resolution remains unchanged. (Courtesy of K. P. J. Braun.)

the steps necessary for metabolite quantification in great detail. Here some specific considerations for MRSI will be highlighted. Despite the fact that MRSI data can be spatially shifted to match MRSI voxels with tissue boundaries, partial volume effects are the rule rather than the exception. It is therefore crucial to acquire the MRSI dataset in conjunction with high-resolution images that allow tissue segmentation. While there are many available methods for tissue segmentation, the requirements for brain MRSI studies are that the MRI method should be fast relative to the MRSI acquisition time and that it allows for a segmentation of gray matter, white matter and CSF. Figure 7.20 shows an example of brain segmentation based on quantitative  $T_1$  mapping ([44], see also Section 4.8.2). Since MRSI voxels are not rectangular, it is important to convolve the segmentation maps with the MRSI PSF (Figure 7.20E) to give the real tissue composition in the corresponding MRSI voxels (Figure 7.20F–H).

## 7.7 Multivolume Localization

In situations where it is required to acquire signal from a small number of voxels, SI does not provide acceptable localization due to the unfavorable PSF associated with limited k-space sampling. The localization can be improved significantly by performing successive single voxel localization experiments at the cost of a severe reduction in signal-to-noise per unit time.

Multivolume localization differs from MRSI in that it is based on single volume methods (i.e. slice selection by RF pulse/gradient combinations) extended for the acquisition of multiple volumes. As such it will have the localization accuracy of single volume methods, but without the cost of reduced sensitivity as signal from multiple volumes is acquired in each scan. Multivoxel localization techniques are naturally divided into methods that acquire signal from several voxels simultaneously [59–67] or sequentially [59, 68, 77].

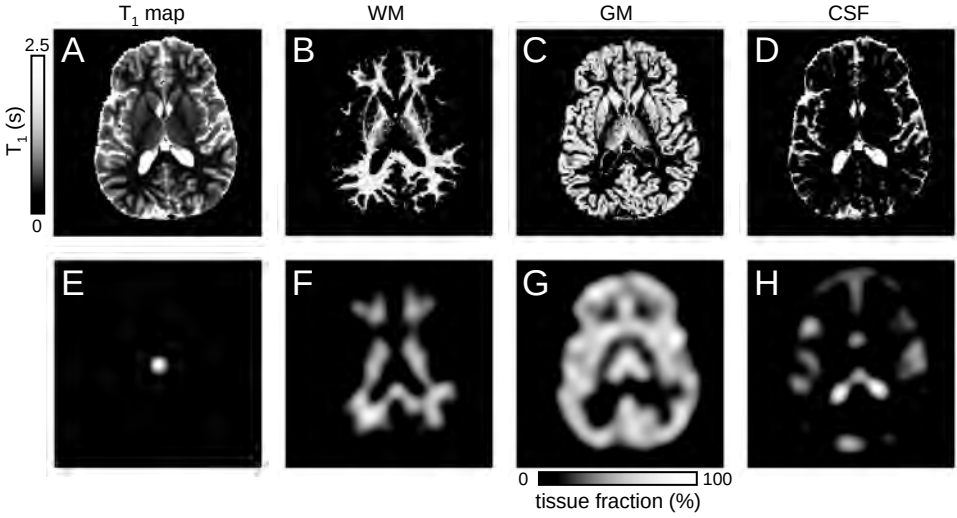


**Figure 7.20** Image segmentation for partial volume correction in MRSI. (A) Quantitative  $T_1$  map from human brain at 2.1 T, together with segmented images for (B) white matter (WM) (C) gray matter (GM) and (D) CSF. In order to account for the limited spatial resolution of the MRSI dataset, the (E) PSF can be convolved with the high-resolution segmented images to produce segmented images at the same resolution as the MRSI dataset (F–H). (Courtesy of G. F. Mason.)

When the signal is acquired simultaneously, the voxels are separated post-acquisition by the Hadamard localization principle as will be discussed next.

### 7.7.1 Hadamard Localization

Simultaneous multivoxel localization is most straightforward when the spatial encoding is performed according to a  $N \times N$  (or  $N \times N \times N$ ) Hadamard matrix, where  $N$  denotes the order of the square matrix. With Hadamard-encoding techniques, the magnetization is encoded along two opposite axes in subsequent experiments. Longitudinal Hadamard encoding [60, 69] can be achieved by storing the magnetization along the  $z$  axis as  $\pm M_z$  with selective inversion in subsequent experiments. With transverse Hadamard encoding [61, 62] the magnetization is encoded as  $\pm M_y$  by inverting the phase of the selective excitation pulse or shifting the phase of the refocusing pulse by  $90^\circ$  in subsequent acquisitions [66].

Before discussing the characteristics of the different Hadamard encoding techniques, the general principles of Hadamard encoding are described. For example, consider the localization of four spatial slices by the following Hadamard matrix:

$$\mathbf{H} = \begin{pmatrix} +1 & +1 & +1 & +1 \\ +1 & +1 & -1 & -1 \\ +1 & -1 & +1 & -1 \\ +1 & -1 & -1 & +1 \end{pmatrix} \quad (7.26)$$

where +1 and -1 correspond to the two possible encoding orientations, e.g. for longitudinal Hadamard encoding +1 and -1 represent no inversion and selective inversion, respectively. In the first experiment (top row) all four slices are acquired with the same phase. In the second experiment the last two slices have an opposite phase relative to the first two slices and so on. Each experiment is stored separately. After completing all four experiments, the individual slices are reconstructed by multiplying the Hadamard encoded slices with an (scaled) inverse Hadamard matrix according to:

$$\mathbf{H} \cdot \mathbf{H}^{-1} = \begin{pmatrix} +1 & +1 & +1 & +1 \\ +1 & +1 & -1 & -1 \\ +1 & -1 & +1 & -1 \\ +1 & -1 & -1 & +1 \end{pmatrix} \begin{pmatrix} +1 & +1 & +1 & +1 \\ +1 & +1 & -1 & -1 \\ +1 & -1 & +1 & -1 \\ +1 & -1 & -1 & +1 \end{pmatrix} = 4 \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix} \quad (7.27)$$

Several pulse sequences exist to generate the required Hadamard matrices in terms of longitudinal or transverse encoded magnetization. Longitudinal Hadamard encoding uses selective inversion in subsequent experiments to encode the longitudinal magnetization along the +z and -z axis, respectively. The selective inversion profiles for multiple slices can be generated either by several individual RF pulses or by one RF pulse exhibiting a multifrequency inversion profile. These so-called multifrequency RF pulses [70–72] simultaneously generate multiple selective excitation or inversion bands and are discussed in Chapter 5. The frequency position of a selective excitation band can, of course, be changed by adjusting the transmitter frequency. However, as an alternative, the pulse can be executed with a linear phase ramp. Furthermore, the relative phase of each band is determined by the phases of the two pulses prior to addition. This principle can easily be extended to multiple bands. The average power of one multifrequency RF pulse and the corresponding number of regular RF pulses is identical. The RF amplitude of multifrequency RF pulses increases linearly (and the RF peak power increases quadratically) with the number of inversion bands (i.e. a multifrequency RF pulse which simultaneously inverts eight frequency bands, requires eight times more RF amplitude). Although several methods have been described to minimize the RF peak power of multifrequency RF pulses [73], this phenomenon limits the applications of multifrequency RF pulses to low-order ( $n \leq 8$ ) Hadamard matrices. Another feature of multifrequency RF pulses is that the frequency bandwidths can not be chosen in the near proximity of each other, since this would distort the frequency bands and consequently the localization accuracy. An elegant solution to this problem was described by Steffen *et al.* [72], in which the RF pulse for one frequency band is adjusted to account for the fact that another RF pulse is simultaneously present at a nearby frequency offset (causing a so-called Bloch–Siegert shift [74], in the which the presence of an intense RF pulse transiently changes the precessional frequency of nearby spins). The problem of slice degradation in adjacent slices is of course eliminated when separate RF pulses for each frequency band are used. Another point of consideration when using multifrequency RF pulses is that the modulation function becomes increasingly complex with increasing Hadamard order. This should be taken into account when the RF waveform generator has a limited digital range or when strong nonlinearities are involved.

A specific problem for longitudinal Hadamard encoding is that a fourth order Hadamard encoding experiment only allows the accurate localization of three voxels (Figure 7.21), when the total localized area is smaller than the entire object. This is because one of the voxel



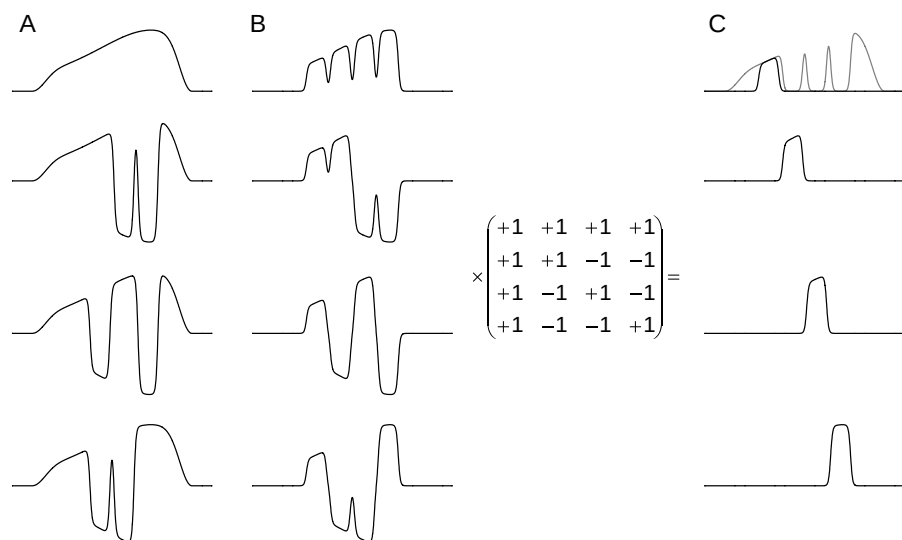


**Figure 7.21** (A) Longitudinal and (B) transverse Hadamard encoding of four spatial slices. Multiplication of the four separately acquired signals with a Hadamard matrix allows (C) the selection of the individual slices. Note that longitudinal Hadamard encoding only allows the detection of three separate slices, whereas the fourth slice is convolved with all other signal from outside the four slices.

positions is not inverted during the entire encoding scheme, such that it is indistinguishable from the unwanted regions of the object. Besides this characteristic problem, longitudinal Hadamard encoding is hampered by the same problems as ISIS localization, i.e. the receiver gain can not be optimized for the localized volume and manual localized shimming is difficult since multiple acquisitions are required to define the total localized volume. Just as with ISIS localization, longitudinal Hadamard encoding schemes can easily be executed with adiabatic RF pulses, an essential point of consideration when surface coils are used for pulse transmission.

Alternatively, Hadamard encoding can be performed in the transverse plane [61, 62, 66]. Discrimination of transverse magnetization is either achieved by changing the phase of the selective excitation pulse [61, 62] or by shifting the phase of the selective refocusing pulse by  $90^\circ$  [66].

Hadamard encoding by itself is not frequently used as a spatial localization technique [75], but the combination of a low-order Hadamard encoding scheme and 2D MRSI is gaining popularity [67, 76]. When a 2D MRSI experiment requires some signal averaging, one can use the additional scans to spatially encode the object under investigation in the third spatial dimension by Hadamard localization, thereby obtaining multiple 2D MRSI datasets with the same sensitivity without increasing the experimental measurement time.

## 7.7.2 Sequential Multivolume Localization

Sequential multivolume localization is a direct extension from multislice imaging, in which slices from other spatial positions can be excited and acquired during the recovery time of

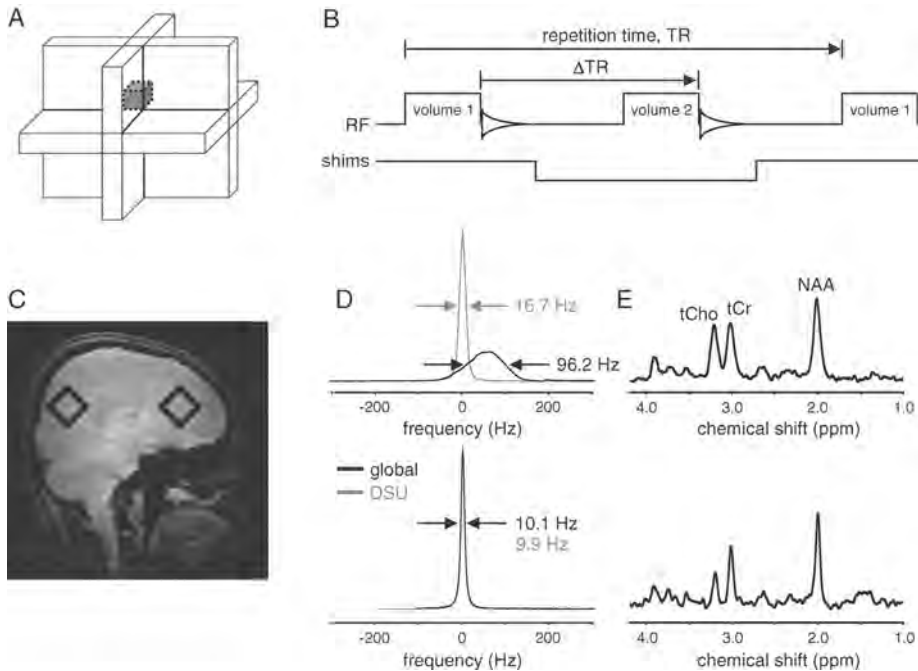


**Figure 7.22** Principle of sequential multivolume localization. (A) A 3D localized volume is formed at the intersection of three orthogonal slices. In order to minimize  $T_1$  saturation between volumes, the spatial position of a given volume should avoid the three slices associated with other volumes. When the volumes are placed along one of the Cartesian axes,  $T_1$  saturation can be minimized by selecting the volume along double-oblique axes (C). (B) Multivolume MRS sequence during which the magnetic field homogeneity of each volume can be optimized by dynamically updating shims (see Chapter 10). (D) Without dynamic shimming (black line) the poor magnetic field homogeneity for the frontal cortex volume makes water-suppressed MRS impossible. However, with dynamic shimming (gray line), the water linewidth in both volumes is acceptable, such that (E) meaningful metabolite  $^1\text{H}$  NMR spectra can be obtained in the same time it would normally take to acquire a NMR spectrum from only one volume.

one slice (see Section 4.3). Regular single volume localization methods like STEAM and PRESS are thus used to acquire signal from multiple volumes with a repetition time that encompasses all volumes (Figure 7.22B). Two potential complications arise from intervoxel saturation effects and intervoxel signal contamination due to incomplete dephasing of unwanted signals.

In multislice MRI it is crucial that all RF pulses are slice selective as to avoid slice-to-slice interference. The same principle applies to multivolume localization with the additional complexity that the slice-to-slice interference must be considered in three dimensions. Figure 7.22A shows the principle of single volume localization by selecting the intersection of three orthogonal slices. When selecting the spatial location of a second volume, the locations on the three orthogonal planes shown in Figure 7.22A should be avoided, as signal in these planes has already been perturbed during the selection of the first volume. This automatically means that, when regular (i.e. nonoblique) x, y and z slices

are used for volume selection, multiple volumes can not be chosen along any of the three orthogonal Cartesian axes. Multiple volumes can be placed along Cartesian axes without inter-volume interference when the slice selection gradients select double-oblique slices, as first pointed out by Ernst and Hennig [59].

In Chapter 6 the dephasing of unwanted coherences in single volume localization was discussed. The effect of coherences generated by scan  $n$  on the signal of scan  $(n + 1)$  was ignored because the repetition time  $TR$  was long relative to  $T_1$  and especially  $T_2$  and  $T_2^*$ . However, while the overall repetition time and thus  $T_1$ -weighting remains constant, the time between the excitation and acquisition of subsequent volumes during a multivolume sequence (denoted by  $\Delta TR$  in Figure 7.22B) becomes smaller as the number of volumes increases. It becomes therefore more likely that coherences from volume  $n$  survive and contribute to the signal from volume  $(n + 1)$ . Transverse coherences are readily destroyed by magnetic field crusher gradients between acquisition of subsequent volume, for example immediately following acquisition. Longitudinal coherences which can potentially generate stimulated echoes can be dephased by having different crusher gradients during the  $TE$  (and  $TM$  periods) of the different volumes.

One unique aspect of sequential over simultaneous multivolume localization is that sequential acquisition allows a greatly improved magnetic field homogeneity when combined with dynamic shim updating (DSU, see Section 10.3.7). Simultaneously acquired data only allows a static shim setting that has been optimized across the *total* localized volume. However, *in vivo* magnetic field inhomogeneity is characterized by many local, higher-order magnetic field gradients introduced by nearby air–brain tissue boundaries, for example around the nasal cavity. A single, static shim setting of low-order shims (typically up to second-order spherical harmonics) is generally incapable of compensating the magnetic field inhomogeneity across the entire sample. However, when the data are acquired sequentially, it is possible to establish optimal shim settings over the much smaller and therefore more manageable localized volumes. Dynamically updating the predetermined shims as the sequence progresses (Figure 7.22B) ensures optimal magnetic field homogeneity for each volume within the limitations imposed by the low-order shim system [77].

## 7.8 Exercises

- 7.1 A** A PRESS localization sequence ( $TR = 20\,000$  ms,  $TE = 5$  ms) is used for volume pre-localization of a  $8 \times 6 \times 1$  cm cubic volume with 5 ms sinc excitation and refocusing pulses ( $R = 6.0$ ). 2D MRSI data are acquired as a  $24 \times 24$  matrix over a FOV of 24 cm at 3 T. For a sample containing 10 mM NAA and 2 mM choline, calculate the NAA methyl-to-choline methyl NMR signal intensity ratio for MRSI voxels in the middle and all four corners of the pre-selected volume. Assume perfect slice and MRSI voxel profiles and an on-resonance condition for the choline methyl resonances. Allow for voxel shifting, such that partial volume effects for choline can be ignored.
- B** Repeat the calculation for a magnetic field strength of 7.05 T.
- C** Calculate the required RF pulse lengths and the corresponding increase in RF power at 3 T to ensure that the NAA methyl-to-choline methyl NMR signal intensity ratio does not change by more than 10 % over the selected volume.

- 7.2** Consider a two-slice OVS method (non-intersecting slices) with 10 ms between the pulses and 20 ms following the last pulse.
- A** In the absence of  $B_1$  magnetic field inhomogeneity, calculate the nutation angles for both pulses to achieve optimal lipid suppression. Assume  $T_{1,\text{lipids}} = 300$  ms and  $TR = 3000$  ms.
  - B** Recalculate the nutation angles when the OVS modules are aimed at removing inhomogeneously broadened water in the nasal cavity. Assume  $T_{1,\text{water}} = 1600$  ms and  $TR = 3000$  ms. Further assume perfect non-selective excitation following OVS.
- 7.3** In a 2D MRSI scan of the human head, extracranial lipid suppression is achieved with a single IR method with  $TR = 2000$  ms and  $TI = 208$  ms.
- A** Calculate the relative signal recoveries for compounds with  $T_1 = 1000, 1300$  and  $1600$  ms. Assume perfect non-selective excitation following OVS.
  - B** In order to avoid saturation effects due to the relative short  $TR$ , the spectroscopist decides to repeat the experiment with  $TR = 6000$  ms. Calculate the relative signal recoveries for compounds with  $T_1 = 1000, 1300$  and  $1600$  ms.
  - C** Based on the calculations performed under (A) and (B) give a recommendation of the sequence timing parameters for quantitative MRSI studies using IR for lipid suppression.
- 7.4** A single-slice 2D MRSI dataset ( $16 \times 16$ ) with a 10 ppm spectral bandwidth can be acquired with a S/N of 10 for NAA in 9 min at 1.5 T (one average).
- A** Calculate the S/N for NAA when the MRSI dataset is acquired with single-shot PEPSI (one average). Assume infinitely short gradient ramp times.
  - B** In order to adequately sample a dynamic time course, the NAA resonance must be sampled with a S/N of 10 and a time resolution of 7 min. Propose at least three solutions to achieve the required sensitivity and time resolution.
- 7.5** Derive an analytical expression for the  $(TR, k)$  curve shown in Figure 7.7A. Hint, combine Equations (7.16) and (7.18) to express the repetition time  $TR$  as a function of the  $k$ -space coordinate  $k$ . Use a modified Hamming function,  $0.75 + 0.25 \cos(\pi k/k_{\text{max}})$  with  $-k_{\text{max}} \leq k \leq +k_{\text{max}}$ , to obtain the curve shown in Figure 7.7A.
- 7.6** Describe an experiment to verify the direction of the ‘half-pixel shift’.
- 7.7** Consider two point samples placed at  $(x_1, y_1)$  and  $(x_2, y_2)$ . Phase-encoded NMR spectra  $P$  are acquired with the phase-encoding gradient in the  $x$  direction, sampling  $k$ -space lines  $k_1$  and  $k_2$ .
- A** Verify that the compartmentalized spectra  $S_1$  and  $S_2$  from the point samples can be uniquely calculated from only two phase-encoded NMR spectra  $P_1$  and  $P_2$ .
  - B** Summarize the exceptions to the answer given under (A).
- 7.8** **A** Calculate the volume sizes for a 2D MRSI experiment ( $16 \times 16$ ) over a FOV = 19.2 cm using the  $k$ -space weighting shown in Figure 7.7 for species with  $T_1 = 2000$  ms and  $T_1 = 1000$  ms. Note, this problem is readily analyzed numerically.
- B** Calculate the scan time reduction for a 2D MRSI experiment ( $32 \times 32$ ) using a Hamming weighting function and  $TR(k=0) = 1500$  ms,  $T_1 = 1500$  ms relative to a nonweighted experiment.
  - C** Repeat the calculation for a Gaussian weighting function [i.e. Equation (7.15)].

- D** Repeat the calculations under (B) and (C) for a 3D acquisition ( $16 \times 16 \times 16$ ).
- E** Derive an expression for the weighting function  $W(k)$  when the weighting is achieved by adjusting the nutation angle rather than the repetition time TR.
- 7.9** In a sequential multivolume experiment a spectroscopist wants to acquire three  $2 \times 2 \times 2 \text{ cm} = 8 \text{ ml}$  volumes.
- A** Under the assumption of non-oblique slice-selection gradients calculate the nearest volume positions that can be used without generating mutual  $T_1$  saturation when the first volume is placed in the magnetic isocenter.
- B** The S/Ns of the NAA resonance in the three volumes after circa 10 min of acquisition ( $TR = 5000 \text{ ms}$ ,  $NA = 128$ ) are 25, 20 and 30, respectively. In the case that multivolume acquisition is not available, calculate the S/Ns for the NAA resonance that would be expected from single volume NMR spectra acquired in separate scans in the same amount of total acquisition time (i.e. all three single volumes acquired in circa 10 min).
- C** For a NAA  $T_1$  relaxation time constant of 1500 ms, calculate the S/Ns for a multivolume acquisition ( $TR = 5000 \text{ ms}$ ,  $NA = 128$ ) when the three volume are placed within each others excitation slices, such that mutual  $T_1$  saturation can not be ignored.
- 7.10** Consider a NMR spectrum acquired from a single volume of volume  $V_{SV}$  with repetition time TR and number of averages  $N_{A,SV}$ .
- A** Derive an expression of the S/N per unit of time for the single volume acquisition. Further consider a 3D MRSI experiment for which  $N_i$  ( $i = 1, 2$  or  $3$ ) phase-encoding increments are acquired over a  $FOV_i$  ( $i = 1, 2$  or  $3$ ). Given a repetition time TR and  $N_{A,MRSI}$  averages per phase-encoding increment.
- B** Derive an expression of the S/N per unit of time for the MRSI acquisition.
- C** When the single volume and nominal MRSI volume sizes are identical, discuss how the S/Ns per unit of time compare.
- D** Discuss the advantage and disadvantages of single volume MRS and MRSI.
- 7.11** MRSI data acquisition time can be significantly reduced through the use of multi-spin-echo methods during which multiple echoes are acquired following a single excitation.
- A** Discuss the temporal aspects (i.e. scan time reduction) when all echoes are sampled at the same k-space coordinate.
- B** Discuss the temporal aspects (i.e. scan time reduction) when subsequent echoes are sampled at different k-space coordinates.
- C** For both methods (A and B) discuss the effects that can arise for scalar-coupled spin systems.

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# 8

## Spectral Editing and Two-dimensional NMR

### 8.1 Introduction

Proton NMR spectra from mammalian tissues *in vivo* hold information on a considerable number of metabolites. Even though the *in vivo* NMR detection limit of  $\sim 100\ \mu\text{M}$  greatly simplifies the spectral appearance, a typical short TE  $^1\text{H}$  NMR spectrum from rat or human brain *in vivo* still contains resonances from more than 15 different metabolites [1, 2]. The abundance of resonances in combination with a small proton chemical shift range leads to significant spectral overlap, thereby complicating unambiguous peak assignment and quantification. Prominent examples can be found for lactate, which overlaps with signals from lipids and macromolecules, the inhibitory neurotransmitter  $\gamma$ -amino butyric acid (GABA) which is obscured by total creatine and other resonances and glutamate and glutamine which overlap with each other at lower magnetic fields. The collective group of techniques that achieves separation of overlapping resonances is often referred to as spectral editing and this chapter describes the principles of homo- and heteronuclear spectral editing together with practical *in vivo* applications.

In the most general sense, spectral editing may include all techniques that can simplify a NMR spectrum in order to limit the detection to specific metabolites. According to this general definition, spectral editing can include water suppression, spatial localization, echo-time variation, use of shift reagents and selective excitation. Because many of these techniques are discussed in separate chapters, a more specific definition will therefore be employed here. Spectral editing is defined as to include all techniques which utilize the scalar coupling between spins to discriminate scalar-coupled from uncoupled spins. This definition includes all 2D NMR techniques which can be seen as the ultimate spectral editing techniques, making no assumptions about the spin system under investigation.

## 8.2 Scalar Evolution

For a proper and complete understanding of spectral editing it is crucial that the coherences of the spin system under investigation can be visualized and quantitatively calculated. The Bloch equations and vector diagrams allow easy visualization and calculation of observable magnetization (also known as single quantum coherences, SQCs), but fall apart when multiple quantum coherences (MQCs) are involved. The density matrix formalism, as will be discussed in Chapter 9, allows a quantitative calculation of the energetic state of a spin system under all conditions, but since the calculations involve energies it does not allow for easy visualization of individual coherences. The product operator formalism [3–5] offers a convenient alternative in that it allows quantitative predictions for weakly coupled spin systems while still allowing a direct visualization of all coherences involved. The product operator formalism is based on the density matrix formalism, under the restriction that the coupled spin system is weakly coupled (i.e. the frequency separation between resonances is much larger than the scalar coupling). Within this restriction the density matrix can be expanded into a linear combination of orthogonal matrices (referred to as product operators), each of which represents an orthogonal component of the magnetization. Various complete orthogonal basis matrix sets can be used, of which product operators and spherical tensor operators are most commonly employed.

Appendix A4 summarizes the complete product operator formalism. Here the product operator formalism is used in several examples. Consider the spin-echo sequence shown in Figure 8.1A and a weakly coupled two-spin system IS. Without loss of generality, only the I spin will (initially) be considered. Prior to excitation, the density matrix  $\sigma$  is equal to the thermal equilibrium magnetization of spin I, given by:

$$\sigma(0) = I_z \quad (8.1)$$

where the argument of  $\sigma$  refers to the time following excitation. All NMR sequences are generally characterized by three elements, namely RF pulses, delays and magnetic field gradients. RF pulses are used to change the coherence order and in the case of excitation (Figure 8.1A) the longitudinal magnetization (while technically not a coherence, it is often assigned coherence order 0) is converted to transverse magnetization (also known as SQC of order  $\pm 1$ ), i.e.:

$$\sigma(+) = I_y \quad (8.2)$$

where  $+$  refers to the time immediately following the excitation pulse. Next, the transverse magnetization will evolve under the influence of chemical shift, scalar coupling and magnetic field inhomogeneity during the evolution time  $TE/2$ . In Chapter 1 it was demonstrated that effects of chemical shift and magnetic field inhomogeneity are refocused by a  $180^\circ$  pulse and are therefore, for sake of clarity, not further considered. The density matrix at  $t = TE/2$  is then given by:

$$\sigma(TE/2) = \left[ I_y \cos\left(\frac{\pi J_{IS} TE}{2}\right) - 2I_x S_z \sin\left(\frac{\pi J_{IS} TE}{2}\right) \right] e^{-TE/2T_2} \quad (8.3)$$

where  $2I_x S_z$  represents so-called antiphase coherence of spin I with respect to spin S. It can be visualized as two opposing vectors along the x axis of the rotating frame (Figure 8.1C). A useful interpretation of the two vectors can be found when the energy-level diagram and corresponding NMR spectrum of Figure 1.15 is considered. For a weakly coupled

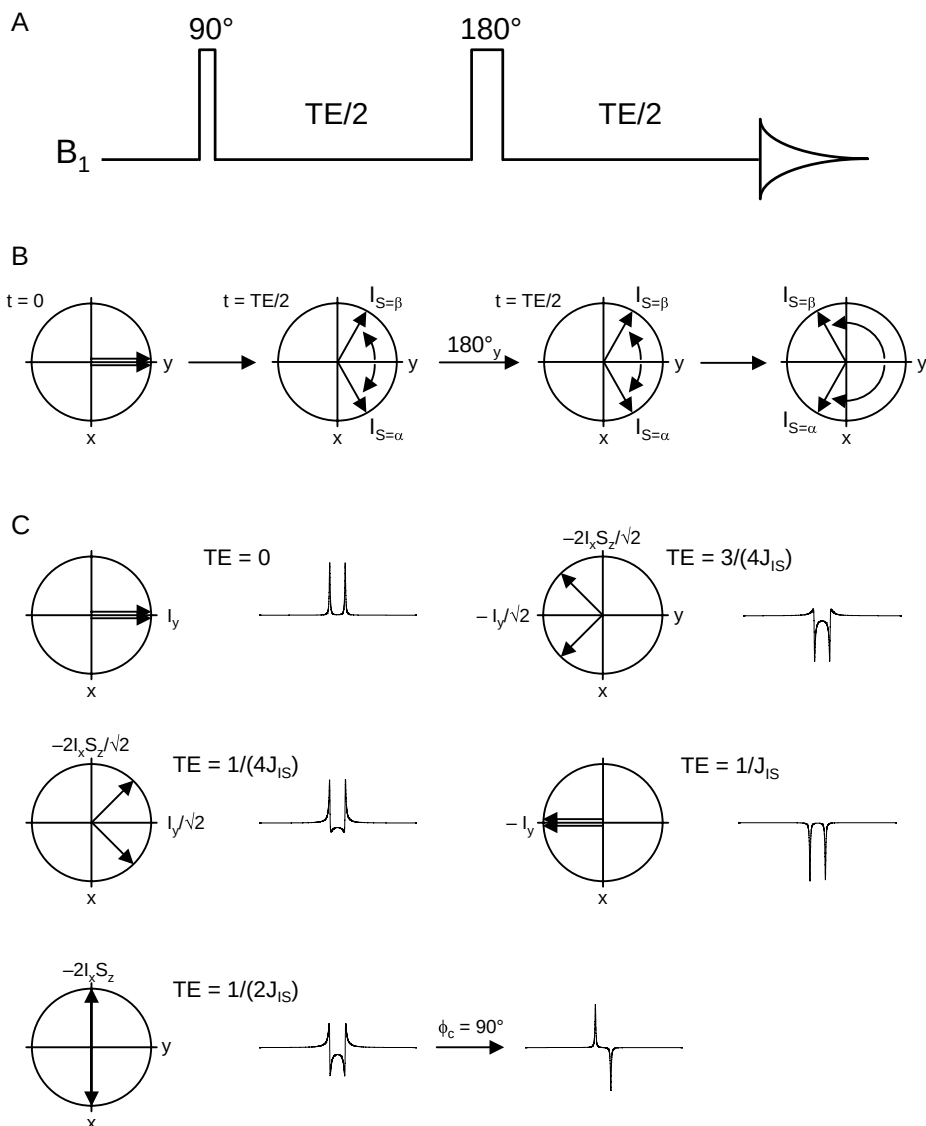


**Figure 8.1** Scalar coupling evolution during (A) a spin-echo sequence for a  $IS$  two-spin system. (B) Following excitation, the two different  $I$ -spin populations acquire an echo-time dependent phase shift. The nonselective  $180^\circ$  refocusing pulse inverts the acquired phase and inverts the  $S$ -spin population. As a result, the two different  $I$ -spin populations will obtain a net phase difference at the top of the spin echo. See text for more details. (C) Transverse coherences and corresponding NMR spectra for the  $I$  spin of a two-spin system as a function of the echo time  $TE$ .

two-spin system the NMR spectrum is characterized by two doublets. The spectral line in the doublet for the I spins that resonates at a lower frequency ( $\nu_I - J_{IS}/2$ ) is characterized by the fact that the attached S spins are in the lower energy  $\alpha$  spin state, while the spectral line at the higher resonance frequency ( $\nu_I + J_{IS}/2$ ) is characterized by I spins attached to S spins in the higher energy  $\beta$  spin state.

The nonselective  $180^\circ$  pulse has two effects on the coherences under investigation. Firstly, the normal action of a  $180^\circ$  pulse, namely resetting the phase evolution  $\phi = \pi(\nu_I \pm J_{IS}/2)TE$  of the I spins to  $-\pi(\nu_I \pm J_{IS}/2)TE$ , would occur. Secondly, since the  $180^\circ$  pulse is nonselective it will simultaneously invert the S spin population, such that I spins attached to S spins in the  $\alpha$  spin state and resonating at the lower frequency ( $\nu_I - J_{IS}/2$ ) before the  $180^\circ$  pulse, are attached to S spins in the  $\beta$  spin state and consequently resonate at the higher frequency ( $\nu_I + J_{IS}/2$ ) following the  $180^\circ$  pulse. In other words, the  $180^\circ$  pulse resets the phases and changes the sense of rotation. The density matrix following the  $180^\circ$  pulse is given by:

$$\sigma(TE/2+) = \left[ I_y \cos\left(\frac{\pi J_{IS}TE}{2}\right) - 2I_x S_z \sin\left(\frac{\pi J_{IS}TE}{2}\right) \right] e^{-TE/2T_2} \quad (8.4)$$

Following an additional delay  $TE/2$ , the density matrix at the start of acquisition is given by:

$$\sigma(TE) = [I_y \cos(\pi J_{IS}TE) - 2I_x S_z \sin(\pi J_{IS}TE)] e^{-TE/T_2} \quad (8.5)$$

where the relation  $4S_z^2 = 1$  has been used to achieve simplification. Equation (8.5) demonstrates that a regular nonselective spin-echo sequence does not refocus the effects of scalar coupling, due to the dual effect of the  $180^\circ$  pulse. Note that for  $TE = 1/(2J_{IS})$  the transverse magnetization is represented by a state of pure antiphase coherence, while for  $TE = 1/J_{IS}$  the in-phase transverse magnetization  $I_y$  is inverted relative to that observed at  $TE = 0$ . Figure 8.1C shows a graphical representation of Equation (8.5) together with simulated NMR spectra. While the S spin was initially ignored, equations similar to those in Equations (8.1)–(8.5) can be constructed for spin S.

Since the spin-echo sequence forms the basis for one of the most common spectral editing techniques, namely J-difference editing, the next section discusses the extension of the spin-echo method to allow spectral editing.

### 8.3 J-difference Editing

Figure 8.2 shows the basic NMR sequence for J-difference editing [6–11]. In the first experiment, the selective  $180^\circ$  pulses are not present and the sequence reduces to a simple spin echo for which the evolution of coherences is given by Equations (8.1)–(8.5). In the second experiment, the selective  $180^\circ$  pulses are present and their effect on the coherences need to be considered. Immediately prior to the first selective  $180^\circ$  pulse, the density matrix is given by:

$$\sigma(t) = [I_y \cos(\pi J_{IS}t) - 2I_x S_z \sin(\pi J_{IS}t)] e^{-t/T_2} \quad (8.6)$$

Unlike a nonselective  $180^\circ$  pulse, the  $180^\circ$  pulses shown in Figure 8.2 are selective for the S spin. They do therefore only have one effect, the inversion of the S spin population, which leads to a reversal of rotation of the scalar coupling evolution of the I spin (i.e. the

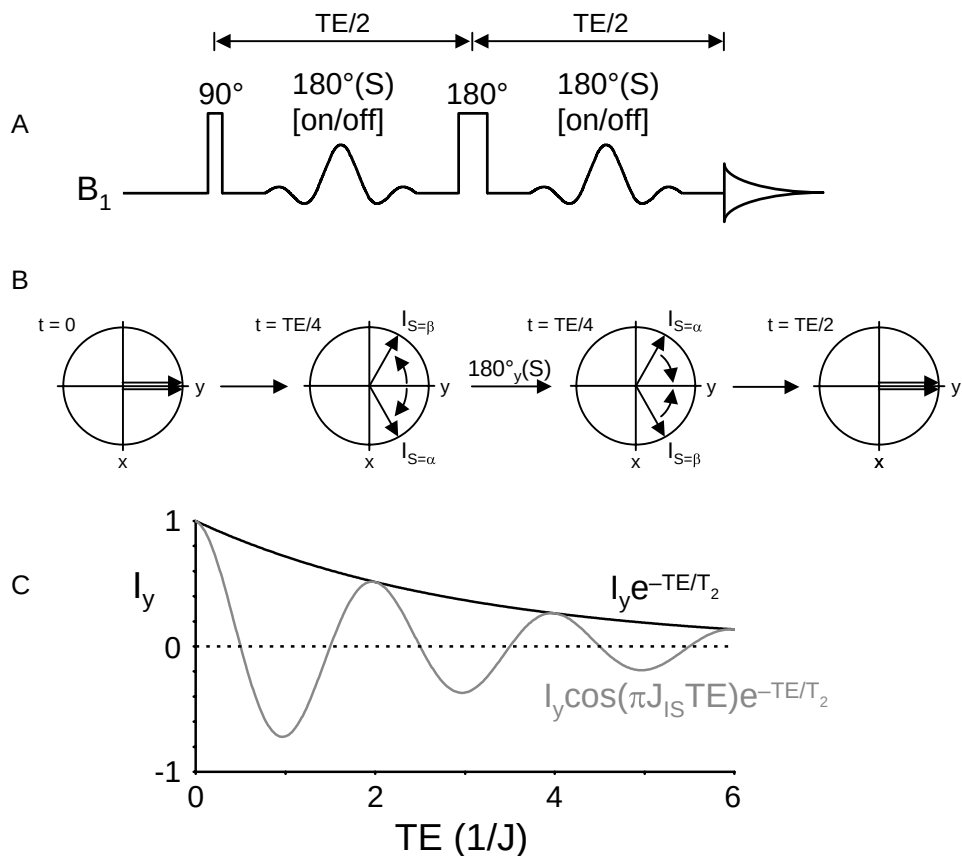


**Figure 8.2** (A) Basic *J*-difference editing sequence. (B) During a 180° refocusing pulse that is selective for the *S* spin, the *S*-spin population is inverted leading to a reversal of the sense of rotation for the *I* spin. However, the selective 180° pulse does not reset the acquired phase of the *I* spin, such that at  $t = TE/2$ , the *I*-spin coherences are perfectly refocused along the *y* axis. (C) *I*-spin signal modulation curves in the absence (gray line) and presence (black line) of selective 180° refocusing pulses in (A).

slower rotating *I* spins attached to *S* spins in the  $\alpha$ -state before the selective 180° pulse become faster rotating *I* spins attached to *S* spins in the  $\beta$ -state after the selective 180° pulse). Therefore, following an equal delay  $TE/4$ , the *I* spin coherences are completely in-phase at  $t = TE/2$  as expressed by the density matrix:

$$\sigma(TE/2) = I_y e^{-TE/2T_2} \quad (8.7)$$

The nonselective 180° pulse simply refocuses phase evolution due to chemical shift and magnetic field inhomogeneity. During the third delay, scalar coupling evolution occurs which will be refocused by the selective 180° pulse, giving rise to the final density matrix:

$$\sigma(TE) = I_y e^{-TE/T_2} \quad (8.8)$$

The principle of J-difference editing can now easily be seen from Equations (8.5) and (8.8), which are graphically depicted in Figure 8.2C. Uncoupled resonances, like water and creatine do not have scalar coupling and their density matrix will be equal in form to Equation (8.8) in the absence and presence of selective  $180^\circ$  pulses. However, when  $TE = 1/J$  the doublet resonance associated with the I spins in a scalar-coupled two-spin system is inverted relative to the uncoupled spins in the selective experiment [see Equation (8.5) and Figures 8.1 and 8.2C]. In the nonselective experiment, the I spin doublet will have the same sign as resonances from uncoupled spins for any echo-time TE.

Subtraction of the two experiments will eliminate all uncoupled resonances, allowing for selective detection of resonances from coupled spins, in this case spin I (Figure 8.3D). Addition of the spectra will give a spectrum devoid of coupled spin resonances (Figure 8.3E). This is one of the advantages of J-difference editing over other spectral editing techniques. The resonance of interest can be selectively observed without eliminating all information about other (uncoupled) resonances. In the particular example given in Figure 8.3, the S spin is no longer visible due to the additional water suppression technique employed. The subtraction (and addition) can be performed by phase cycling or during post-processing if the two datasets are stored separately. Obviously, phase cycling only allows subtraction or addition.

The sequence shown in Figure 8.2A is one of many possibilities to achieve J-difference editing. Other variants mainly differ in the manner by which selective refocusing is achieved [6–11]. The advantage of the sequence in Figure 8.2A is that the symmetry of the  $180^\circ$  pulses ensures complete refocusing of RF-induced phases. As such the selective  $180^\circ$  pulses can even be executed with AFP pulses (see Sections 5.7.2 and 6.3.3). Even though a single AFP pulse introduces significant  $B_1$  and offset-dependent phase modulations, the second AFP pulse completely refocuses those effects. Similar arguments have been used in the development of spin-echo water suppression techniques with negligible spectral phase distortion [12].

So far only two-spin systems have been considered in which the scalar coupling modulation was governed by Equation (8.1). For more extended spin systems, the scalar coupling modulation will be more complicated. Repeated application of Equation (8.1) yields for an  $IS_2$  spin system:

$$I_x \xrightarrow{2\pi J_{IS}t(S_{1z}+S_{2z})} I_x \cos^2(\pi J_{IS}t) + 2I_y(S_{1z} + S_{2z}) \sin(\pi J_{IS}t) \cos(\pi J_{IS}t) - 4I_x S_{1z} S_{2z} \sin^2(\pi J_{IS}t) \quad (8.9)$$

and

$$I_y \xrightarrow{2\pi J_{IS}t(S_{1z}+S_{2z})} I_y \cos^2(\pi J_{IS}t) - 2I_x(S_{1z} + S_{2z}) \sin(\pi J_{IS}t) \cos(\pi J_{IS}t) - 4I_y S_{1z} S_{2z} \sin^2(\pi J_{IS}t) \quad (8.10)$$

and for an  $IS_3$  spin system:

$$I_x \xrightarrow{2\pi J_{IS}t(S_{1z}+S_{2z})} I_x \cos^3(\pi J_{IS}t) + 2I_y(S_{1z} + S_{2z} + S_{3z}) \sin(\pi J_{IS}t) \cos^2(\pi J_{IS}t) - 4I_x(S_{1z}S_{2z} + S_{1z}S_{3z} + S_{2z}S_{3z}) \sin^2(\pi J_{IS}t) \cos(\pi J_{IS}t) - 8I_y S_{1z} S_{2z} S_{3z} \sin^3(\pi J_{IS}t) \quad (8.11)$$

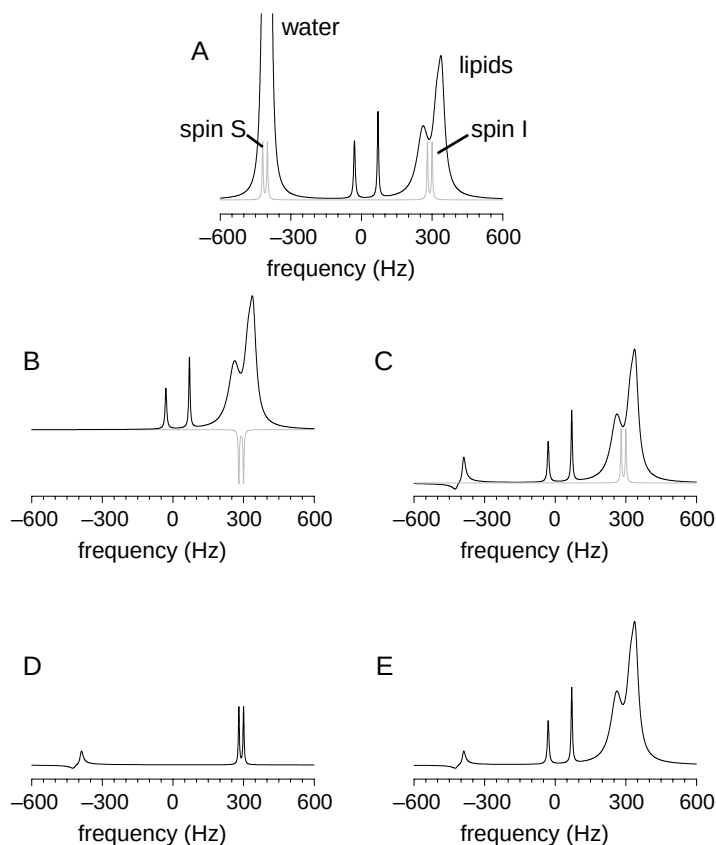


**Figure 8.3** Principle of *J*-difference spectral editing. (A) Simulated, nonedited  $^1\text{H}$  NMR spectrum. The resonances of interest (gray, dotted lines) are both overwhelmed by other resonances (water and lipids). (B) Using the sequence in Figure 8.2A without the selective  $180^\circ$  RF pulses yields a NMR spectrum in which the resonance from the *I* spins appears inverted relative to uncoupled spins. The water and the underlying resonance from the *S* spins are suppressed by a nonspecified water suppression method. (C) In the presence of the two selective  $180^\circ$  refocusing pulses, the resonance of the *I* spins appears in-phase with the other resonances from uncoupled metabolites. (D) Subtraction of (B) and (C) will give the coupled resonance, while (E) the summation will give the uncoupled resonances.

and

$$I_y \xrightarrow{2\pi J_{IS}t(S_{1z}+S_{2z})} I_y \cos^3(\pi J_{IS}t) - 2I_x(S_{1z} + S_{2z} + S_{3z}) \sin(\pi J_{IS}t) \cos^2(\pi J_{IS}t) \\ - 4I_y(S_{1z}S_{2z} + S_{1z}S_{3z} + S_{2z}S_{3z}) \sin^2(\pi J_{IS}t) \cos(\pi J_{IS}t) \\ + 8I_xS_{1z}S_{2z}S_{3z} \sin^3(\pi J_{IS}t) \quad (8.12)$$

where the relation  $4S_{1z}^2 = 4S_{2z}^2 = 4S_{3z}^2 = 1$  has been used repeatedly to achieve simplification. The individual terms in Equations (8.9)–(8.12) are graphically shown in Figure 8.4

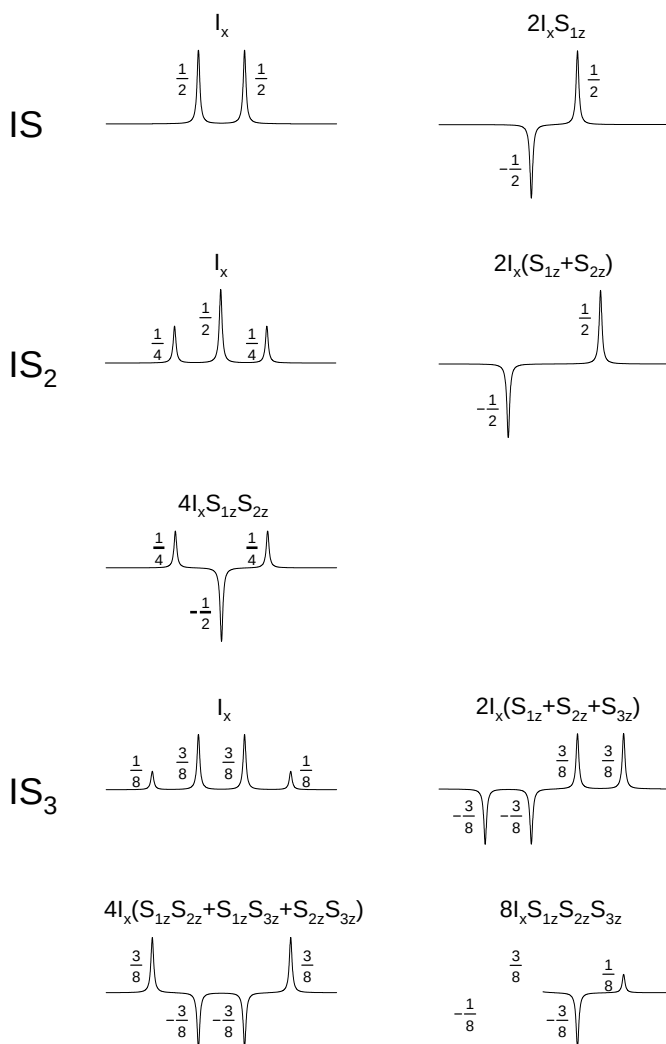


**Figure 8.4** Graphical representation of different coherences for  $IS$ ,  $IS_2$  and  $IS_3$  spin systems. Only  $I_x$  containing coherences are shown.  $I_y$  containing coherences are  $90^\circ$  out of phase with the coherences shown. The absolute sum of the resonances in an  $IS_3$  spin system can be  $>1$  for some coherences, since it is not possible to detect these coherences at 100 % efficiency.

assuming a phase-sensitive detection of  $I_x$  containing operators. The operators containing  $I_y$  lead to dispersive contributions with the same multiplet structure.

As with a simple  $IS$  spin system, selective refocusing of one spin in an  $IS_N$  ( $N = 1, 2$ , or  $3$ ) spin system, leads to complete refocusing of scalar coupling evolution. Therefore, for an  $IS_3$  spin system, the J-difference experiment is virtually identical as for an  $IS$  spin system, i.e. in the selective experiment the resonance is positive ( $+I_y e^{-TE/T_2}$ ) and in the nonselective experiment at  $TE = 1/J$  the resonance is inverted ( $-I_y e^{-TE/T_2}$ ). Subtraction



gives the resonance of interest. However, for  $IS_2$  spin systems a different situation arises. Due to the  $\cos^2(\pi J_{IS})$  dependence of the  $I_y$  operator, scalar coupling modulation will not lead to inversion of the I resonance relative to uncoupled spins. Nevertheless, by taking  $TE = 1/(2J_{IS})$ , partial editing can be achieved. With selective  $180^\circ$  pulses, the scalar coupling evolution is refocused leading to a density matrix at the start of acquisition given by Equation (8.8). Without selective refocusing, the antiphase term  $-4I_yS_{1z}S_{2z}$  is observed. Subtraction of the two spectra gives the two outer resonances of the antiphase triplet. The inner resonance is lost, since it is positive in both experiments. Under *in vivo* conditions (i.e. relatively short  $T_2^*$  relaxation times and, therefore, broad resonances), the two outer resonances will appear as a broadened single resonance line. Therefore, J-difference editing achieves 100, 50 and 100 % signal recovery for  $IS$ ,  $IS_2$  and  $IS_3$  spin systems, respectively. The complex scalar coupling evolution of  $IS_2$  and  $IS_3$  spin systems will lead to significant signal loss with other editing techniques like polarization transfer.

## 8.4 Practical Considerations of J-difference Editing

In the previous section the theoretical principles of J-difference editing have been outlined. However, a successful implementation of J-difference editing requires attention to detail ranging from the obvious (the need for spatial localization) to the not so obvious (frequency drift correction). One of the most popular applications of *in vivo* J-difference editing is the detection of cerebral GABA [11, 13–15]. GABA is the major inhibitory neurotransmitter in the mammalian central nervous system and alterations in GABA concentration have been found in epilepsy, depression and schizophrenia. However, the detection of GABA by *in vivo* NMR spectroscopy is not straightforward, as all three resonances of GABA are overlapping with other resonances, making direct observation impossible. Here, the selective detection of GABA by J-difference editing will be used to illustrate the requirements of successful spectral editing.

Figure 8.5 shows the three GABA resonances in relation to other commonly observed resonances. The GABA-H2 methylene protons at 2.28 ppm partially overlap with glutamate-H4 protons at 2.34 ppm as well as with broad macromolecular resonances at 2.29 ppm (see also Figure 8.8). The broad GABA-H3 multiplet at 1.89 ppm partially overlaps with macromolecular resonances at 2.05 and 1.72 ppm, as well as with the broad base of the large NAA methyl resonance at 2.01 ppm. The GABA-H4 methylene protons at 3.01 ppm overlap completely with a combined resonance from creatine and phosphocreatine. The first decision that needs to be made is on which resonance to focus in the spectral editing process. Detection of GABA-H3 is not desirable due to the multiple coupling partners and the reduced intensity of the multiplet. Detection of GABA-H2 is not desirable because of the close proximity of glutamate-H3 to GABA-H3. Selective refocusing of GABA-H3 (which is the coupling partner of GABA-H2) will certainly affect glutamate-H3, leading to partial co-editing of glutamate-H4 and hence partial overlap of glutamate-H4 and GABA-H2. To a first approximation GABA-H4 only overlaps with singlet resonances and is therefore typically the choice for spectral editing methods. Since in this case GABA-H3 is also the coupling partner, spectral editing of GABA-H4 typically leads to near-complete GABA-H2 recovery as well (with a small amount of co-edited glutamate-H4).

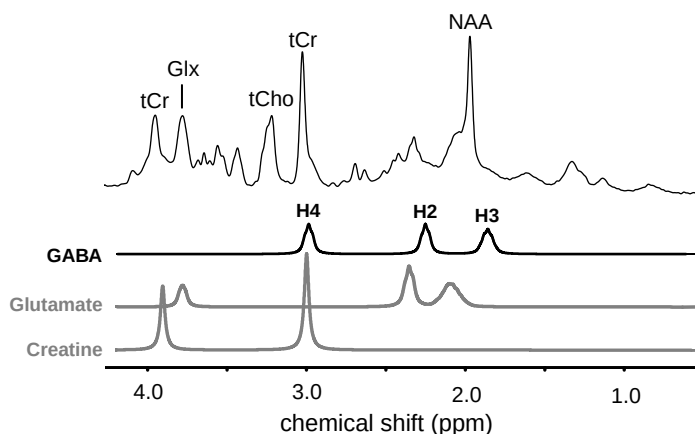


**Figure 8.5**  $^1\text{H}$  NMR of GABA showing the three resonances in relation to other proton resonances, including those from creatine and glutamate.

The next consideration concerns the extension of the sequence with spatial localization. As detailed in Chapter 6, spatial localization is required for all applications of *in vivo* NMR spectroscopy, in order to (1) eliminate unwanted resonances (e.g. extracranial lipids), (2) accurately define the origins of the detected signal and (3) improve the  $B_0$  and  $B_1$  magnetic field homogeneity over the detected volume. Many spectral editing techniques utilize the ISIS method (or one of the many variants) for spatial localization. The main advantage of ISIS is that the magnetization of interest remains along the longitudinal axis, such that any kind of MR sequence can follow the localization part. The main drawback of ISIS is that it requires a minimum of eight acquisitions to completely localize a 3D volume. This prevents a scan-by-scan evaluation of the data for system instabilities or subject motion. Since these considerations become more important with higher precision measurements and higher resolution data acquired at high magnetic fields (see next section), the need for single-scan localization methods becomes more urgent. For the basic J-difference editing as shown in Figure 8.6A it is relatively simple to replace the nonselective  $90^\circ$  and  $180^\circ$  pulses with spatially selective variants. Complete single-scan 3D localization can, for example, be achieved by adding an additional, spatially selective  $180^\circ$  pulse immediately following the  $90^\circ$  pulse, as shown in Figure 8.6B. It should be realized however, that the evolution delays surrounding the first  $180^\circ$  pulse do not contribute to the editing process and should therefore be minimized.

A second sequence extension over the basic J-difference editing sequence shown in Figure 8.6A is concerned with water suppression. Even though all uncoupled resonances, including water, are eliminated in the subtraction process, J-difference editing still requires excellent water suppression for two reasons. Firstly, while the edited resonance (GABA in this case) is the prime focus, the other resonances that can be observed in the two subspectra are often also important. Total creatine (creatine + phosphocreatine) is often used as an internal concentration reference, while glutamate and glutamine provide complementary information on neurotransmitter metabolism. Reliable detection of the nonedited

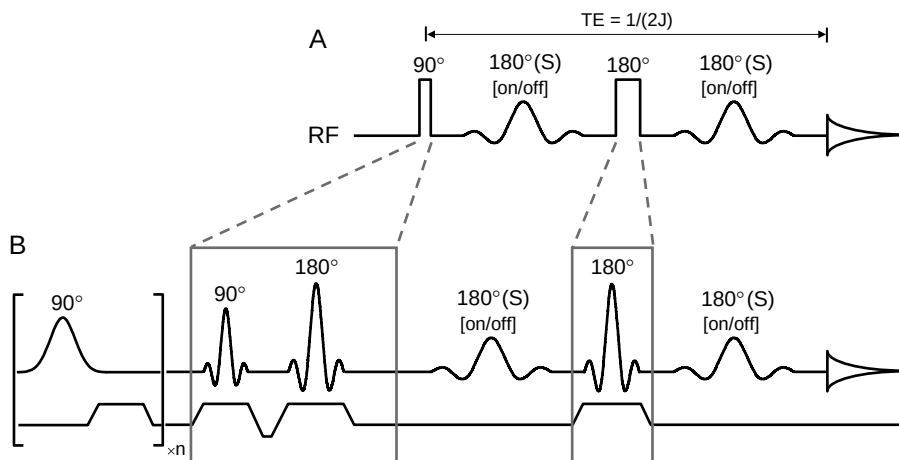


**Figure 8.6** Required modifications to convert (A) the basic J-difference editing sequence into (B) a 3D localized, water-suppressed editing method.

metabolites requires excellent water suppression, since the large water resonance baseline and vibration-induced sidebands (see Section 6.3 and Figure 6.21) can obscure the small metabolite resonances. Secondly, small perturbations in the water resonance by motion, magnetic field drift, or other system instabilities can result in a large water subtraction artifact in the GABA edited spectrum.

Chemical shift selective (CHESS) water suppression [16], or any of the many variations, can always be used for water suppression since it only excites the water and leaves the metabolites unperturbed prior to excitation. In cases where CHESS does not provide adequate suppression (e.g. in the presence of strong  $B_1$  field inhomogeneity or very short  $T_1$  relaxation times), water suppression based on spin-echo dephasing can be used. In particular the MEGA method [12] can be combined with the selective 180° editing pulses to provide simultaneous water suppression and spectral editing [11]. A realistic version of J-difference editing is shown in Figure 8.6B. Using this sequence on human brain at 4.0 T allows the detection of cerebral GABA levels with a reproducibility in the order of 10–15 % (Figure 8.7).

However, the observation of a well-isolated signal with high sensitivity, good water suppression and a flat baseline, as shown in Figure 8.7, is not a guarantee for successful GABA detection. While the mentioned observations are important for reliable GABA detection, there is still the possibility that the observed resonance is contaminated by co-edited resonances and incompletely subtracted overlapping resonances. During the design of a spectral editing experiment it should be determined which metabolites can potentially be co-edited and contaminate the edited resonances. For GABA-H4 detection the prime candidates for co-editing are glutamate, homocarnosine and macromolecular resonances. From Figure 8.7 it is clear that glutamate co-editing does occur, as evidenced by the glutamate-H2 resonance ( $\sim 3.74$  ppm) in the edited spectrum. However, since glutamate-H2 does not overlap with GABA-H4 this form of co-editing is of no concern. Homocarnosine is a dipeptide of histidine and GABA and occurs in the human brain at a concentration of

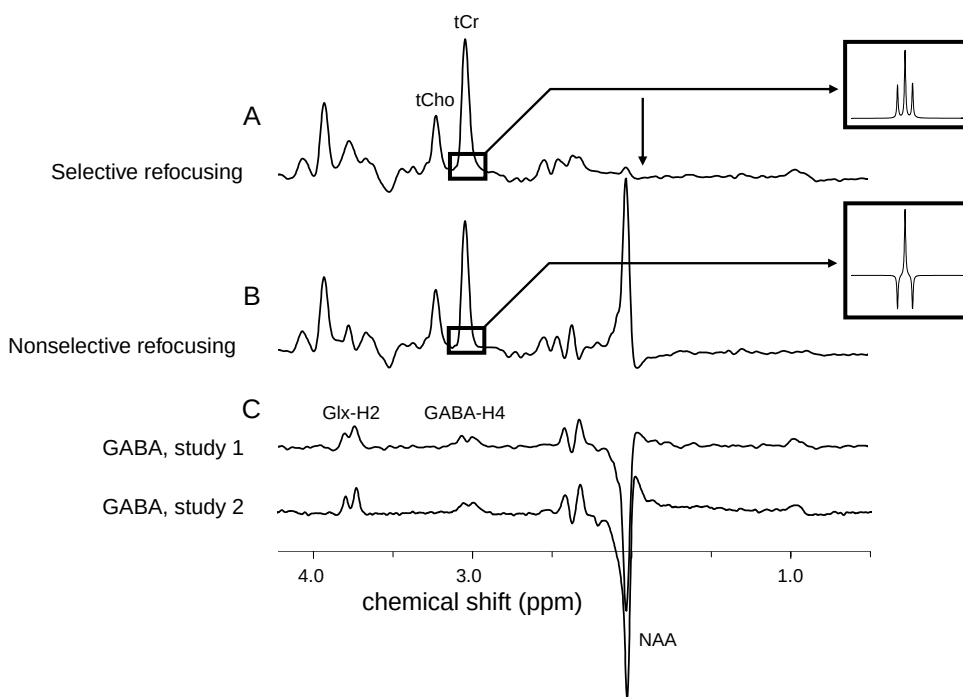


**Figure 8.7** GABA editing on human brain at 4.0 T. Localized  $^1\text{H}$  NMR spectra (12 ml, TR/TE = 3000/68 ms) acquired (A) with and (B) without selective refocusing of the GABA-H3 resonance. (C) Difference spectra obtained from the same subject on separate occasions, showing the reproducibility of GABA-H4 detection.

circa 0.5 mM. Since the spin system of the GABA moiety of homocarnosine is essentially identical to that of GABA, the edited signal observed *in vivo* is typically the sum of GABA and homocarnosine (which is sometimes referred to as ‘total GABA’). This form of complete co-editing can be a problem when GABA and homocarnosine need to be separated. Fortunately, in this particular case, homocarnosine can be detected separately through the histidine–imidazole proton resonances at 7.1 and 8.1 ppm observable in short echo time  $^1\text{H}$  NMR spectra [17].

The contamination of edited GABA by macromolecular resonances is more complicated and depends on the magnetic field strength, the editing pulse shape and duration and the specific editing strategy employed. The macromolecular resonances of importance are M4 at 1.72 ppm and M7 at 3.00 ppm with a scalar coupling constant  $J_{\text{M4-M7}} = 7.3$  Hz (see Figure 8.8). The echo-time of the J-difference editing sequence is dictated by  $1/(2J)$  which equals 68 ms. With two spatially selective  $180^\circ$  pulses and a number of magnetic field crusher gradients within the total echo-time, a realistic maximum duration of the two editing pulses is circa 20 ms. Because the spectral selectivity needs to be as high as possible, most J-difference editing sequences employ Gaussian  $180^\circ$  pulses because Gaussian pulses have among the lowest product of bandwidth and pulse length (i.e. R-value, see

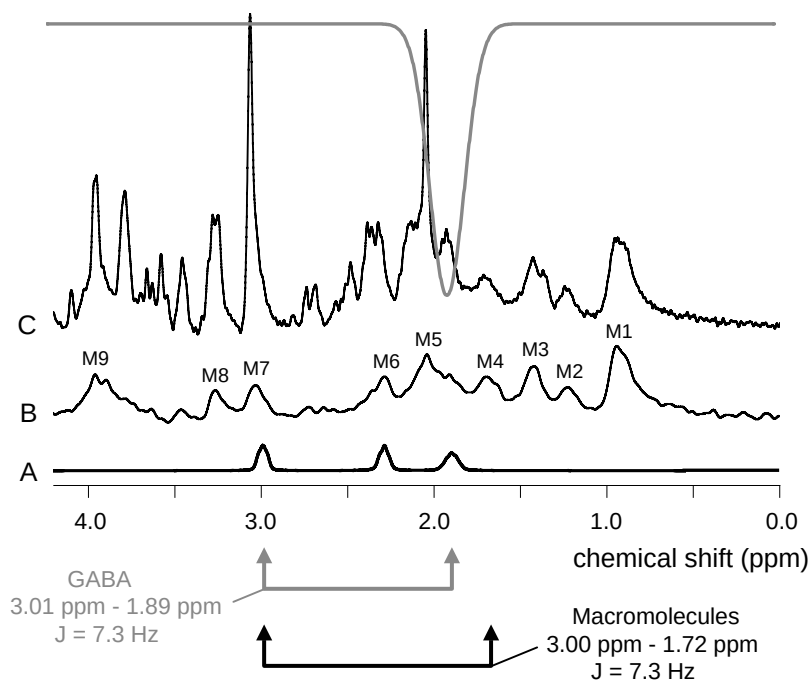


**Figure 8.8** (A) *In vitro*  $^1\text{H}$  NMR spectrum of GABA showing the three resonances relative to (B) macromolecular and (C) other proton NMR resonances at 9.4 T. The macromolecular NMR spectrum in (B) was obtained with a double inversion recovery ( $TR/TI_1/TI_2 = 3250/2100/630$  ms) which leads to a selective ‘nulling’ of the metabolite signals. The close proximity of the GABA-H3 and macromolecular M4 resonances can lead to co-editing of the macromolecular M7 resonance, depending on the bandwidth of the selective refocusing pulses.

Section 5.3). A 20 ms Gaussian  $180^\circ$  pulse (truncated at 1 %) will have a full bandwidth at half maximum of 75 Hz. Given that the frequency separation between GABA-H3 and M4 is only 0.17 ppm (= 11 Hz, 29 Hz and 68 Hz at 1.5 T, 4.0 T and 9.4 T, respectively), the editing pulses will certainly affect M4 at low magnetic fields, leading to partial co-editing of M7 underlying the GABA-H4 resonance. Three strategies to account for or minimize the contribution of macromolecules are in common use. Firstly, the nonselective experiment can be modified to apply frequency selective  $180^\circ$  pulses at a frequency mirrored around the M4 resonance (i.e. at  $1.72 - 0.17 = 1.55$  ppm). The M4 will be affected in both experiments by the symmetric editing pulses, but because the effect is identical in both experiments the M7 resonance will be subtracted from the edited spectrum [18]. While this method works well at medium to high magnetic field strengths, it may not perform satisfactorily at lower magnetic fields due to asymmetry in the M4 resonance.

Secondly, the macromolecular contribution can be minimized by utilizing the large  $T_1$  difference between metabolites and macromolecules [19–21]. Applying a nonselective inversion pulse prior to excitation will ‘null’ the longitudinal magnetization of macromolecules when the recovery delay is adjusted to  $T_{1,M7}\ln(2)$ . The metabolites, with a much

longer  $T_1$  relaxation constant, have only partially recovered and can be excited and detected without macromolecule contamination. While this is a simple and effective method, it does not allow for absolute quantification of GABA unless the GABA  $T_1$  relaxation constant is known. As an alternative to macromolecule nulling, the recovery delay can also be adjusted to achieve metabolite nulling. Due to the larger spread of metabolite  $T_1$  relaxation times, a double inversion recovery typically gives better metabolite suppression [21]. The observed macromolecular resonances can then be observed, quantified and subtracted from the edited GABA resonance.

A final consideration for successful *in vivo* spectral editing is system and subject stability. All MR systems have a basic magnetic field drift by which the magnetic field slowly decreases over time, typically less than  $10 \text{ Hz h}^{-1}$ . In addition, ultra-long  $B_0$  eddy currents and heating of passive shims can also lead to temporal magnetic field variations [22]. Besides system-related variations, subject motion can introduce temporal alterations of the magnetic field. The main requirement for successful J-difference editing is spectral stability between the two acquisitions. Any variation can potentially lead to incomplete subtraction of overlapping resonances, resulting in an overestimation of the edited resonance intensity. For most applications, significant signal averaging (e.g.  $N$  averages) is required to achieve sufficient SNR of the edited resonances. Rather than acquiring two spectra with  $N/2$  averages each, it is preferable to acquire  $N$  spectra of 1 average each. This allows frequency alignment of the spectra before summation, thereby significantly reducing the effects of magnetic field drifts on the final edited spectrum. In the practical example shown in Figure 8.9, ignoring magnetic field drifts resulted in a circa 50 % overestimation of the edited GABA resonance.

## 8.5 Multiple Quantum Coherence Editing

J-difference editing as detailed in the previous section is a valuable and popular editing method that performs well over a wide range of conditions. However, the requirement for two separate acquisitions is not always desirable and as a result a wide range of spectral editing methods have been developed, including polarization transfer [23–32], longitudinal scalar-order-based editing [33–35], Hartmann–Hahn transfer [36, 37] and MQC-based editing [38–45]. While each editing method has its own merits, the present discussion will be limited to MQC-based spectral editing since these methods achieve single scan editing with excellent water suppression.

Figure 8.10 shows a number of MQC-based spectral editing sequences. All methods start with a general preparation block consisting of  $90^\circ - t_1/2 - 180^\circ - t_1/2 - 90^\circ$  which, depending on the phase of the second  $90^\circ$  pulse generates MQCs or longitudinal scalar order or achieves polarization transfer. Using the product operator formalism, the density matrix for an IS spin system immediately prior to the second  $90^\circ$  pulse (ignoring relaxation) is given by:

$$\sigma(t_1) = I_y \cos(\pi J_{IS} t_1) - 2I_x S_z \sin(\pi J_{IS} t_1) \quad (8.13)$$

which for  $t_1 = 1/(2J_{IS})$  reduces to:

$$\sigma(t_1) = -2I_x S_z \quad (8.14)$$

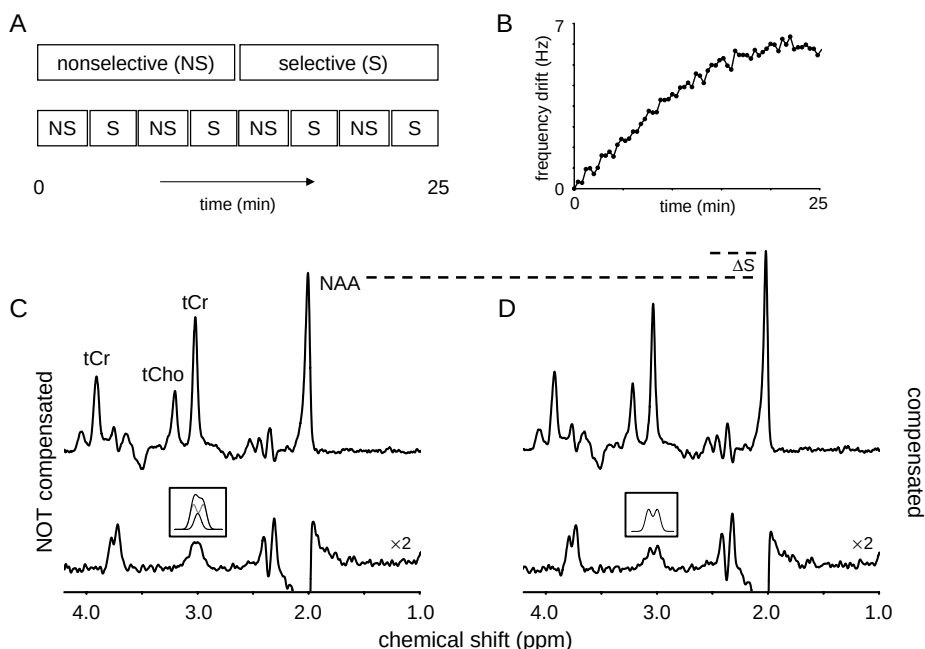


**Figure 8.9** (A) When averaging over longer time spans, the effect of frequency drifts can be minimized by acquiring and storing data in smaller blocks (bottom). (B) Exemplary frequency drift on rat brain in vivo at 9.4 T over a time span of 25 min. (C) When *J*-difference editing data are acquired in large blocks (A, top), frequency drifts lead to line broadening and incomplete signal subtraction. The observed signal at  $\sim 3.0$  ppm is readily modeled as edited GABA-H4 signal superimposed on a contamination from the overlapping creatine resonance. (D) Acquisition of *J*-difference editing data in small blocks allows post-acquisition frequency drift correction, leading to narrower resonance lines and improved editing performance.

As will be discussed in Section 8.7, polarization transfer ( $-2I_xS_z \rightarrow 2I_xS_x$ ) will occur when the second  $90^\circ$  pulse is applied along the *y* axis. Longitudinal scalar order ( $2I_zS_z$ ) will be generated when the second  $90^\circ$  pulse is selective for spin *I* and applied along the *y* axis. MQCs are generated when the second  $90^\circ$  pulse is applied along the  $-x$  axis, transforming the density matrix into:

$$\sigma(t_1+) = -2I_xS_y \quad (8.15)$$

While not obvious from product operators in a Cartesian basis, the density matrix in Equation (8.15) represents the sum of zero (ZQCs) and double quantum coherences (DQCs). Expressing the density matrix in terms of shift operators or spherical tensor operators has the advantage that each operator is uniquely associated with a particular coherence level or coherence order of the spin system. The Cartesian components of angular momentum operators can be transformed to shift operator components according to:

$$I^+ = I_x + iI_y \quad (8.16)$$

$$I^- = I_x - iI_y \quad (8.17)$$

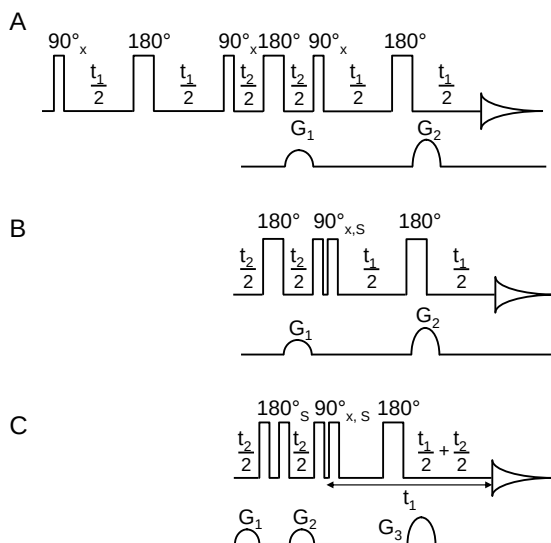


**Figure 8.10** MQC editing methods for (A) the detection and (B) the enhanced detection of signal arising from DQCs during  $t_2$  and (C) the detection of signal from both ZQCs and DQCs during  $t_2$ . For (B) and (C) the preparation part (i.e. first three pulses) is identical to that shown in (A). For (A) and (B) the gradient combinations must obey  $G_2 = 2G_1$ , whereas (C) requires the gradient combination  $G_3 = 2G_1 = 2G_2$ .

Substituting Equations (8.16) and (8.17) into Equation (8.15) gives:

$$\sigma(t_1+) = \frac{i}{2} (I^+S^+ - I^+S^- + I^-S^+ - I^-S^-) \quad (8.18)$$

Since the total coherence order is equal to the sum of the coherence levels of the individual shift operators, it is immediately obvious that Equation (8.18) is composed of ZQCs ( $I^+S^-$  and  $I^-S^+$ ) and DQCs ( $I^+S^+$  and  $I^-S^-$ ). Following the evolution delay  $t_2$  and the magnetic field gradient  $G_1$  the density operator is given by:

$$\sigma(t_1 + t_2) = \frac{i}{2} (I^+S^+e^{-2i\phi_1} - I^+S^- + I^-S^+ - I^-S^-e^{2i\phi_1}) \quad (8.19)$$

where

$$\phi_1 = \gamma r \int_0^{T_1} G_1(t) dt$$

is the phase accumulation due to frequency offset and the magnetic field gradient  $G_1$  of length  $T_1$ . Note that the homonuclear ZQCs are not affected by the magnetic field gradient, while DQCs are dephased by twice the amount of regular transverse magnetization. Further note that there is no scalar coupling evolution during the  $t_2$  time period. After the MQCs have been converted to single quantum coherences by the third  $90^\circ$  pulse, a second magnetic



field gradient  $G_2$  is applied resulting in a density operator at time  $2t_1 + t_2$  given by:

$$\begin{aligned} \sigma(2t_1 + t_2) = & \frac{i}{8} (I^+ e^{+i(2\phi_1 - \phi_2)} - I^- e^{-i(2\phi_1 - \phi_2)}) + \frac{i}{8} (I^+ e^{-i(2\phi_1 + \phi_2)} - I^- e^{+i(2\phi_1 + \phi_2)}) \\ & + \frac{i}{4} (I^+ e^{-i\phi_2} - I^- e^{+i\phi_2}) \end{aligned} \quad (8.20)$$

In order to understand which coherences can be detected for a particular  $G_1$  and  $G_2$  gradient combination, it is convenient to introduce the coherence transfer pathway formalism. Recall that regular magnetization was associated with an energy level difference corresponding to a change in spin quantum number  $\Delta m = \pm 1$ . As a result, regular transverse magnetization is also referred to as SQC or coherence of order 1. It has two associated coherence levels  $\pm p$ , depending on whether  $I^+$  or  $I^-$  is detected [see also Equations (8.16) and (8.17)]. Double and triple quantum coherences have coherence orders of 2 and 3, respectively. ZQC corresponds to a transition in which the spin quantum number does not change, making the coherence order 0. Even though longitudinal magnetization is not a coherence, it is often assigned coherence order 0, also because it has some properties in common with ZQC. The detectable magnetization is usually assigned coherence level  $-1$ , since NMR phase-sensitive quadrature detection is obtained as  $I^- = I_x - iI_y$ . Alternatively,  $I^+$  could be detected.

Returning now to Equation (8.20) it follows that for  $G_2 = 0$ , the pathway  $\mathbf{p}(+1, -1, 0, +1, -1)$  (i.e. the ZQCs) is refocused, while  $G_2 = \pm 2G_1$  refocuses the pathway  $\mathbf{p}(+1, -1, \pm 2, +1, -1)$  (i.e. the DQCs), where the coherence orders refer to the coherence levels during the delays ( $t_1/2, t_1/2, t_2, t_1/2, t_1/2$ ) as shown in Figure 8.10.

The result indicates that the intensities of the ZQC and DQC edited signals are only 50 % and 25 % of the total observed magnetization, respectively. 50 % signal reduction is caused by the conversion of SQCs into 50 % ZQCs and 50 % DQCs. For DQC edited signals an additional 50 % signal reduction is observed when gradient pulses are applied, since only one of the two possible coherence transfer pathways is refocused. Since homonuclear ZQCs are not affected by the magnetic field gradients, signals due to both coherence pathways are preserved during  $t_2$ . Note that ZQC based editing has the disadvantage that signal from uncoupled spins (e.g. water) is not suppressed. Coherences of uncoupled spins are along the longitudinal axis during the multiple quantum evolution period  $t_2$ , and therefore they do not experience the magnetic field gradient pulse  $G_1$ . Because no second gradient pulse  $G_2$  is required for the selection of homonuclear ZQCs, unwanted resonances are not dephased.

Several groups [42, 43] have described how the intensity of the DQC signal can be doubled. This is done by using a frequency selective excitation RF pulse on the I spin to convert the DQCs into antiphase SQCs (Figure 8.10B). Application of the second gradient results in:

$$\sigma(2t_1 + t_2) = \frac{i}{4} (I^+ e^{+i(2\phi_1 - \phi_2)} - I^- e^{-i(2\phi_1 - \phi_2)}) + \frac{i}{4} (I^+ e^{-i\phi_2} - I^- e^{+i\phi_2}) \quad (8.21)$$

The selective 'read' pulse therefore results in a total signal recovery of 50 %.

Trimble *et al.* [42] have described a method by which the signal intensity can be increased to 100 % of the total observable magnetization. This is achieved by simultaneously 'encoding' the ZQCs and DQCs during  $t_2$ . The pulse sequence is shown in Figure 8.10C. Up to the point of the selective  $180^\circ$  pulse in the middle of the  $t_2$  period, the sequence

is identical to those in Figure 8.10A and B and therefore the density matrix is given by Equation (8.19). The selective  $180^\circ$  pulse on spin I interconverts ZQCs and DQCs (e.g.  $I^+S^+ \rightarrow I^-S^+$ ). Application of a second magnetic field gradient dephases the generated DQCs, while the ZQCs (originating from the already dephased DQCs) remain unaffected. At the end of the  $t_2$  period both ZQCs and DQCs are completely dephased. The density operator prior to the selective read pulse on the I spin is given by:

$$\begin{aligned} \sigma(t_1 + t_2) = & \frac{i}{2} (I^+S^-e^{-2i\phi_1} - I^+S^+e^{-2i\phi_2}) e^{-i\omega_1 t_2} \\ & + \frac{i}{2} (I^-S^-e^{+2i\phi_2} - I^-S^+e^{+2i\phi_1}) e^{+i\omega_1 t_2} \end{aligned} \quad (8.22)$$

Application of a third magnetic field gradient gives the density operator at the end of the sequence:

$$\begin{aligned} \sigma(2t_1 + t_2) = & \frac{i}{4} I^+e^{+i(2\phi_1 - \phi_3)} + \frac{i}{4} I^+e^{+i(2\phi_2 - \phi_3)} \\ & - \frac{i}{4} I^-e^{-i(2\phi_1 - \phi_3)} - \frac{i}{4} I^-e^{-i(2\phi_2 - \phi_3)} \end{aligned} \quad (8.23)$$

Therefore by choosing  $G_1 = G_2 = 0.5G_3$ , complete signal recovery is obtained. The chemical shift evolution of spin I during  $t_2$  [as can be seen from Equation (8.22)] can be refocused by shifting the last  $180^\circ$  pulse over  $t_2/2$  towards the selective  $90^\circ$  pulse. Figure 8.11 shows a typical *in vivo* application of homonuclear, gradient-enhanced MQC-based spectral editing (geMQC) to selectively observe lactate.

In principle, MQC-based spectral editing sequences are not limited to ZQC and DQCs only, but can be constructed to selectively observe higher-order coherences. For instance, Wilman and Allen [46] have used triple quantum coherence filtering to selectively observe GABA (a  $A_2M_2X_2$  spin system) in rat brain extracts. However, in general it can be stated that spectral editing aimed at the detection of higher order coherences is prone to increased signal loss, since it is almost impossible to refocus all coherence pathways.

MQC-based spectral editing can, besides the methods shown in Figure 8.10, be achieved with a wide variety of pulse sequences (e.g. [42–45]). Each method will have considerations concerning spectral selectivity and co-editing, signal recovery and sensitivity to experimental imperfections. However, all MQC-based sequences are based on the same principle in that scalar-coupled spins can form MQCs which are dephased by magnetic field gradients. After conversion to SQCs, the magnetization is refocused by magnetic field gradients of appropriate amplitude. Uncoupled spins can not form MQCs, such that the combination of magnetic field gradients will lead to dephasing of transverse magnetization.

While MQC-based spectral editing methods are typically very robust and give excellent suppression of unwanted resonances, they have their own drawbacks, typically in terms of signal quantification. Since all uncoupled resonances are dephased in the editing process, they can not be used as an internal reference unless a separate, nonedited experiment is performed. Furthermore, frequency drift correction as outlined in Figure 8.9 may be difficult as the single scan SNR of the edited signal is typically insufficient. Fortunately, the performance of MQC-based spectral editing is not sensitive to small frequency drifts.

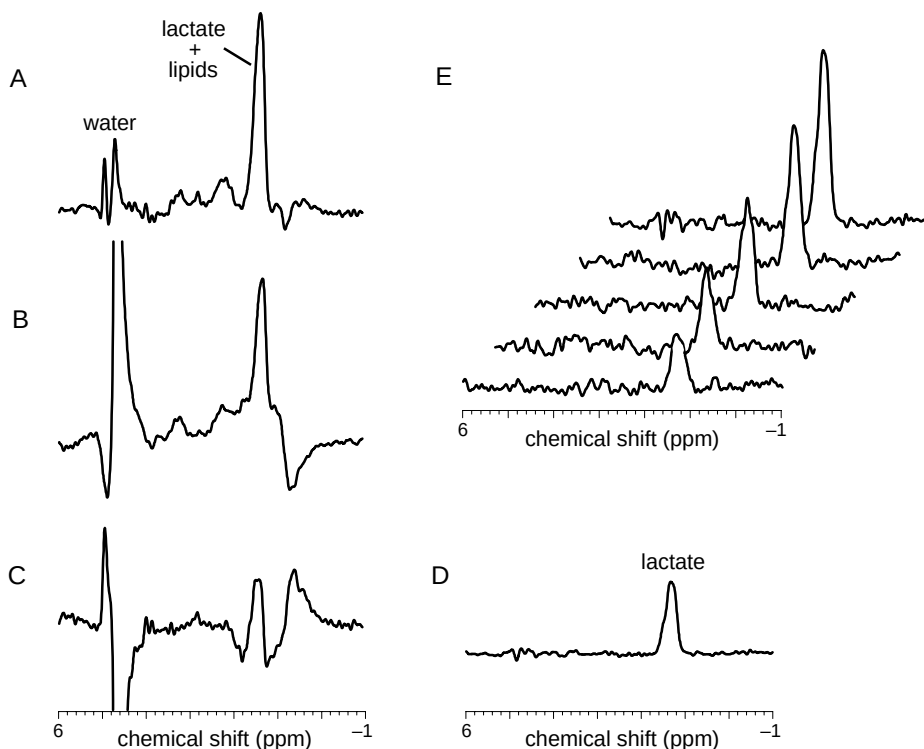


**Figure 8.11** Spectral editing of lactate in a RG2 glioma implanted on the flank of a rat *in vivo*. (A) Localized  $^1\text{H}$  NMR spectra (4.7 T, TR/TE = 3000/144 ms, 512  $\mu\text{l}$ ) with selective refocusing of the lactate-H2 resonance at 4.10 ppm, leading to a positive lactate-H3 resonance at 1.31 ppm, overlapping with signals from mobile lipids. (B)  $^1\text{H}$  NMR spectra without selective refocusing, such that lactate-H3 appears negative. The large positive resonance at  $\sim 1.3$  ppm indicates the presence of large amounts of lipids. (C) Difference of (A) and (B). Lactate is visible, but the spectrum is heavily contaminated with lipids, which is probably caused by motion of the tumor by breathing. (D) geMQC edited  $^1\text{H}$  NMR spectrum of lactate. Note the excellent water suppression and clean selection of lactate. (E) The unambiguous detection of lactate allows one to perform more sophisticated experiments, like lactate diffusion measurements. Diffusion sensitization was achieved during an additional (selective) spin echo following the geMQC spectral editing sequence. Diffusion sensitizing gradients generated  $b$ -values of 171, 682, 1538, 2734 and 4272  $\text{s mm}^{-2}$  ( $\delta = 5$  ms,  $\Delta = 35$  ms), respectively, resulting in a calculated ADC of  $0.24 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$ .

## 8.6 Heteronuclear Spectral Editing

As detailed in Chapter 3, the differentiation of  $^{13}\text{C}$ -labeled compounds from their unlabeled counterparts is important for the measurement of metabolic fluxes *in vivo*. Direct  $^{13}\text{C}$  NMR spectroscopy can reliably detect the  $^{13}\text{C}$ -labeled compounds, but has an inherently low NMR sensitivity. While this can be improved through the use of heteronuclear polarization transfer methods, as will be described in Section 8.7, the sensitivity of

proton detection will typically be higher. Furthermore, proton NMR allows the detection of  $^{13}\text{C}$ -labeled metabolites, as well as the total ( $^{12}\text{C} + ^{13}\text{C}$ ) metabolite pools, leading to a straightforward determination of  $^{13}\text{C}$  fractional enrichments. However, especially in the stages of low fractional enrichment, the proton resonances from  $^{13}\text{C}$ -labeled compounds are overwhelmed by resonances from unlabeled compounds. Separation of these resonances is a task ideally suited for heteronuclear spectral editing techniques that utilizes the heteronuclear scalar coupling between protons and  $^{13}\text{C}$  atoms.

Heteronuclear spectral editing methods are almost identical to the homonuclear techniques described in the previous sections, with several noticeable exceptions. Figure 8.12A shows a basic heteronuclear J-difference editing sequence. The application of a  $^1\text{H}$  RF pulse is automatically selective for a heteronuclear spin system, as the bandwidth of a RF pulse is typically orders of magnitude smaller than the frequency separation between protons and carbon-13 nuclei. Therefore, in the absence of RF pulses on the carbon-13 channel, heteronuclear scalar coupling is always refocused and the proton resonances from  $^{13}\text{C}$ -labeled metabolites appear in phase with the rest of the  $^1\text{H}$  NMR spectrum. A nonselective experiment is created when in a second, separate experiment a  $^{13}\text{C}$   $180^\circ$  RF pulse is applied in conjunction with a  $^1\text{H}$   $180^\circ$  RF pulse. In this case, the phase of the proton magnetization is reset, while the  $^{13}\text{C}$  spin populations are inverted, leading to evolution of the scalar coupling according to Equation (8.5). When the echo time TE is chosen as  $1/J_{\text{IS}}$ , where  $J_{\text{IS}}$  is the heteronuclear scalar coupling, the proton resonances from  $^{13}\text{C}$ -labeled metabolites appear inverted relative to resonances from protons attached to carbon-12 nuclei. Therefore, subtraction of the two spectra will result in the selective observation of protons attached to carbon-13 nuclei. Note that since the heteronuclear scalar coupling constant  $J_{\text{IS}}$  is on the order of 125–140 Hz, the echo-time of the proton-observed, carbon-edited (POCE) sequence is only 7–8 ms. In analogy to homonuclear spectral editing sequences, the POCE sequence shown in Figure 8.12A requires spatial localization and water suppression to allow meaningful spectral editing *in vivo*. Figure 8.12B shows a practical POCE or  $^1\text{H}$ - $^{13}\text{C}$  NMR sequence based on the LASER localization method (see Section 6.2.7). The carbon-13  $180^\circ$  inversion pulse should not be executed during a magnetic field gradient, as this could lead to incomplete inversion due to severe chemical shift displacement, thus leading to the specific timings shown in Figure 8.12B (see also Exercise 8.6). Besides spatial localization and water suppression,  $^1\text{H}$ - $^{13}\text{C}$  NMR sequences are almost always executed with heteronuclear broadband decoupling during acquisition in order to increase the sensitivity and decrease the spectral complexity. Details on heteronuclear decoupling can be found in Section 8.9.

Figure 8.13 shows a typical  $^1\text{H}$ - $^{13}\text{C}$ -NMR spectrum acquired from rat brain at 9.4 T, circa 90 min following the onset of  $[1,6-^{13}\text{C}_2]\text{glucose}$  infusion. The total proton spectrum, acquired in the absence of a  $^{13}\text{C}$   $180^\circ$  inversion pulse, allows the detection and quantification of cerebral metabolites, like glutamate and glutamine. The edited difference spectrum exhibits large resonances from  $[4-^{13}\text{C}]\text{glutamate}$  and  $[4-^{13}\text{C}]\text{glutamine}$ , in close analogy to the spectra acquired with direct  $^{13}\text{C}$  NMR (Figures 3.31 and 3.32). Noticeable differences are that the proton spectrum has a greatly increased sensitivity, albeit a decrease in spectral resolution. However, at higher magnetic field ( $>7$  T), the spectral resolution is sufficient to separate and quantify resonances from the most important molecules, like glutamate, glutamine, GABA and lactate. Further note, that both the  $^1\text{H}$  and  $^1\text{H}$ - $^{13}\text{C}$  NMR spectra hold resonances from glucose and  $[1-^{13}\text{C}]\text{glucose}$ , respectively, thereby allowing the direct

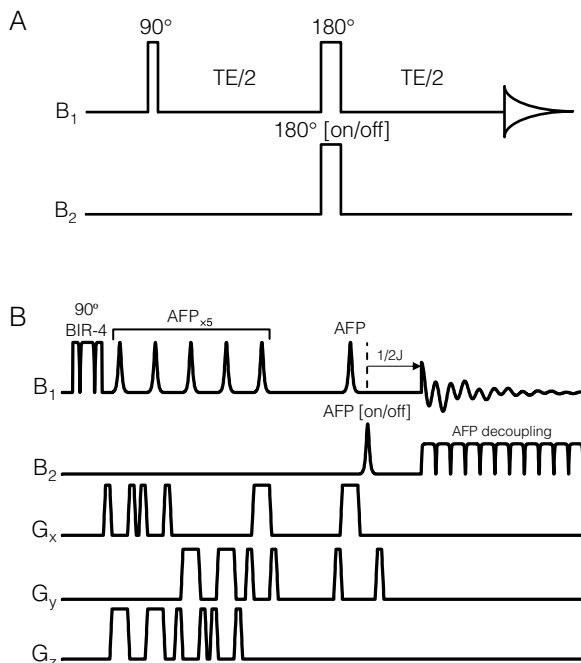


**Figure 8.12** (A) Basic heteronuclear J-difference editing, or proton-observed, carbon-edited (POCE) sequence and (B) 3D localized POCE sequence based on the adiabatic LASER method extended with adiabatic editing and decoupling.

detection of cerebral glucose turnover and thus bypassing possible assumptions underlying the blood-to-brain transport.

Besides the J-difference method shown in Figures 8.12 and 8.13, essentially all homonuclear spectral editing methods have a heteronuclear counterpart including those based on MQCs [47–50]. Heteronuclear methods based on polarization transfer could also be classified as spectral editing techniques. However, since the prime purpose of polarization transfer techniques is sensitivity enhancement over direct heteronuclear NMR detection they will be discussed separately in the next section.

## 8.7 Polarization Transfer – INEPT and DEPT

Polarization transfer methods are normally not classified as spectral editing methods in the *in vivo* NMR community. Instead these methods are used to enhance the sensitivity of the insensitive (low-abundance) nucleus in a heteronuclear NMR experiment [51–56]. The simplest heteronuclear polarization transfer sequence is shown in Figure 8.14A and is referred to as INEPT (insensitive nuclei enhanced by polarization transfer) [51]. Consider (for simplicity) a two-spin system IS, where I and S refer to the sensitive (i.e.  $^1\text{H}$ ) and insensitive (e.g.  $^{13}\text{C}$ ) nuclei, respectively. The equilibrium density operator, expressed in

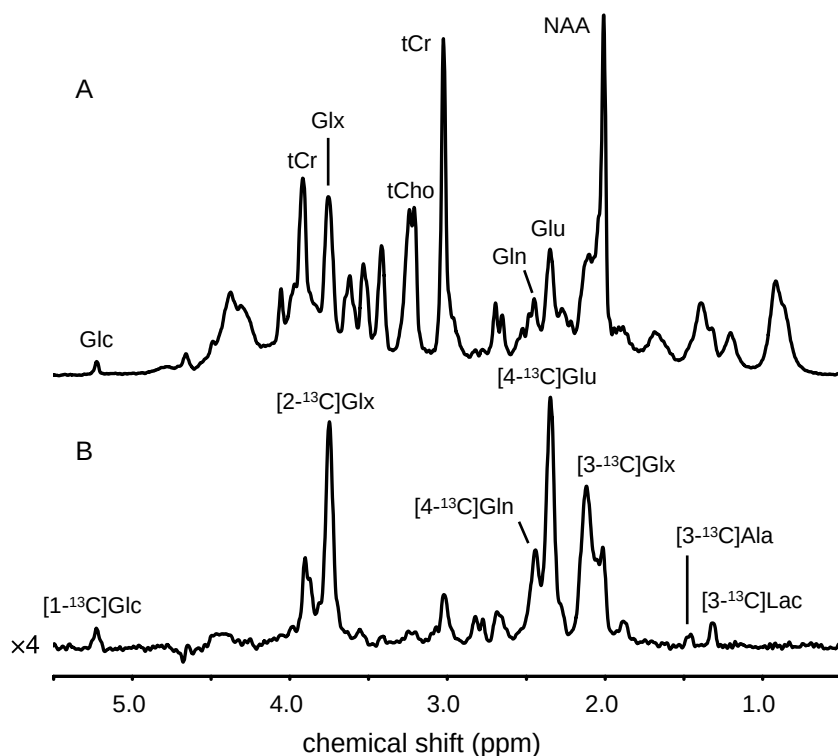


**Figure 8.13** (A)  $^1\text{H}$  and (B)  $^1\text{H}$ - $^{13}\text{C}$  edited NMR spectra acquired from rat brain ( $180\ \mu\text{l}$  volume,  $\text{TR}/\text{TE} = 4000/8.5\ \text{ms}$ ,  $\text{NA} = 512$ ) at 9.4 T. Data acquisition began circa 90 min after the start of an intravenous  $[1,6\text{-}^{13}\text{C}_2]\text{glucose}$  infusion. Adiabatic decoupling based on AFPST4 pulses ( $T = 1.2\ \text{ms}$ ,  $R = 10$ ) was applied during the entire acquisition period (102 ms) with  $B_{2\text{max}} = 1800\ \text{Hz}$ . The  $^{13}\text{C}$  labeling patterns in the  $^1\text{H}$ - $^{13}\text{C}$  NMR spectrum are similar to those obtained during direct  $^{13}\text{C}$  NMR detection (compare to Figure 3.31).

terms of spin operators, is given by:

$$\sigma(0) = I_z + \left( \frac{\gamma_S}{\gamma_I} \right) S_z \quad (8.24)$$

where  $\gamma_I$  and  $\gamma_S$  are the gyromagnetic ratios of the I and S spins, respectively. After excitation (which is automatically selective for spin I) along the positive x axis, the density operator is equal to:

$$\sigma(+) = -I_y + \left( \frac{\gamma_S}{\gamma_I} \right) S_z \quad (8.25)$$

During the following delay  $t_1$ , the coherence  $I_y$  evolves under chemical shifts and scalar coupling. The simultaneous application of  $180^\circ$  pulses on both the I and S spins, refocuses chemical shifts and magnetic field inhomogeneity, but not scalar coupling evolution, such

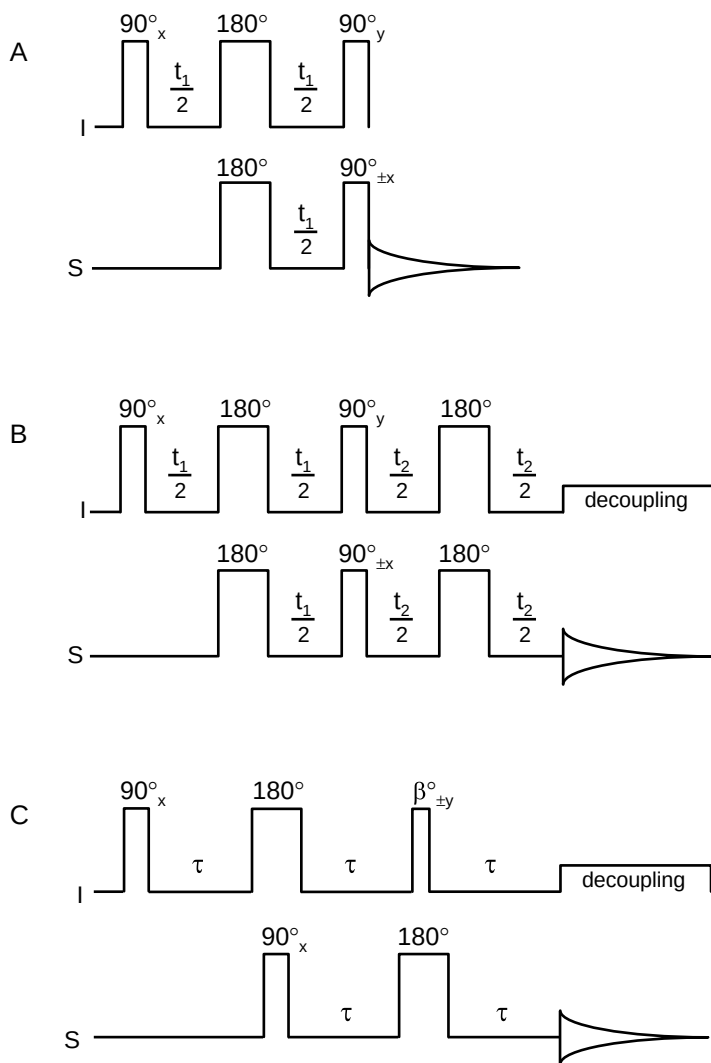


**Figure 8.14** Heteronuclear polarization transfer pulse sequences. (A) INEPT, (B) refocused INEPT and (C) DEPT. Refocused INEPT and DEPT allow decoupling during signal acquisition.

that the density operator prior to the second  $90^\circ$  pulse is given by:

$$\sigma(t_1) = -I_y \cos \pi J_{IS} t_1 + 2I_x S_z \sin \pi J_{IS} t_1 - \left( \frac{\gamma_S}{\gamma_I} \right) S_z \quad (8.26)$$

Application of a  $90^\circ_y$   $^1\text{H}$  (I) pulse and a  $90^\circ_x$   $^{13}\text{C}$  (S) pulse, converts Equation (8.26) to:

$$\sigma(t_1+) = -I_y \cos \pi J_{IS} t_1 + 2I_z S_y \sin \pi J_{IS} t_1 + \left( \frac{\gamma_S}{\gamma_I} \right) S_y \quad (8.27)$$

The final term represents transverse  $^{13}\text{C}$  magnetization generated by direct excitation (i.e. not generated through polarization transfer). Since this term originates from the entire sample (localization is typically performed on the  $^1\text{H}$  channel) and is influenced by  $^{13}\text{C}$   $T_1$  relaxation it is generally desirable to remove this term. By performing a two-step phase cycle on the last  $90^\circ$   $^{13}\text{C}$  pulse (e.g.  $90^\circ_x$  and  $90^\circ_{-x}$ ) and adding the two experiments, only one term survives that is given by:

$$\sigma(t_1+) = 2I_zS_y \sin\pi J_{IS}t_1 \quad (8.28)$$

which reduces to  $2I_zS_y$  for  $t_1 = 1/2J$ . Therefore, the acquired signal is enhanced by a factor  $(\gamma_I/\gamma_S)$  (3.976 for  $^1\text{H}/^{13}\text{C}$ ) with respect to the native ( $^{13}\text{C}$ ) magnetization of  $(\gamma_S/\gamma_I)S_y$ . And since the acquired signal depends on the transfer of coherence, rather than excited magnetization from a thermal equilibrium situation, the repetition time of the experiment is dominated by the  $T_1$  relaxation time of protons, which is normally shorter than the  $T_1$  relaxation time of the insensitive spin. The main disadvantage of the basic INEPT sequence of Figure 8.14A is that the S spin appears in anti-phase (Figure 8.15A). This excludes the use of proton decoupling during  $^{13}\text{C}$  acquisition, a technique commonly employed to further enhance the signal intensity of  $^{13}\text{C}$  resonances (see Section 8.9).

A modified version of INEPT, commonly referred to as refocused INEPT, delays the acquisition of the  $^{13}\text{C}$  FID such that the anti-phase magnetization has evolved to in-phase magnetization (Figures 8.14B and 8.15B). For spin systems containing a range of scalar coupling constants and for  $I_2S$  and  $I_3S$  spin systems, perfect refocusing of scalar coupling evolution into in-phase components is not possible. For  $I_NS$  ( $N = 1, 2$  or  $3$ ) spin systems the amount of in-phase S signal at the start of acquisition is given by:

$$\sigma_{IS}(t_1 + t_2) = S_x \sin\pi J_{IS}t_1 \sin\pi J_{IS}t_2 \quad (8.29)$$

$$\sigma_{I_2S}(t_1 + t_2) = 2S_x \sin\pi J_{IS}t_1 \sin\pi J_{IS}t_2 \cos\pi J_{IS}t_2 \quad (8.30)$$

$$\sigma_{I_3S}(t_1 + t_2) = 3S_x \sin\pi J_{IS}t_1 \sin\pi J_{IS}t_2 \cos^2\pi J_{IS}t_2 \quad (8.31)$$

or for a general  $I_NS$  spin system:

$$\sigma_{I_NS}(t_1 + t_2) = NS_x \sin\pi J_{IS}t_1 \sin\pi J_{IS}t_2 \cos^{N-1}\pi J_{IS}t_2 \quad (8.32)$$

For all  $N$ , maximum polarization transfer efficiency is achieved when  $t_1 = 1/(2J)$ . However, the optimal  $t_2$  delay varies for different  $N$  and is given by  $t_2 = 1/(2J)$ ,  $1/(4J)$  and  $\sim 1/(5J)$  for  $N = 1, 2$  and  $3$ , respectively. While these delays maximize the amount of detectable (in-phase) signal, there will nevertheless be large amounts of anti-phase coherences [for  $I_2S$  and  $I_3S$  spin systems and for  $IS$  spin systems when  $t_2 \neq 1/(2J)$ ], which distorts the phase of the observed multiplets as demonstrated in Figure 8.15B. These antiphase coherences are readily removed by a  $90^\circ_x(^1\text{H})$  ‘purging’ pulse immediately prior to acquisition, a sequence modification known as refocused INEPT<sup>+</sup>. The effect of the purging pulse is that all antiphase coherences [of the form  $(I_z)_NS_x$ ] are being converted to unobservable MQCs [of the form  $(I_y)_NS_x$ ], leaving only the in-phase  $S_x$  coherences (Figure 8.15C).

However, most *in vivo* applications of polarization transfer are executed with proton decoupling during acquisition in order to (1) simplify the  $^{13}\text{C}$  NMR spectrum and (2)



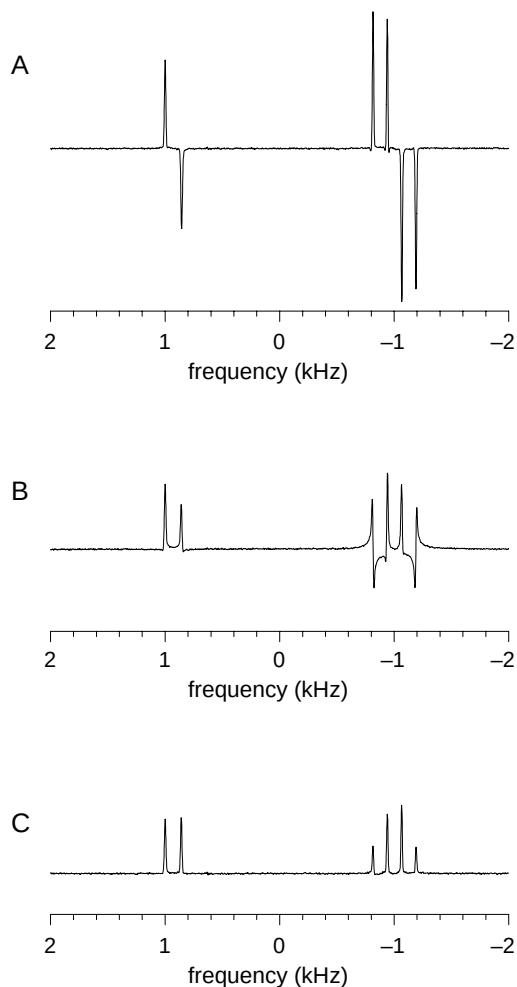


**Figure 8.15** Experimental  $^{13}\text{C}$  NMR spectra of 2-propanol ( $\text{CH}_3\text{CHOHCH}_3$ ) acquired with different polarization transfer sequences, namely (A) INEPT, (B) refocused INEPT and (C) INEPT $^+$ . All spectra were acquired with  $t_1 = 3.57$  ms ( $= 1/2J$ ). Spectra (B) and (C) were acquired with  $t_2 = 2.30$  ms ( $\sim 0.32/J$ ), which is suboptimal for either resonance. For the doublet resonance, 100 % signal recovery would have been obtained for  $t_2 = 3.57$  ms. Note the absence of dispersive components in (C) while present in (B).

increase the sensitivity. The proton decoupling sequence then automatically removes any antiphase coherences, thereby eliminating the need for a purging pulse.

An alternative to INEPT was proposed by Doddrell *et al.* [53] under the acronym DEPT (distortionless enhancement by polarization transfer). DEPT offers a fundamental improvement over INEPT in that the polarization transfer can be achieved without the generation of anti-phase coherences at the start of acquisition (for spin systems with equal scalar coupling constants). Therefore, a regular  $^{13}\text{C}$  quartet resonance with relative

intensities 1:3:3:1 will appear as an intensity-enhanced multiplet with the same relative intensities, as opposed to INEPT where the multiplet will appear as an anti-phase doublet of doublets (Figure 8.15A). The basic DEPT pulse sequence is shown in Figure 8.14C. Following excitation of the I spin, the density matrix for a simple IS spin system is given by:

$$\sigma(+) = -I_y + \left( \frac{\gamma_S}{\gamma_I} \right) S_z \quad (8.33)$$

The longitudinal magnetization of the S spin can be ignored, as it will be removed during a two-step phase cycle of the last pulse on the I spin. During the following delays, chemical shift evolution will be ignored, as it will be refocused for both spins at the start of acquisition. Therefore, following a delay  $\tau = 1/2J$ , the density matrix prior to the S spin excitation pulse is:

$$\sigma(\tau) = 2I_x S_z \quad (8.34)$$

Following S spin excitation, I spin refocusing and a subsequent delay  $\tau$ , during which no scalar coupling evolution occurs, the density matrix is represented by:

$$\sigma(2\tau) = 2I_x S_y \quad (8.35)$$

The final part of the polarization transfer occurs when the I spin  $\beta$  pulse converts the MQCs to S spin transverse magnetization. The S spin  $180^\circ$  pulse simply refocuses chemical shift evolution of the S spin. Following a delay  $\tau = 1/2J$ , the final density matrix at the start of acquisition is:

$$\sigma(3\tau) = \pm S_x \sin\beta \quad (8.36)$$

Where the  $\pm$  sign originates from whether the  $\beta$  pulse was applied along the  $+y$  or  $-y$  axis. Subtraction of the two experiments cancels out the magnetization coming directly from the S spin polarization. For  $I_2S$  and  $I_3S$  spin systems the observed signal is proportional to:

$$\sigma_{I_2S}(3\tau) = \pm S_x \sin\beta \cos\beta \quad (8.37)$$

$$\sigma_{I_3S}(3\tau) = \pm S_x \sin\beta \cos^2\beta \quad (8.38)$$

Maximum signal enhancements for IS,  $I_2S$  and  $I_3S$  spin systems therefore occur for  $\beta = \pi/2$ ,  $\pi/4$  and  $\sim\pi/5$ , respectively. For a 'mismatched' DEPT sequence (i.e. when  $\tau \neq 1/2J$  for some or all metabolites) phase anomalies will appear similar to those seen in refocused INEPT spectra. Several extensions to the basic DEPT sequence have been proposed, like DEPT<sup>+</sup> and DEPT<sup>++</sup>, to eliminate such phase anomalies [54].

For *in vivo*  $^{13}\text{C}$  NMR spectroscopy, the INEPT and DEPT methods are typically extended with ISIS localization, possibly in combination with outer volume suppression (OVS). Furthermore, because *in vivo* acquisitions are typically acquired with surface coils, both sequences are often executed with adiabatic RF pulses ([55,56], see also Section 5.7).

## 8.8 Sensitivity

In Chapter 1 it was shown that the thermal equilibrium magnetization  $M_0$  is proportional to  $\gamma^2 B_0$  [i.e. see Equation (1.27)]. One  $\gamma$  term originated from the intrinsic magnetic moment,

while another  $\gamma$  term (as well as  $B_0$ ) entered the equation due to the energy level difference between the two spin states. The corresponding electromotive force (emf) is, through the principle of reciprocity, given by  $\omega_0 M_0$ , making the final detected signal  $S$  proportional to  $\gamma^3 B_0^2$ . It is well-known that the noise voltage arising from conducting samples increases linearly with frequency [57]. In the case that all the noise originates from the sample, as is the case for an ideal probe and spectrometer, the relative sensitivity SNR is proportional to  $\gamma^2 B_0$ . When the relative sensitivity is expressed as SNR per (time)<sup>1/2</sup> the longitudinal relaxation and linewidths (or  $T_2^*$ ) must be taken into account, leading to:

$$\text{SNR} \propto \gamma^2 B_0 \sqrt{\frac{T_2^*}{T_1}} \quad (8.39)$$

Using Equation (8.39) the relative sensitivities of different nuclei can be calculated. For example, the relative sensitivity between  $^1\text{H}$  detection and  $^{13}\text{C}$  detection of the same compound can be expressed as:

$$\frac{\text{SNR}_\text{H}}{\text{SNR}_\text{C}} = \left(\frac{\gamma_\text{H}}{\gamma_\text{C}}\right)^2 \left(\frac{N_\text{H}}{N_\text{C} n\text{Oe}}\right) \left(\frac{T_2^*}{T_1}\right)_\text{H}^{1/2} \left(\frac{T_1}{T_2^*}\right)_\text{C}^{1/2} \quad (8.40)$$

where  $N_\text{H}$  and  $N_\text{C}$  are the number of nuclei observed by proton and carbon-13 NMR, respectively.  $n\text{Oe}$  is the nuclear Overhauser enhancement which, in the best case, is equal to  $(1 + \gamma_\text{H}/2\gamma_\text{C}) = 2.988$ . Equation (8.40) ignores noise contributions from the probe and spectrometer or sequence-specific parameters like echo-time TE.

Ignoring relaxation and  $n\text{Oe}$ , proton detection provides 16 times higher SNR than direct  $^{13}\text{C}$  detection for equal number of nuclei. Including realistic values for  $T_1$  and  $T_2^*$  at 9.4 T ( $T_{1\text{H}} = 1600$  ms,  $T_{2\text{H}}^* = 32$  ms,  $T_{1\text{C}} = 1200$  ms,  $T_{2\text{C}}^* = 65$  ms) reduces the SNR advantage to circa 10. The inclusion of  $n\text{Oe}$  reduces the SNR advantage of  $^1\text{H}$  detection to circa 3.5 for equal number of nuclei.

However, with most modern  $^{13}\text{C}$  NMR experiments, signal detection is performed with polarization transfer in which the  $^{13}\text{C}$  signal is enhanced by a factor  $(\gamma_\text{H}/\gamma_\text{C})$  (see Section 8.7). As a result, the SNR comparison between  $^1\text{H}$  detection and  $^{13}\text{C}$  detection becomes:

$$\frac{\text{SNR}_\text{H}}{\text{SNR}_\text{C}} = \left(\frac{\gamma_\text{H}}{\gamma_\text{C}}\right) \left(\frac{N_\text{H}}{N_\text{C}}\right) \left(\frac{T_{2\text{H}}^*}{T_{2\text{C}}^*}\right)^{1/2} \left(\frac{e^{-TE/T_{2\text{H}}}}{e^{-TE1/T_{2\text{H}}} e^{-TE2/T_{2\text{C}}}}\right) \quad (8.41)$$

Note that the proton  $T_1$  is eliminated from Equation (8.41) since both the direct  $^1\text{H}$  as well as the polarization transfer  $^{13}\text{C}$  NMR experiments are influenced by proton  $T_1$  relaxation. When assuming equal total echo-times of  $1/J$  ( $= 7.1$  ms for  $^1J_\text{CH} = 140$  Hz), proton detection gives 2.7 times higher SNR than carbon-13 detection by polarization transfer for equal number of nuclei. Further note that Equation (8.41) only holds for two-spin-systems. For more than two spins (i.e. more than one proton), the polarization transfer efficiency will be lower than 100 % (see Section 8.7) and the signal detected by direct  $^1\text{H}$  NMR will increase proportionally.

## 8.9 Broadband Decoupling

The purpose of decoupling is to remove the effects of heteronuclear scalar coupling from NMR spectra for two primary reasons, namely (1) spectral simplification and (2) increased sensitivity. Comparing Figures 8.13 and 8.16 illustrates the utility of broadband decoupling in  $^1\text{H}$ - $^{13}\text{C}$  NMR spectroscopy. In the absence of decoupling (Figure 8.16), the edited  $^1\text{H}$ - $^{13}\text{C}$  difference spectrum is dominated by doublet resonances as a result of splitting by the single bond heteronuclear scalar coupling. Heteronuclear scalar coupling over more than one chemical bond (see Table 2.5) leads to broadening of resonances. Applying broadband decoupling effectively merges the doublet resonances into a single resonance line, thereby increasing the sensitivity and simplifying the spectrum (Figure 8.13). Note that in the absence of decoupling, the total spectrum is now a sum of doublet resonances originating from the  $^{13}\text{C}$ -labeled metabolites in addition to the regular resonances from the unlabeled metabolites. While the different chemical shifts of proton resonances attached to  $^{12}\text{C}$  and  $^{13}\text{C}$  nuclei have been used to observe  $^{13}\text{C}$  label turnover without the use of a

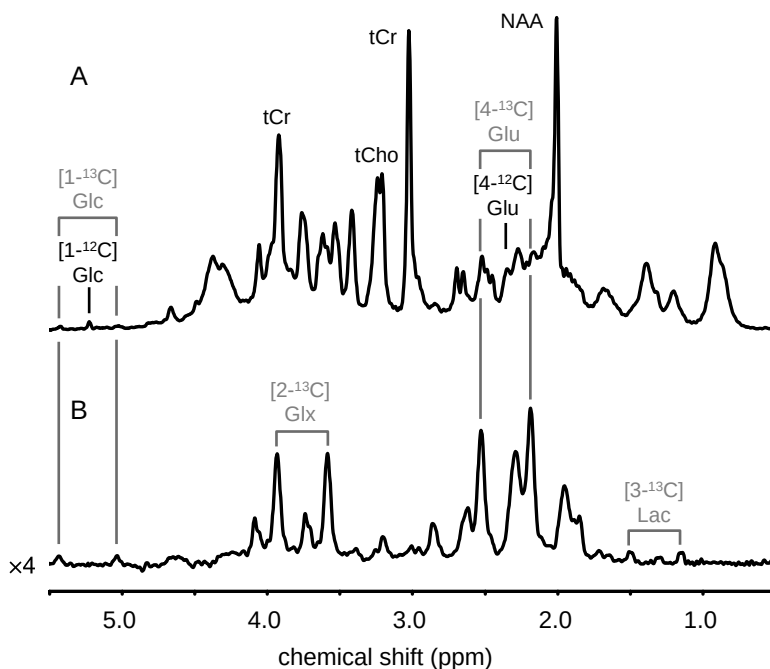


**Figure 8.16** (A)  $^1\text{H}$  and (B)  $^1\text{H}$ - $^{13}\text{C}$  edited NMR spectra acquired from rat brain ( $180\ \mu\text{l}$  volume,  $TR/TE = 4000/8.5\ \text{ms}$ ,  $NA = 512$ ) at 9.4 T without broadband decoupling during acquisition. Data acquisition began circa 90 min after the start of an intravenous  $[1,6-^{13}\text{C}_2]\text{glucose}$  infusion. Note that the fractional enrichment of  $\alpha\text{H1-glucose}$  can be directly obtained from the unedited  $^1\text{H}$  NMR spectrum. However, the presence of splittings due to heteronuclear scalar couplings generally leads to more complicated spectral patterns and a reduction of the obtainable SNR.

second decoupling channel [58], it is in general an undesirable complication that can be removed through broadband decoupling (compare Figures 8.13A and 8.16A).

The basic principle of decoupling is similar to that already discussed in Section 8.2 for homonuclear J-difference editing, namely that selective refocusing of one spin in a multi-spin system leads to an inhibition of scalar coupling involving that spin. Figure 8.17A and B show the theoretical implementation of this principle for heteronuclear NMR, in this case  $^1\text{H}$  detection with  $^{13}\text{C}$  decoupling, i.e.  $^1\text{H}\text{-}[^{13}\text{C}]$  NMR. On the proton channel no RF pulses are applied, such that the signal can be acquired while evolving under the effects of chemical shift, magnetic field inhomogeneity and homonuclear as well as heteronuclear scalar coupling. When a short, intense  $^{13}\text{C}$   $180^\circ$  inversion pulse is applied midway between the first and second acquisition points, the effects of heteronuclear scalar coupling will be completely refocused at the time of the second acquisition point. This principle can be continued by applying short, intense  $^{13}\text{C}$   $180^\circ$  inversion pulses in between all following acquisition points, such that even though heteronuclear scalar coupling evolution occurs during the dwell time  $\Delta\tau$ , it appears constant ('frozen') at the time of data acquisition. As a result, the Fourier transformation of a FID with constant heteronuclear scalar coupling evolution at all data points will result in a spectrum devoid of resonance splitting due to heteronuclear coupling. While theoretically sound, the approach depicted is experimentally not feasible because of RF power restrictions, both in terms of unrealistic RF peak power requirements and excessive RF power deposition (see Section 5.9). Fortunately, the typical heteronuclear scalar coupling evolution ( $^1J_{\text{CH}} = 120\text{--}170$  Hz) is much slower than the data acquisition sampling rate, such that scalar coupling evolution during one dwell time is very small. For example, for a spectral bandwidth of 5000 Hz and  $^1J_{\text{CH}} = 140$  Hz, the in-phase component of the transverse magnetization has decreased by less than 0.5 % over one dwell time  $\Delta\tau$  and less than 3.5 % over three dwell times. Therefore, the  $^{13}\text{C}$   $180^\circ$  inversion pulses can be stretched out over several dwell times (Figure 8.17C) without disastrous effects on the decoupling performance. When the interpulse delay becomes zero, the sequence is referred to as continuous wave decoupling [59]. While continuous wave decoupling is experimentally feasible in terms of RF power requirements and deposition, it has one serious drawback in that the decoupling performance is only adequate when the  $^{13}\text{C}$  inversion pulses are applied close to on-resonance. For selected applications where only a single resonance requires decoupling (e.g.  $^{13}\text{C}$  glycogen detection) continuous wave decoupling is a feasible option. However, for most applications in which multiple metabolites at different chemical shifts require simultaneous decoupling, this approach will fail. Continuous wave decoupling does therefore not belong to the class of broadband decoupling techniques that will be discussed next.

Broadband decoupling methods utilize the same principle as continuous wave decoupling, i.e. the decoupling pulse can stretch over several dwell times, with the crucial difference that the regular 'hard'  $180^\circ$  inversion pulses have been replaced with composite or adiabatic RF pulses that achieved a net rotation angle of  $180^\circ$  over a wider bandwidth. In Section 5.6 composite RF pulses were described as  $180^\circ$  pulses with a built-in compensation towards parameters like RF inhomogeneity and frequency offsets. Figure 5.17 summarizes the performance of the two composite  $180^\circ$  pulses most commonly used for broadband decoupling, namely MLEV  $90^\circ_x 180^\circ_y 90^\circ_x$  [60–62] and WALTZ  $90^\circ_x 180^\circ_{-x} 270^\circ_x$  [59, 63, 64]. It follows that while a regular 'hard'  $180^\circ$  pulse only achieves inversion close to on-resonance, MLEV and WALTZ achieve near-complete inversion over a bandwidth

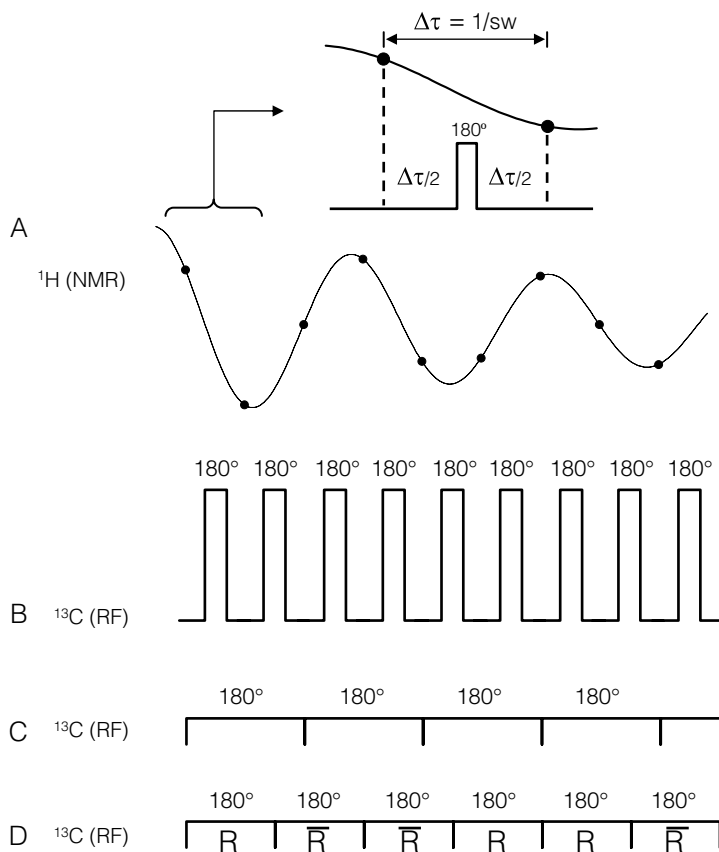


**Figure 8.17** Principle of heteronuclear decoupling during  $^1\text{H}$ - $^{13}\text{C}$  NMR. (A) A continuous  $^1\text{H}$  time-domain signal is sampled at discrete points separated by the dwell time  $\Delta\tau$ . (B) The application of short  $180^\circ$  pulses on the  $^{13}\text{C}$  channel in the middle of each dwell time would lead to complete refocusing of heteronuclear scalar coupling evolution at each data acquisition point and theoretically to perfect decoupling. (C) RF power restrictions necessitate lengthening of the  $180^\circ$   $^{13}\text{C}$  RF pulses over several dwell times, which would lead in the extreme case to continuous wave decoupling. (D) In order to improve the off-resonance performance, the regular  $180^\circ$  pulses are typically substituted with pulse combinations, composite or adiabatic RF pulses (denoted R, where the overbar represents a  $180^\circ$  phase inversion) and placed inside decoupling super cycles.

slightly over  $2B_{2\text{max}}$ , where  $B_{2\text{max}}$  is the applied decoupling RF amplitude. However, the performance of these pulses is surprisingly poor when executed as a continuous train for broadband decoupling (Figure 8.18A). The origin of the strong oscillations in Figure 8.18A can be found in the imperfect inversion profile of the basic WALTZ element. Despite the greatly improved inversion profile of a WALTZ pulse, the inversion is not perfect at all frequencies (e.g. at frequency offset  $\Delta\Omega = 0.325B_2$  WALTZ achieves a net rotation of only  $155^\circ$ ). These minor imperfections propagate through a decoupling pulse train as shown in

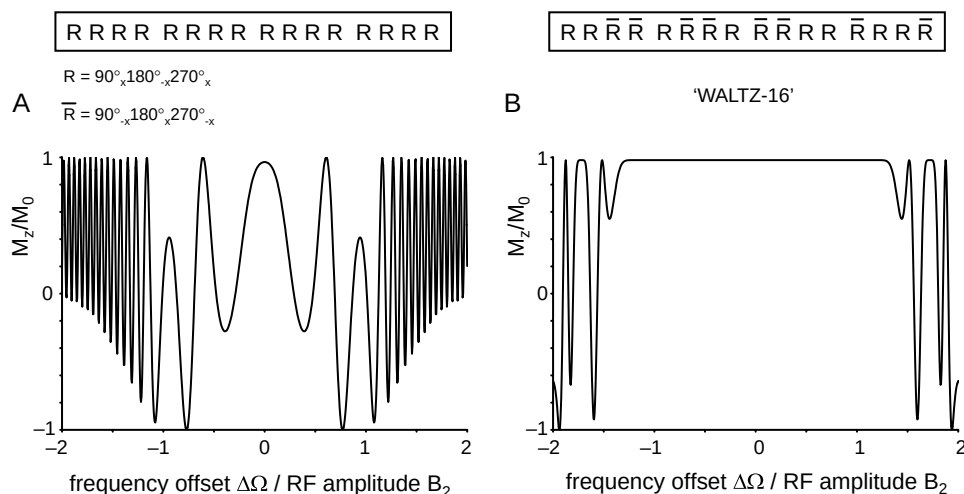


**Figure 8.18** Effect of decoupling super cycles on the off-resonance performance of longer pulse trains. (A) Without any interpulse phase cycling, small errors in an individual pulse  $R$  will propagate through a 16-pulse train leading to poor off-resonance performance. (B) With a WALTZ-16 interpulse phase cycling method, small errors in individual pulses  $R$  do not propagate leading to a greatly enhanced off-resonance performance. Note that since  $R$  represents an inversion pulse, an even number of pulses  $R$  should ideally lead to a net rotation of  $0^\circ$ . This result is closely approximated in (B) for  $|\Delta\Omega| < \sim |B_2|$ .

Figure 8.18A, giving rise to the oscillatory frequency profile. Since minor imperfections are unavoidable, specific pulse phase schemes have been designed to minimize or compensate error propagation throughout a longer pulse train. These so-called decoupling super cycles are essential to broadband decoupling. The specific 16-step decoupling super cycle that is used in combination with WALTZ pulses gives rise to the so-called WALTZ-16 decoupling scheme [63, 64], with greatly enhanced performance (Figure 8.18B) over the decoupling scheme in the absence of a super cycle (Figure 8.18A). Shorter, but less effective cycles are used in WALTZ-4 and WALTZ-8 decoupling [59].

Up to this point, the fact that decoupling pulses are allowed to run over several dwell times has been ignored, given the fact that the scalar coupling evolution is a relatively slow process compared to the data acquisition rate. However, restrictions on RF power deposition limit the available RF amplitude typically leading to a lengthening of the RF pulse duration. For example, the RF amplitude for human decoupling at 4 T may be limited to 500 Hz, making an individual WALTZ element 3.0 ms in duration. During this relatively long pulse, the coherences will evolve under the effects of heteronuclear scalar coupling giving rise to small signal oscillations in the detected NMR signal, which in turn lead to so-called decoupling or cycling sidebands [65] in the NMR spectrum (Figure 8.19). The decoupling side bands have two undesirable effects on the appearance of the NMR spectrum. Firstly, the multiple decoupling sidebands increase the effective noise level, leading to a reduced SNR. Secondly, the intensity of the main resonance is redistributed across multiple smaller decoupling sidebands, leading to a reduction in the main decoupled

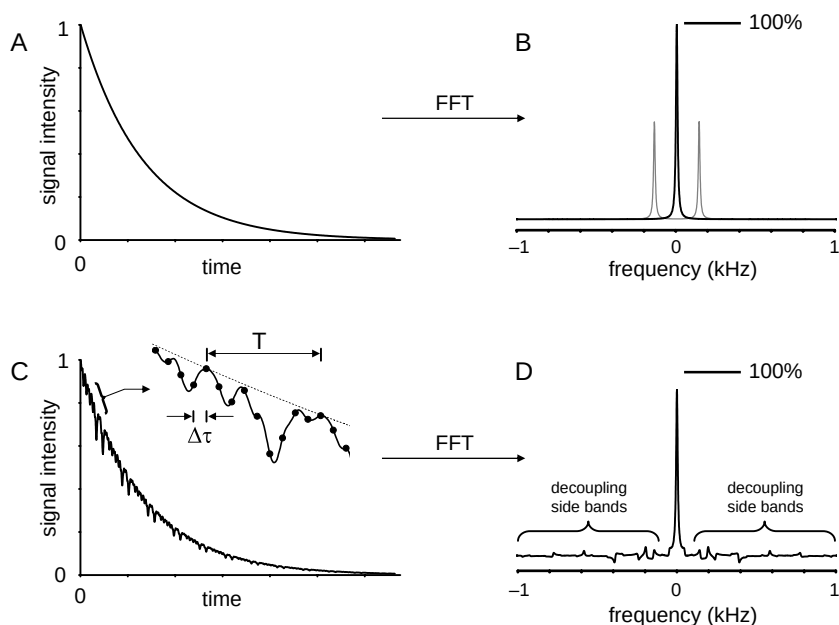


**Figure 8.19** Time- and frequency-domain features of ideal and realistic decoupling. (A) In the case of ideal decoupling (see Figure 8.17B), heteronuclear scalar coupling evolution would be refocused at each data acquisition point, leading to (B) a single resonance line following Fourier transformation, with a peak height that is exactly twice that of the undecoupled resonances (for a two-spin system). (C) In the case of realistic decoupling with RF pulses of length  $T$  that span several dwell times, the effects of heteronuclear scalar coupling evolution are not refocused until the end of the pulse, such that part of the scalar coupling evolution is captured by the data acquisition points covering the length of the RF pulse. (D) These small modulations give rise to so-called decoupling or modulation side bands following Fourier transformation. Furthermore, the peak height of the main decoupled resonance is also reduced.

signal. It is therefore desirable to minimize the decoupling sidebands and maximize the main decoupled resonance. Besides these two parameters, there are other often conflicting considerations including the minimum decoupling bandwidth, the allowable RF power deposition, the maximum available RF peak power and the sensitivity towards experimental imperfections like RF inhomogeneity. These parameters can be optimized and/or balanced with four basic variables, namely the decoupling pulse length (relative to the heteronuclear scalar coupling  $J_{\text{HX}}$ ), amplitude, shape and decoupling scheme. A rigorous comparison and evaluation of decoupling methods most commonly used for *in vivo* NMR has been given by de Graaf [66]. Table 8.1 summarizes the results of those comparisons. Most noticeably, for RF amplitudes suitable for human applications at 4.0 T (i.e.  $B_{2\text{max}} = 500$  Hz), the classic MLEV-16 and WALTZ-16 provide the best performance with an effective decoupling bandwidth of circa  $1.7B_{2\text{max}}$  for a 90 % decoupling efficiency (i.e. the main decoupled resonance is at least 90 % of a perfectly decoupled resonance). Furthermore, at higher RF amplitudes suitable for animal applications, many other decoupling methods become available [67–71]. However, without reservation it can be stated that for



**Table 8.1** Relative decoupling bandwidths ( $BW_{90}/B_{2\max}$ ) as a function of  $B_{2\max}$  or  $B_{2\text{rms}}$ .

| $B_{2\max}$ (kHz)       | 0.50              | 0.75 | 1.0  | 1.5  |
|-------------------------|-------------------|------|------|------|
| WALTZ-16                | 1.64 <sup>b</sup> | 2.27 | 2.37 | 2.40 |
| MLEV-16                 | 1.74              | 2.05 | 2.18 | 2.21 |
| F2                      | 1.34              | 2.68 | 2.74 | 2.76 |
| F3                      | 0                 | 3.21 | 4.13 | 4.17 |
| FP2                     | 1.38              | 2.12 | 2.17 | 2.20 |
| FP3                     | 1.22              | 2.61 | 2.92 | 3.30 |
| GARP                    | 0                 | 0    | 1.65 | 4.96 |
| PBAR                    | 0.52              | 0.65 | 0.66 | 0.68 |
| AFPSTn <sup>a</sup>     | 0                 | 0.45 | 3.44 | 5.40 |
| AFPTT <sup>a</sup>      | 0                 | 1.96 | 2.72 | 3.55 |
| $B_{2\text{rms}}$ (kHz) | 0.50              | 0.75 | 1.0  | 1.5  |
| PBAR                    | 0.66              | 0.67 | 0.68 | 0.68 |
| AFPSTn <sup>a</sup>     | 1.48              | 3.36 | 4.36 | 7.53 |
| AFPTT <sup>a</sup>      | 0                 | 2.15 | 2.82 | 3.67 |

<sup>a</sup>The performance at different  $B_2$  field strengths may correspond to AFP pulses with different R values, pulselengths or modulation functions.

<sup>b</sup>The boxed values indicate the highest bandwidths at the given  $B_{2\max}$  and  $B_{2\text{rms}}$  values.

$B_{2\text{rms}} > 500$  Hz it is always possible to design a decoupling method based on adiabatic RF pulses [66, 69–71] that outperforms all other methods.

## 8.10 Two-dimensional NMR Spectroscopy

The previous sections described the principles of spectral editing to differentiate scalar-coupled from noncoupled spin systems by using several pulse sequence elements like frequency selective RF pulses and magnetic field crusher gradients. While spectral editing methods often lead to the unambiguous detection of one specific compound, much of the information on other compounds is lost in the process. A more general and arguably more intelligent method to manipulate NMR data can be achieved by separating the various characteristics of resonances along orthogonal axes, giving rise to multidimensional NMR. Although high-resolution liquid-state NMR spectroscopy can generate 3D or 4D spectra, the discussion given here will be limited to two dimensions as most commonly encountered for *in vivo* NMR. This section will only review the basic principles of 2D NMR spectroscopy; for more in-depth information the reader is referred to a wide range of texts on multidimensional NMR spectroscopy [72–75].

The first 2D NMR experiment was suggested by Jeener in 1971 during a summer school on NMR given in Yugoslavia [76]. This experiment, which is now known as homonuclear correlation spectroscopy or COSY, was designed to obtain information about homonuclear coupling connectivities for the identification of scalar-coupled spin systems. In 1976, Ernst and coworkers gave a complete theoretical description of COSY, together with important

generalizations about 2D NMR which opened the way to develop multidimensional NMR to the important technique it is today [73, 77]. Besides homonuclear COSY, many other 2D NMR techniques have been developed and have found routine use in organic chemistry, biochemistry and structural biology. For *in vivo* NMR, 2D NMR has not yet found widespread applications, mainly due to the long measurement times required to record a 2D NMR spectrum. However, 2D NMR has great potential for specific *in vivo* applications, where it can mainly be used to unambiguously observe, identify and study coupled resonances, which would be unobservable (due to spectral overlap) in regular 1D NMR spectra.

### 8.10.1 Correlation Spectroscopy (COSY)

The general idea of 2D experiments is to generate a second frequency axis by introducing an evolution delay (and at least one additional pulse) into a pulse sequence, during which the transverse magnetization precesses at a different frequency than during signal acquisition. The COSY experiment (Figure 8.20) can therefore be seen as an extension of a simple  $90^\circ$ -acquisition experiment, in that an additional delay  $t_1$  and a second  $90^\circ$  pulse follow the excitation pulse [73, 77]. Signal acquisition is performed during  $t_2$ . Some of the basic aspects of 2D NMR will first be discussed for uncoupled spins.

*Uncoupled Spins.* The initial  $90^\circ_x$  pulse excites the longitudinal magnetization onto the transverse plane (in this case along the  $-y'$  axis). In 2D NMR the  $90^\circ$  pulse and the preceding delay are commonly referred to as the preparation period. The system then evolves in the transverse plane under the effects of chemical shift (and magnetic field inhomogeneity) during the evolution period  $t_1$ . During  $t_1$  the magnetization evolves through an angle  $\omega_1 t_1$  with respect to the  $-y$  axis, where  $\omega_1$  is the angular resonance frequency of the magnetization under investigation during evolution period  $t_1$ . At the end of the evolution period, a  $90^\circ_x$  pulse rotates the magnetization into the  $xz$  plane. The transverse component of the magnetization after the second  $90^\circ$  pulse, which is referred to as the mixing pulse, is proportional to  $\cos(\omega_1 t_1)$  and coincides with the  $x$  axis. During the detection period  $t_2$ , a signal (i.e. FID)  $S(t_1, t_2)$  is detected which is given by:

$$S(t_1, t_2) = M_0 \sin(\omega_1 t_1) e^{i\omega_2 t_2} e^{-t_1/T_{2,1}} e^{-t_2/T_{2,2}} \quad (8.42)$$

where quadrature detection during the detection period  $t_2$  is assumed.  $\omega_1$  and  $\omega_2$  are the angular resonance frequencies during the  $t_1$  and  $t_2$  periods, respectively.  $T_{2,1}$  and  $T_{2,2}$  are the transverse relaxation times during  $t_1$  and  $t_2$ , respectively.  $M_0$  is the thermal equilibrium magnetization. For uncoupled spins,  $\omega_1 = \omega_2$  and  $T_{2,1} = T_{2,2}$ , but as will become apparent in the next section this is not true for coupled spins per se. A 2D data matrix is created by incrementing  $t_1$  in subsequent acquisitions. This is schematically drawn in Figure 8.20 for uncoupled spins. Fourier transformation of the acquired FIDs with respect to  $t_2$  gives a set of spectra given by:

$$S(t_1, \omega_2) = M_0 [A(\omega_2) + iD(\omega_2)] \sin(\omega_1 t_1) e^{-t_1/T_{2,1}} \quad (8.43)$$

where  $A(\omega_2)$  and  $D(\omega_2)$  are the absorption and dispersion lineshapes of the resonance centered about  $\omega_2$ .  $A(\omega_2)$  and  $D(\omega_2)$  are given by Equations (1.56) and (1.57), respectively. Figure 8.20 shows five of these spectra for different  $t_1$  evolution times. Fourier transformation of these so-called interferograms with respect to  $t_1$  gives the complete 2D spectrum. Although not shown in Figure 8.20C, it is impossible to tell from Equation (8.43) [or

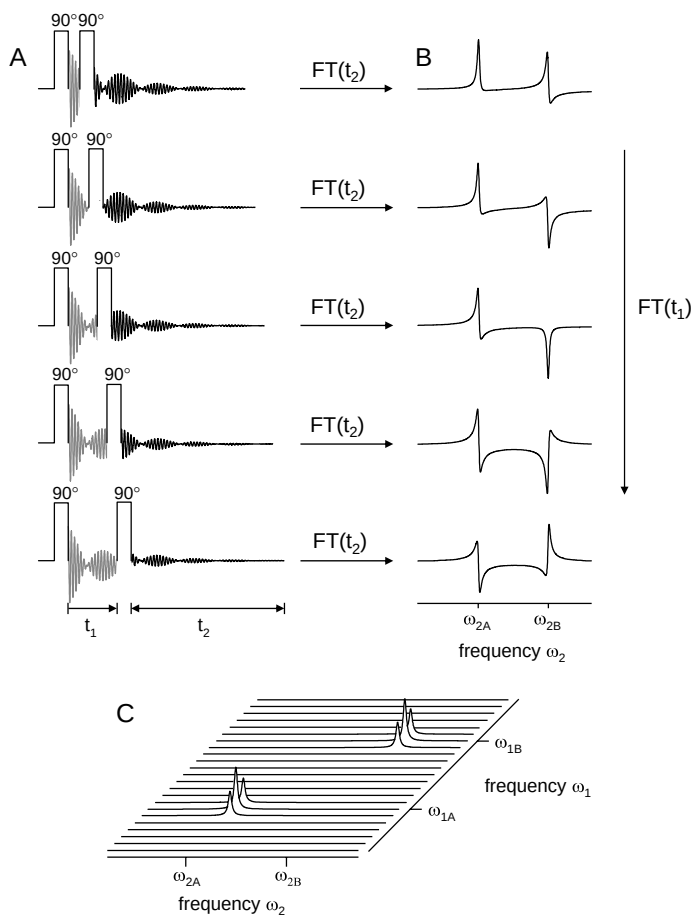


**Figure 8.20** Principle of 2D NMR spectroscopy for uncoupled spin systems. (A) After excitation, the transverse magnetization is allowed to evolve under chemical shifts  $\omega_1$  during the evolution period  $t_1$ . Following the second  $90^\circ$  pulse, the magnetization is evolving under chemical shifts  $\omega_2$  during the acquisition period  $t_2$ . The precession in the evolution period  $t_1$  determines the phase of the coherences during the detection period  $t_2$ , as can be judged from (B) the Fourier transformed spectra  $S(t_1, \omega_2)$ . (C) Incrementing the evolution period reveals the frequencies  $\omega_1$  during  $t_1$  according to  $\omega_1 = (d\phi/dt)$ , where  $\phi$  is the phase of the coherences at  $t_2 = 0$ . For uncoupled spins the resonance frequency is identical during the evolution and detection period (i.e.  $\omega_1 = \omega_2$ ), giving rise to diagonal peaks in a 2D NMR spectrum. For coupled spin systems, additional off-diagonal (or cross) peaks will arise which indicate the connectivities between the diagonal peaks.

from the Fourier transformation of Equation (8.43)], whether the modulation frequency  $\omega_1$  during the evolution period is positive or negative. This problem was also encountered in 1D NMR, when only one component (e.g.  $x$ ) of the FID was sampled. One possible solution is to position the transmitter frequency on one side of the spectrum, such that all resonance frequencies have the same sign. However, this solution is very inefficient in terms of sensitivity and data storage. A more favorable solution is offered by quadrature

detection in which two orthogonal components  $x$  and  $y$  are detected. This principle can also be applied for the  $t_1$  dimension in 2D NMR by performing a second experiment in which the mixing pulse (i.e. the second  $90^\circ$  pulse) is phase-shifted by  $90^\circ$  relative to the first experiment. The signal detected in the second experiment is now along the  $y$  axis and is given by:

$$\begin{aligned} S'(t_1, t_2) &= M_0 \cos(\omega_1 t_1) e^{i(\omega_2 t_2 + \pi/2)} e^{-t_1/T_{2,1}} e^{-t_2/T_{2,2}} \\ &= iM_0 \cos(\omega_1 t_1) e^{i\omega_2 t_2} e^{-t_1/T_{2,1}} e^{-t_2/T_{2,2}} \end{aligned} \quad (8.44)$$

In the early days of 2D NMR, the signals given by Equations (8.42) and (8.44) were added together, giving:

$$S^+(t_1, t_2) = M_0 e^{i\omega_1 t_1} e^{i\omega_2 t_2} e^{-t_1/T_{2,1}} e^{-t_2/T_{2,2}} \quad (8.45)$$

Complete 2D Fourier transformation of Equation (8.45) gives the 2D NMR spectrum with quadrature detection in both  $t_1$  and  $t_2$  dimensions:

$$S^+(\omega_1, \omega_2) = M_0 [A(\omega_1)A(\omega_2) - D(\omega_1)D(\omega_2)] + iM_0 [A(\omega_1)D(\omega_2) + D(\omega_1)A(\omega_2)] \quad (8.46)$$

Clearly, both the real and imaginary component of the 2D resonance are a mixture of absorption  $A$  (Figure 8.21A) and dispersion  $D$  (Figure 8.21B) lineshapes. This leads to so-called ‘phase-twisted’ lineshapes, which cannot be phased to a pure absorption mode (Figure 8.21C). This can be remedied with an absolute value representation, according to Equation (1.61) (Figure 8.21D). However, just as with absolute value spectra in 1D NMR, the lines are broadened, leading to decreased spectral resolution in both dimensions. Although such tailing can be suppressed by the use of appropriate 2D apodization functions (see Section 7.3), the approach to achieve quadrature detection in the  $t_1$  dimension as outlined above generally suffers from decreased sensitivity and resolution.

By appropriate manipulation of the acquired data, States *et al.* [78] were able to obtain pure 2D absorption resonances. Instead of adding the results of the two independent

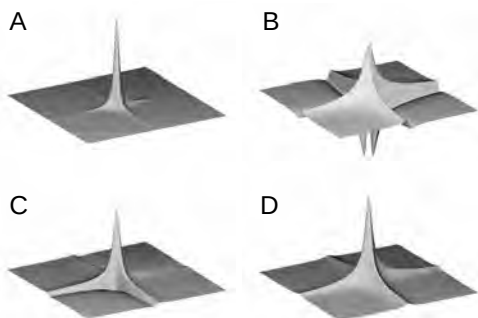


**Figure 8.21** Lorentzian lineshapes encountered in 2D NMR spectra. (A) Pure absorption and (B) pure dispersion resonances. (C) Phase-twisted resonance holding both absorption and dispersion components. This type of lineshape can never be phased to a pure absorption or dispersion lineshape. (D) Magnitude representation of a 2D resonance line. Note the increased ‘tails’ of the magnitude representation when compared with a pure absorption resonance line.

measurements, given by Equations (8.42) and (8.44), the acquired FIDs are stored separately in memory. The crucial ‘trick’ proposed by States *et al.* [78] is to replace the imaginary part of  $S(t_1, \omega_2)$  (the first experiment) by the real part of  $S'(t_1, \omega_2)$  (the second experiment) yielding:

$$S_{\text{HC}}(t_1, \omega_2) = M_0 A(\omega_2) e^{i\omega_1 t_1} e^{-t_1/T_{2,1}} \quad (8.47)$$

Complex Fourier transformation of Equation (8.47) with respect to  $t_1$  will give a pure 2D absorption mode resonance:

$$S_{\text{HC}}(\omega_1, \omega_2) = M_0 A(\omega_1) A(\omega_2) \quad (8.48)$$

The method of States *et al.* [78] is sometimes referred to as the hypercomplex method and is the common method to acquire phase-sensitive 2D NMR spectra.

As was already graphically demonstrated in Figure 8.20, the acquired signal holds information on the frequencies during the  $t_1$  and  $t_2$  periods. Fourier transformation of the FIDs (after multiple  $t_1$  increments) gives the frequencies during  $t_1$  and  $t_2$ . For uncoupled spins these frequencies are identical in both periods (see Figure 8.20). Therefore, even though a 2D data matrix has been created, this has not led to any additional information for uncoupled resonances in terms of spectral resolution enhancement, since all resonances occur on the diagonal of the 2D NMR spectrum.

**Coupled Spins.** A different situation arises for scalar-coupled spins. As was described Section 8.7, scalar-coupled spin systems are capable of polarization transfer between two coupled nuclei, such that the frequency during  $t_1$  no longer equals the frequency during  $t_2$  per se. Therefore, for coupled spin systems resonances can occur at off-diagonal positions  $(\omega_1, \omega_2)$  and  $(\omega_2, \omega_1)$  where  $\omega_1 \neq \omega_2$ . This gives direct information on the coupling pattern within a spin system, even when the spins are not resolved in the 1D spectrum. Unfortunately, polarization transfer which will lead to the formation of off-diagonal peaks can not be readily explained in a graphical manner as was used for the explanation of diagonal peaks in Figure 8.20. For a quantitative description of polarization transfer, one has to resort to the quantum mechanical machinery of density matrix calculations. For weakly coupled spins (i.e. for which the frequency difference is much larger than the scalar coupling constant  $J$ ), which are becoming more common as magnetic field strength increases, the complicated density matrix calculations can be simplified by following the product operator formalism (see also Appendix A4). Consider a weakly coupled IS spin system, with coupling constant  $J_{\text{IS}}$ , resonating at frequencies  $\omega_I$  and  $\omega_S$ , respectively. Following a  $90^\circ_x$  pulse and a delay  $t_1$ , the transverse magnetization is given by:

$$\begin{aligned} I_z + S_z &\xrightarrow{90^\circ_x} -I_y - S_y \xrightarrow{t_1} \\ &- [I_y \cos(\pi J_{\text{IS}} t_1) - 2I_x S_z \sin(\pi J_{\text{IS}} t_1)] \cos(\omega_I t_1) \\ &+ [I_x \cos(\pi J_{\text{IS}} t_1) + 2I_y S_z \sin(\pi J_{\text{IS}} t_1)] \sin(\omega_I t_1) \\ &- [S_y \cos(\pi J_{\text{IS}} t_1) - 2I_z S_x \sin(\pi J_{\text{IS}} t_1)] \cos(\omega_S t_1) \\ &+ [S_x \cos(\pi J_{\text{IS}} t_1) + 2I_z S_y \sin(\pi J_{\text{IS}} t_1)] \sin(\omega_S t_1) \end{aligned} \quad (8.49)$$

Immediately after the second  $90^\circ_x$  pulse, the coherence can be described by:

$$\begin{aligned}
 \xrightarrow{90^\circ_x} & -[I_z \cos(\pi J_{IS}t_1) + 2I_x S_y \sin(\pi J_{IS}t_1)]\cos(\omega_I t_1) \\
 & +[I_x \cos(\pi J_{IS}t_1) - 2I_z S_y \sin(\pi J_{IS}t_1)]\sin(\omega_I t_1) \\
 & -[S_z \cos(\pi J_{IS}t_1) + 2I_y S_x \sin(\pi J_{IS}t_1)]\cos(\omega_S t_1) \\
 & +[S_x \cos(\pi J_{IS}t_1) - 2I_y S_z \sin(\pi J_{IS}t_1)]\sin(\omega_S t_1)
 \end{aligned} \quad (8.50)$$

During the acquisition period  $t_2$ , each of these terms (except  $I_z$  and  $S_z$ ) will evolve under the influence of chemical shift and scalar coupling. However, since not all operators in Equation (8.50) are directly observable, not all operators need to be considered. Four different types of operators can be recognized from Equation (8.50):

- (1)  $I_z$  and  $S_z$  represent longitudinal coherence of spins I and S, respectively, and since no excitation pulses follow the second  $90^\circ$  pulse, they do not lead to observable signal and can be omitted in further calculations.
- (2)  $I_x$  and  $S_x$  represent in-phase x coherence of spins I and S, respectively, and will lead to observable magnetization during the detection period.
- (3)  $2I_y S_z$  and  $2I_z S_y$  represent anti phase y coherence of spins I and S, respectively, with respect to the coupling partner. These operators will also lead to observable signal during  $t_2$ .
- (4)  $2I_x S_y$  and  $2I_y S_x$  represent a combination of ZQCs and DQCs which remain invisible during the detection period. These terms can be made observable in so-called double-quantum-filtered COSY [79, 80].

Therefore, Equation (8.50) can be simplified to:

$$\begin{aligned}
 & +[I_x \cos(\pi J_{IS}t_1) - 2I_z S_y \sin(\pi J_{IS}t_1)]\sin(\omega_I t_1) \\
 & +[S_x \cos(\pi J_{IS}t_1) - 2I_y S_z \sin(\pi J_{IS}t_1)]\sin(\omega_S t_1)
 \end{aligned} \quad (8.51)$$

During the detection period  $t_2$ , the terms in Equation (8.51) evolve under chemical shifts and scalar coupling to:

$$\begin{aligned}
 \xrightarrow{t_2} & +[(I_x \cos(\omega_I t_2) - I_y \sin(\omega_I t_2))\cos(\pi J_{IS}t_2) \\
 & +2(I_y \cos(\omega_I t_2) + I_x \sin(\omega_I t_2))S_z \sin(\pi J_{IS}t_2)]\sin(\omega_I t_1)\cos(\pi J_{IS}t_1) \\
 & +[(S_x \cos(\omega_S t_2) - S_y \sin(\omega_S t_2))\cos(\pi J_{IS}t_2) \\
 & +2(S_y \cos(\omega_S t_2) + S_x \sin(\omega_S t_2))I_z \sin(\pi J_{IS}t_2)]\sin(\omega_S t_1)\cos(\pi J_{IS}t_1) \\
 & +[(S_x \cos(\omega_S t_2) - S_y \sin(\omega_S t_2))\sin(\pi J_{IS}t_2) \\
 & -2(S_y \cos(\omega_S t_2) + S_x \sin(\omega_S t_2))I_z \cos(\pi J_{IS}t_2)]\sin(\omega_I t_1)\sin(\pi J_{IS}t_1) \\
 & +[(I_x \cos(\omega_I t_2) - I_y \sin(\omega_I t_2))\sin(\pi J_{IS}t_2) \\
 & -2(I_y \cos(\omega_I t_2) + I_x \sin(\omega_I t_2))S_z \cos(\pi J_{IS}t_2)]\sin(\omega_S t_1)\cos(\pi J_{IS}t_1)
 \end{aligned} \quad (8.52)$$

At first glance, Equation (8.52) may appear very complicated, but a closer examination shows that only four relevant terms are involved. Just as in 1D spectra, the first point of the FID determines the phase (and amplitude) of the spectrum, while the following points determine the frequency and linewidth of the resonances. From Equation (8.52) it follows that the 2D NMR spectrum of a weakly coupled IS spin system consists of four resonances at  $(\omega_I, \omega_I)$  [first two terms in Equation (8.52)], at  $(\omega_S, \omega_S)$  (third and fourth terms) and at  $(\omega_I, \omega_S)$  and  $(\omega_S, \omega_I)$  (last four terms). The 2D resonances at  $(\omega_I, \omega_I)$  and  $(\omega_S, \omega_S)$  are generally referred to as diagonal peaks (or resonances), while  $(\omega_I, \omega_S)$  and  $(\omega_S, \omega_I)$  are known as cross peaks. The cross peaks originate from anti phase coherence in the  $t_1$  period, since only anti phase coherence can undergo polarization transfer (i.e.  $2I_x S_z \rightarrow -2I_z S_x$ ). From Equation (8.52) it can also be deduced that the cross peaks are always  $90^\circ$  out of phase with the diagonal peaks, such that resonances in a COSY spectrum can never be all simultaneously 'in phase'. Figure 8.22 shows a theoretical COSY spectrum for a IS spin system according to Equation (8.52). Besides the four principle resonance frequencies as predicted by Equation (8.52), each resonance has a fine structure due to scalar coupling evolution of each term during  $t_1$  and  $t_2$ . Both the diagonal and cross peaks consist of four individual resonances which can be attributed to two doublets along either frequency axis. In Figure 8.22 the cross peaks are in an anti phase coherence state, while the diagonal peaks are in an in-phase coherence state,  $90^\circ$  out of phase relative to the cross peaks.

Note that it is customary in NMR to refer to all peaks in a cross or diagonal multiplet collectively as a single resonance.

Additional resonances in the spectrum may arise when magnetization is detected during  $t_2$  which has not been frequency labeled in the evolution period  $t_1$ . This can for instance occur when magnetization significantly recovers along the  $z$  axis during the  $t_1$  period due to longitudinal relaxation. The second  $90^\circ$  (mixing) pulse converts it to transverse magnetization which will be detected at a frequency  $\omega_I = 0$ . These so-called axial peaks can be suppressed by a simple two-step phase cycle. Table 8.2 gives a four-step phase cycle most commonly employed for phase-sensitive 2D NMR with quadrature detection in both dimensions without artifacts from axial peaks. Quadrature detection is achieved according to the hypercomplex method by cycling the first (or second)  $90^\circ$  through  $\phi_1 = +x, +y$  and storing the two experiments in separate memory blocks. Axial peaks are easily eliminated by cycling the second  $90^\circ$  pulse through  $\phi_2 = +x, -x$ , such that coherence during  $t_2$  arising from longitudinal magnetization prior to the mixing pulse is cancelled. To eliminate imperfections in the quadrature detection system of the spectrometer, the four-step phase cycle of Table 8.2 is often combined with a four-step CYCLOPS phase cycle [81], giving a minimum of sixteen acquisitions per  $t_1$  increment.

For a simple IS spin system, the cross peak observed in a COSY spectrum between the I and S spins is a relatively simple anti phase doublet in both dimensions. However, pure two-spin systems are rarely encountered in biological NMR. In order to be able to predict the appearance of cross peaks for more complicated spin systems, a three-spin AMX system is considered. The large difference in letter notation for the individual spins is indicative for large frequency differences between the resonances and hence indicates a weakly coupled spin system (see Chapter 1). It is assumed that all three spins are mutually coupled with scalar coupling constants  $J_{AM}$ ,  $J_{AX}$ , and  $J_{MX}$ . Now consider the AM spin subsystem, in which the X spins can be considered a passive spin. The coherence between A and M, which leads to the cross peak, is not affected by X. However, as was already shown for spectral

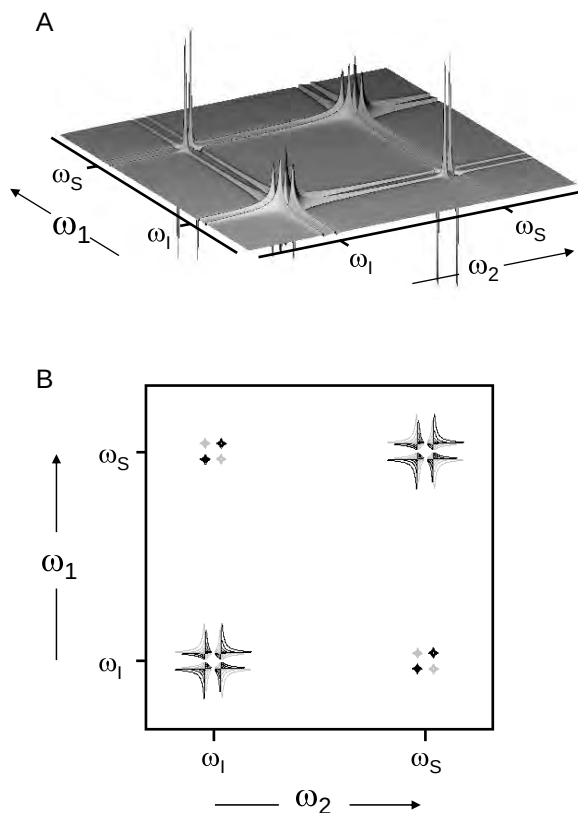


**Figure 8.22** (A) Theoretical 2D COSY spectrum for a weakly coupled IS spin system. The dispersive, in-phase diagonal peaks at  $(\omega_I, \omega_I)$  and  $(\omega_S, \omega_S)$  are  $90^\circ$  out of phase with the antiphase cross peaks at  $(\omega_I, \omega_S)$  and  $(\omega_S, \omega_I)$ . (B) More commonly used 2D contour plot of the spectrum shown in (A). Black and gray lines indicative positive and negative signal intensities, respectively.

**Table 8.2** Phase cycle for COSY with 2D quadrature detection and elimination of axial peaks.

| Scan | $\phi_1$ | $\phi_2$ | Receiver |
|------|----------|----------|----------|
| 1    | +x       | +x       | +x       |
| 2    | +x       | -x       | +x       |
| 3    | +y       | +x       | +x       |
| 4    | +y       | -x       | +x       |

$\phi_1$  and  $\phi_2$  are the phases for the first and second  $90^\circ$  pulses, respectively.



editing with MQC filtering (Section 8.5), the X spin contributes a cosine modulation to the cross peak via the scalar coupling to spins A and M leading to the observation that the AMX cross peak no longer resembles a pure antiphase doublet in both dimensions, because it is complicated by in-phase components caused by the X spin. More explicitly, the anti phase peak in the  $\omega_1$  (or F1) dimension is extended with regular in-phase splitting due to  $J_{AX}$  and the peak in the  $\omega_2$  (or F2) dimension is extended with in-phase  $J_{MX}$  splitting. Essentially, the passive X spins affect the 2D cross peak as if it was a regular 1D spectrum. This result can be extended to include more than one passive spin and hence the cross peaks of more complicated spin systems can be calculated. Figure 8.23 shows a 2D NMR spectrum of an  $AMX_2$  spin system and in particular the cross peak for the AM interaction. It can be seen that the effect of the two passive X spins can be calculated by performed two successive in-phase splittings in both dimensions starting from an anti phase doublet.

Detection of complicated cross peaks as the one shown in Figure 8.23 is essentially impossible under *in vivo* conditions. In order to adequately describe the cross peak, a very high spectral resolution in both dimensions is required. This can only be achieved by increasing the number of  $t_1$  increments in the F1 dimension, directly leading to increased measurement times. Another disadvantage of extended 2D cross peaks is that the total signal intensity of the cross peak is distributed over the smaller 2D resonances, leading to a reduced SNR. Besides these effects, the problem most often encountered in *in vivo* 2D NMR is that the  $R_2^*$  relaxation rate is large compared with the scalar coupling constants. This will lead to overlap of resonances within the cross peak and undoubtedly to signal loss when positive and negative resonances cancel each other out.

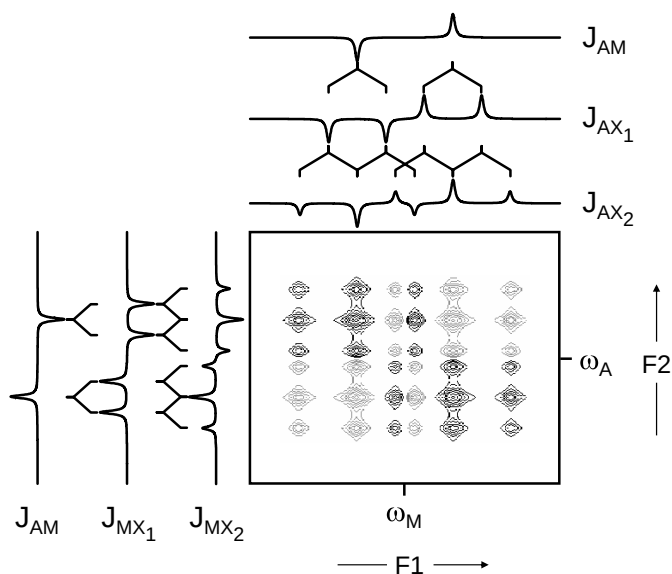


**Figure 8.23** Construction of the AM cross peak of a  $AMX_2$  spin system. Successive splitting of the basic, anti phase AM cross peak with respect to the X nucleus gives the total cross peak consisting of 36 individual resonances. Black and gray lines indicate positive and negative signal intensities, respectively.

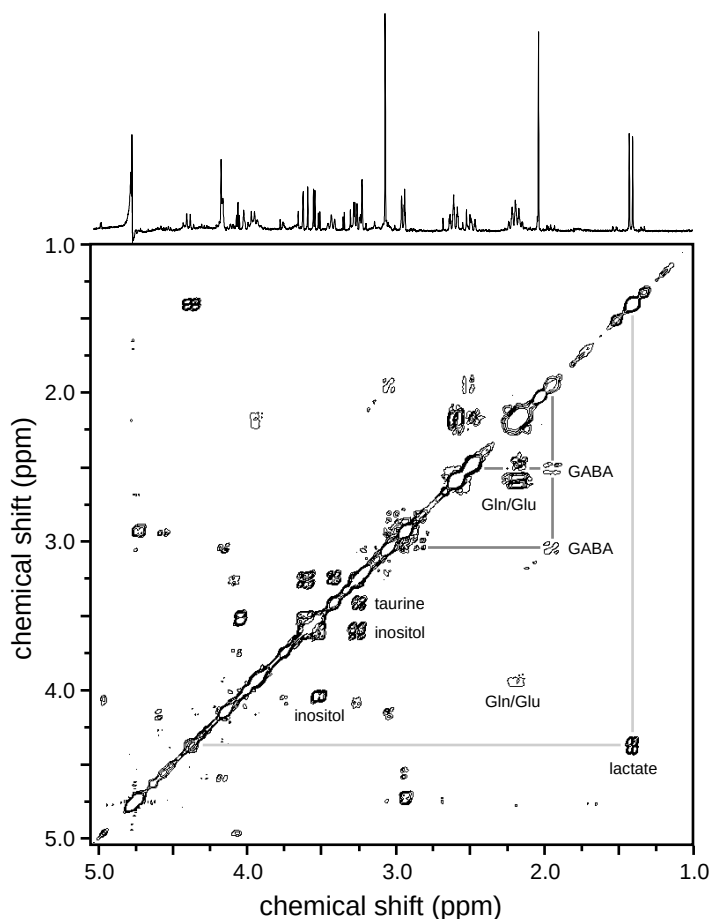


**Figure 8.24** 2D COSY NMR spectrum (absolute value) of PCA extract from rat brain dissolved in  $D_2O$  at 298 K, acquired at 300 MHz. Besides the diagonal resonances, a substantial number of cross peaks is observed, which indicate the connectivities between resonances in coupled spin systems. As an example, the connectivities for GABA and lactate are indicated. Connectivities for glutamate, glutamine, inositol, NAA and taurine are also readily observed.

Figure 8.24 shows a 2D COSY obtained from a perchloric acid (PCA) extract from rat brain at 300 MHz. The spectrum is represented in absolute value. Besides the many resonances present on the diagonal, the spectrum also exhibits a large number of cross peaks arising from coupled spin systems. The connectivities for lactate (an  $AX_3$  spin system) and GABA (an  $A_2M_2X_2$  spin system) are indicated. Note that the COSY spectrum is symmetrical with respect to the diagonal, a fact which can be used by symmetrization algorithms to distinguish low intensity resonances from noise and improve the suppression of spurious (water) signals. Many of the connectivities visible in Figure 8.24 have been listed in Table 2.1.

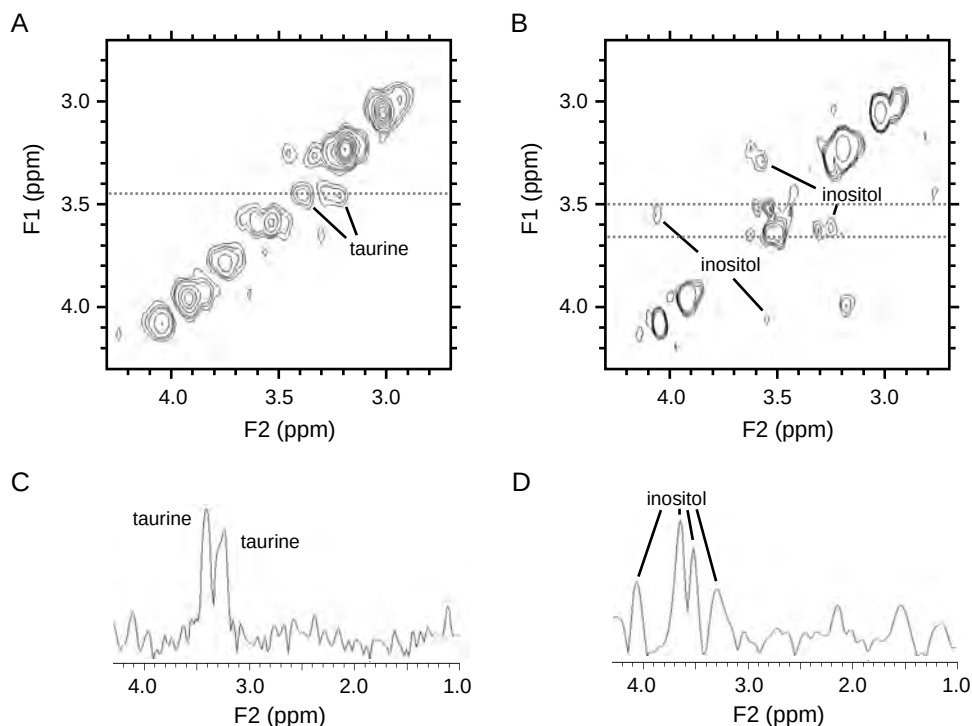


**Figure 8.25** In vivo correlated spectroscopy on a F98 glioma in rat brain with a constant time (CT) COSY sequence. The sequence consist of  $90^\circ - (t_a/2) - 180^\circ - (t_a/2) - (t_b - t_1)/2 - 180^\circ - (t_b + t_1)/2 - 90^\circ$  - acquisition, where the  $90^\circ$  RF pulses and the first  $180^\circ$  RF pulse are spatially selective to select a  $100\ \mu\text{l}$  voxel. The second  $180^\circ$  RF pulse is nonselective. The advantages of CT-COSY over regular COSY are threefold. First, CT-COSY allows single-scan spatial localization. Second, the delay  $t_c = t_a + t_b$  can be optimized for a specific metabolite, thereby maximizing the cross peak intensity. Third, the CT character (i.e.  $t_c = \text{constant}$ , irrespective of  $t_1$ ) gives an effectively decoupled  $^1\text{H}$  NMR spectrum along F1. The absolute value CT-COSY spectra were acquired with 128  $t_1$  increments ( $-17\ \text{ms} \leq t_1 \leq +17\ \text{ms}$ ),  $\text{TR} = 2000\ \text{ms}$  and two accumulations (making the experiment duration 8.5 min). Taurine and inositol detection is optimized for (A)  $t_c = 51\ \text{ms}$  and (B)  $t_c = 85\ \text{ms}$ . (C) The projection onto F2 at  $F1 = 3.44\ \text{ppm}$  gives the taurine resonances, while (D) the projection onto F2 for  $3.50\ \text{ppm} \leq F1 \leq 3.66\ \text{ppm}$  gives the inositol resonances. (Courtesy of W. Dreher.)

COSY is readily employed *in vivo*. In most cases, the basic COSY sequence is extended to allow spatial localization or increase the spectral resolution or sensitivity [82–88]. Figure 8.25 shows an *in vivo* example of (constant-time) COSY that allowed the unambiguous detection of taurine and inositol in a F98 glioma in rat brain. In 1D  $^1\text{H}$  NMR spectra, taurine and inositol are overlapped by glucose and glycine, respectively, making identification of resonances difficult. The cross peaks in a COSY experiment eliminate any ambiguity concerning resonance assignment.

### 8.10.2 Spin-echo or J-resolved NMR

The purpose of homo- and heteronuclear 2D J-resolved NMR experiments is to resolve the scalar coupling fine structure of resonances and their chemical shifts in two orthogonal directions. This type of experiment is referred to as spin-echo or J-resolved NMR spectroscopy [89–96]. Compared with COSY or other 2D correlation spectroscopy methods, 2D J-resolved spectroscopy is a much simpler experiment, as is the basic pulse sequence (Figure 8.26A). The sequence is essentially a spin-echo sequence in which the echo-time equals the evolution period  $t_1$ . The key principle of 2D J-resolved spectroscopy lies in the concept of scalar coupling evolution. Following excitation and the evolution period  $t_1$ , the coherences (in a weakly coupled IS spin system) at the beginning of the acquisition period  $t_2$  are given by:

$$\begin{aligned}\sigma(t_1) = & -I_y \cos(\pi J_{IS}t_1) + 2I_x S_z \sin(\pi J_{IS}t_1) \\ & -S_y \cos(\pi J_{IS}t_1) + 2I_z S_x \sin(\pi J_{IS}t_1)\end{aligned}\quad (8.53)$$

The combination of in-phase and anti phase components implies that 2D J-resolved spectra are composed of the well-known phase-twisted lineshape (see Figure 8.21). It is therefore not possible to obtain pure absorption mode lineshapes. As a result, 2D J-resolved spectra are always presented in an absolute value mode. As can be seen from Equation (8.53), the  $t_1$  period does not introduce any information on chemical shifts, since these are refocused by the  $180^\circ$  pulse. During the  $t_2$  acquisition period, both chemical shift and scalar coupling evolution are operative. This gives rise to the theoretical spectrum [for a weakly coupled  $AM_2X$  spin system ( $J_{AX} = 0$ )] shown in Figure 8.26B.

2D J-resolved NMR spectra exhibit some interesting features. The spectral bandwidth in the  $\omega_1$  (or F1) dimension (which is associated with the  $t_1$  period) needs only to be in the order of 50 Hz to cover all homonuclear scalar couplings. The digital resolution in  $\omega_1$  can therefore be made very high, such that the scalar coupling constant can be accurately measured. The spectral resolution can be especially high due to the fact that the lines in the  $\omega_1$  dimension have their natural linewidths (governed by  $T_2$  neglecting diffusion effects), and are not broadened by  $T_2^*$  effects. However, this advantage is somewhat reduced by the fact that the resonances are presented in absolute value mode which decreases the spectral resolution. Due to signal cancellation, the individual multiplet lines lie along a line which is  $45^\circ$  tilted with respect to the  $\omega_2$  axis. Therefore, the multiplet structures are preserved in the  $\omega_2$  dimension. However, a calculated NMR spectrum along this tilted axis can be obtained, in which all multiplets are collapsed to singlets. As a result, a homonuclear decoupled  $^1H$  NMR spectrum is obtained, which gives a substantial improvement of the spectral resolution in the  $\omega_2$  dimension. The calculation of a proton-decoupled  $^1H$  NMR spectrum is readily achieved with ‘tilting’ or ‘rotation’ algorithms, after which the projections (or the summation) onto the  $\omega_2$  axis gives the desired, decoupled spectrum (Figure 8.26C). Unfortunately, because chemical shift information of strongly coupled spin systems is not completely refocused by a simple  $180^\circ$  pulse, this procedure only works for weakly coupled spin systems. For strongly coupled spin systems, spurious resonance lines will be observed making spectral interpretation difficult [92, 94]. To optimize the detection of specific metabolite resonances, a so-called constant-time J-resolved experiment can be performed of which the sequence is shown in Figure 8.26D. Signal acquisition is started after a constant time  $t_{1,max}$  following signal excitation. A  $180^\circ$  refocusing pulse is systematically shifted

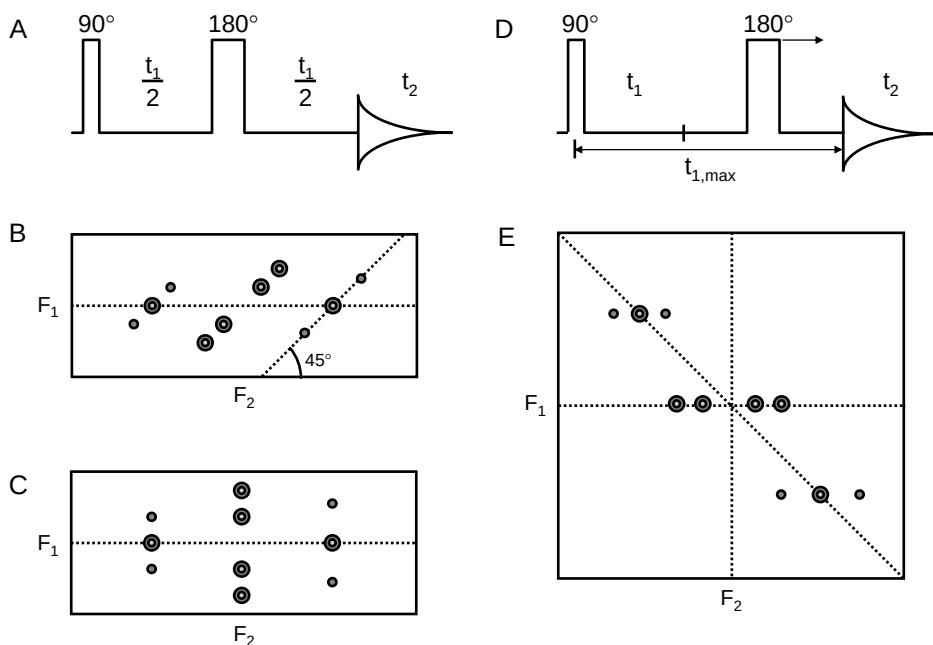


**Figure 8.26** (A) Pulse sequence for J-resolved NMR spectroscopy. The echo-time equals the evolution time  $t_1$ . (B) Theoretical 2D J-resolved NMR spectrum for an  $AM_2X$  spin system. Due to signal cancellation, the resonances lay on a 45° tilted axis. (C) Tilting or rotating algorithms allow the generation of a nontilted spectrum, from which an effectively proton-decoupled  $^1H$  NMR spectrum can be calculated as the projection onto the  $\omega_2$  (or  $F_2$ ) axis. (D) Constant-time J-resolved NMR pulse sequence and (E) corresponding NMR spectrum for an  $AM_2X$  spin system. The 180° pulse is shifted within the constant delay  $t_{1,max}$  to encode chemical shift information during  $t_1$ . Since J-evolution is constant during  $t_1$ , an effectively proton-decoupled  $^1H$  NMR spectrum can be calculated as the projection onto  $\omega_1$ .

within this interval from experiment to experiment. Since homonuclear scalar couplings are not affected by the position of the 180° pulse, the  $t_1$ -modulation is purely determined by chemical shifts (note that  $T_2$  relaxation decay is also constant for each  $t_1$  increment). The theoretical spectrum for a weakly coupled  $AM_2X$  spin system is shown in Figure 8.26E. The projections onto the  $\omega_1$  and  $\omega_2$  axes correspond to the coupled and homonuclear decoupled NMR spectra, respectively. Note that constant-time J-resolved spectroscopy can only be optimal for one scalar coupling constant [i.e. the signal intensity of an IS spin system in the decoupled spectrum is proportional to  $\cos(\pi J t_{1,max})$ ], making the choice of  $t_{1,max}$  critical. Due to signal loss related to  $T_2$  relaxation, the maximum value of  $t_{1,max}$  is limited.

2D J-resolved spectroscopy can be a simple method to assist resonance assignment, determine scalar coupling constants and improve spectral resolution as shown in Figure 8.27 for PCA extract of rat brain. An additional feature is shown in the bottom trace in Figure 8.27. Coupled resonances (e.g. GABA) which are overlapped by other

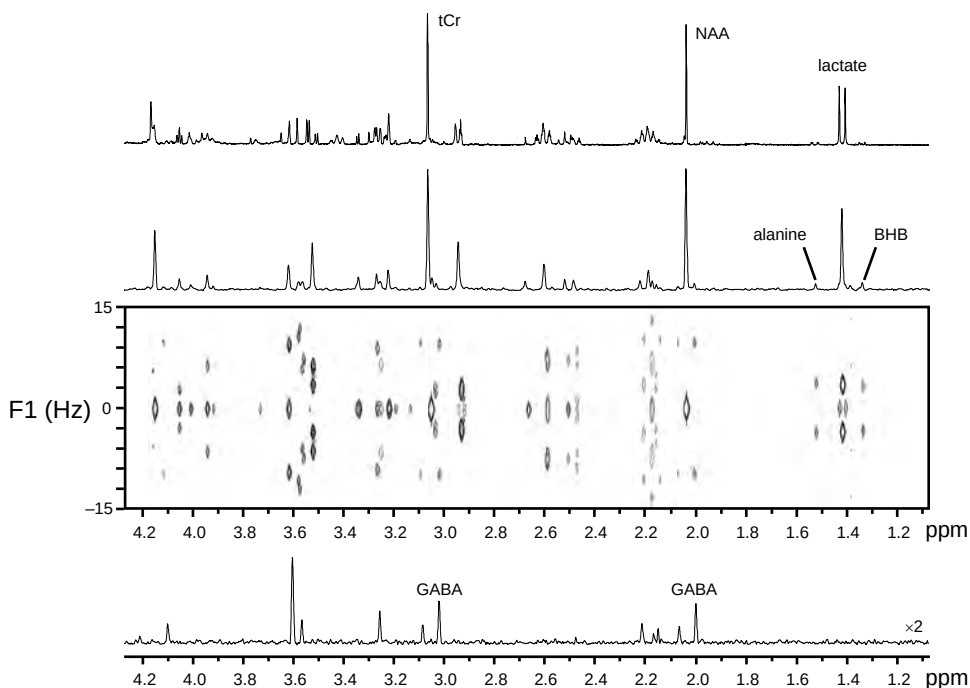


**Figure 8.27** 2D J-resolved NMR spectrum (absolute value) of PCA extract from rat brain dissolved in  $D_2O$  at 298 K, acquired at 300 MHz. The proton-decoupled  $^1H$  spectrum (middle trace), obtained from the projection of the 2D NMR spectrum onto the F2 axis, has a significantly increased resolution when compared with a regular (nondecoupled)  $^1H$  NMR spectrum (top trace). The 2D J-resolved dataset also allows the construction of traces at  $F1 \neq 0$  to selectively observe specific metabolites as shown for GABA (bottom trace, calculated for  $9 \text{ Hz} < F1 < 15 \text{ Hz}$ ).

(uncoupled) resonances (e.g. PCr/Cr) can be selectively observed by calculating a trace for that particular compound at  $\omega_1 \neq 0$ .

2D J-resolved spectroscopy is readily performed *in vivo* [94–96], for example by a double spin-echo sequence. The PRESS sequence achieves spatial localization and allows the incrementation of the echo-time (which equals the evolution period  $t_1$ ). Several *in vivo* feasibility studies have shown the utility of this sequence for *in vivo* 2D J-resolved spectroscopy. Figure 8.28 shows a typical *in vivo* example of localized, J-resolved spectroscopy on a F98 glioma in rat [95].

### 8.10.3 Two-dimensional Exchange Spectroscopy

The chemical exchange between phosphocreatine and ATP through the creatine kinase enzyme can be studied with selective magnetization transfer experiments as described in Section 3.3.1. As a more general alternative, (chemical) exchange reactions can be studied with 2D exchange spectroscopy. Unlike the previously discussed correlation spectroscopy

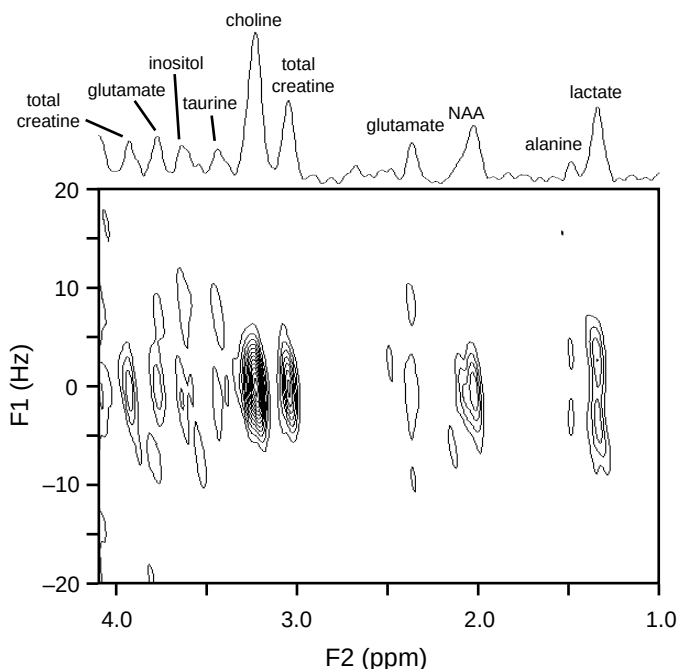


**Figure 8.28** In vivo 2D  $J$ -resolved  $^1\text{H}$  NMR spectrum obtained from a F98 glioma in rat brain. Spatial localization ( $64\ \mu\text{l}$ ) was achieved with PRESS, in which the second echo-time (surrounding the second  $180^\circ$  pulse) equals the  $t_1$  evolution period.  $t_1$  was incremented as  $n \cdot 17\ \text{ms}$  ( $n = 0, 1, \dots, 16$ ). The spectrum was tilted by  $45^\circ$  after which the projection (summation) of the effectively  $^1\text{H}$ -decoupled NMR spectrum onto the  $F2$  axis could be calculated. Although the spectral resolution is lower than for the in vitro spectrum as shown in Figure 8.27, many resonances can be clearly distinguished, like alanine from lactate. (Courtesy of W. Dreher.)

techniques, 2D exchange NMR [97–99] does not require that the spins under investigation are scalar-coupled. They merely have to be coupled through chemical exchange and/or cross-relaxation. For this reason the principles of 2D exchange NMR experiments are readily explained by a classical vector model, as shown in Figure 8.29B. Figure 8.29A shows the 2D exchange NMR [or nuclear Overhauser effect spectroscopy (NOESY)] sequence. Prior to excitation, two spins A (black line) and B (gray line) are in thermal equilibrium (Figure 8.29B). Following excitation, the spins precess at their own Larmor frequencies  $\omega_A$  and  $\omega_B$  during the evolution period  $t_1$ . The second  $90^\circ$  RF pulse returns part of the transverse magnetization to the  $z$  axis. The other part remains in the transverse plane and can be removed by phase cycling or magnetic field gradients. During the mixing period  $\tau_m$ , the longitudinal components of A and B can exchange coherence by chemical exchange and/or cross-relaxation. Following the last  $90^\circ$  pulse, transverse magnetization will be detected during the  $t_2$  period. Coherences which have not been exchanged during  $\tau_m$ , will have the same resonance frequencies during  $t_1$  and  $t_2$  and give rise to diagonal peaks. Coherences which have exchanged from A to B during  $\tau_m$ , will have different resonance frequencies during  $t_1$  and  $t_2$  and give rise to a cross peak at  $(\omega_A, \omega_B)$ . Similarly,

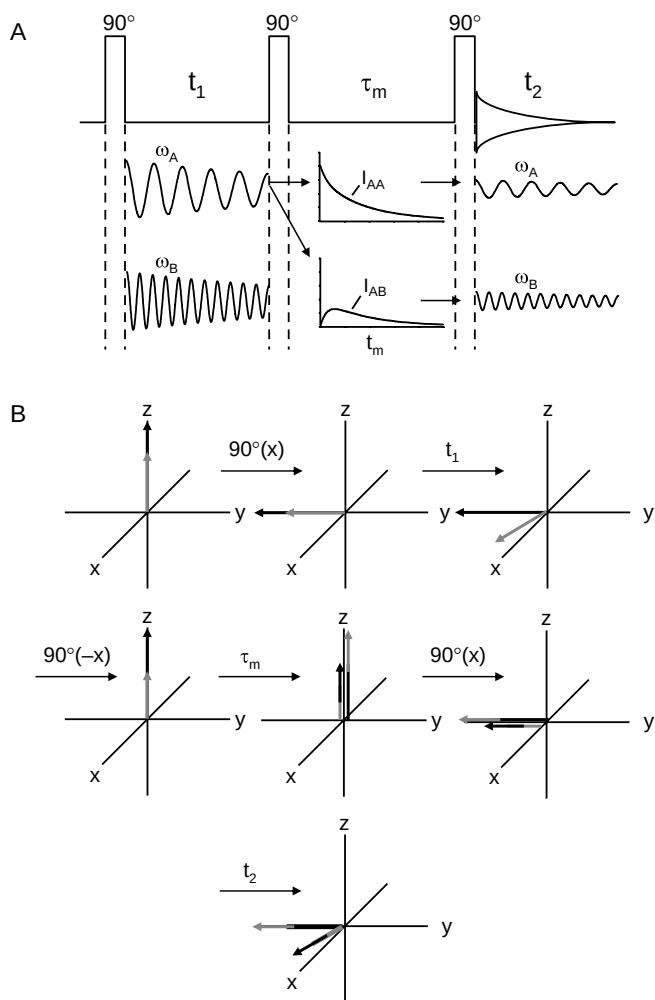


**Figure 8.29** (A) Transfer of frequency-labeled longitudinal magnetization in 2D exchange spectroscopy for a two-sided (A, B) system. After excitation, the spins evolve in the transverse plane at their own Larmor frequency,  $\omega_A$  or  $\omega_B$ , during  $t_1$ , i.e. the spins are frequency-encoded during  $t_1$ . During  $\tau_m$ , the amplitudes of the coherences which give rise to diagonal peaks,  $I_{AA}$  and  $I_{BB}$ , decay bi-exponentially due to exchange and longitudinal relaxation. The amplitudes of the coherences which give rise to cross peaks,  $I_{AB} = I_{BA}$ , first increase due to exchange before decaying because of spin-lattice relaxation. During  $t_2$ , the coherences are again frequency-encoded. Coherences which have undergone exchange during  $\tau_m$  have different frequencies during  $t_1$  and  $t_2$  and therefore give rise to cross peaks at  $(\omega_A, \omega_B)$  and  $(\omega_B, \omega_A)$ . (B) Graphical representation of exchange during 2D exchange NMR spectroscopy. During the evolution period  $t_1$  the spins are frequency labeled, after which they are rotated along the longitudinal axis. During the  $\tau_m$  period, the exchange partners (black and gray lines for A and B, respectively) exchange polarization, which will be frequency labeled for the second time during  $t_2$ . The  $t_1$  and  $t_2$  frequencies are not the same for nuclei that have undergone exchange and will thus give rise to cross peaks.



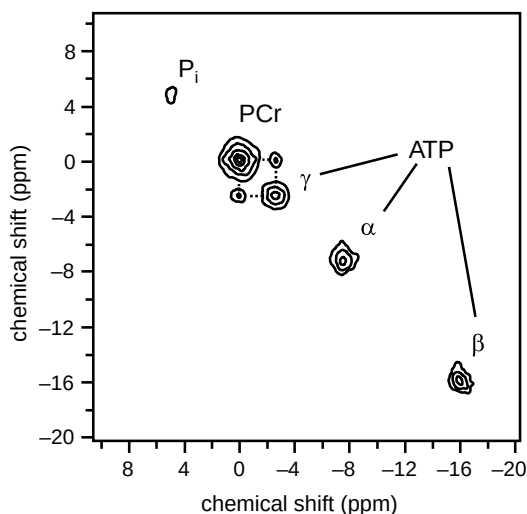


**Figure 8.30** 2D exchange spectrum obtained from rat muscle *in vivo* at 4.7 T. The mixing time  $\tau_m$  was 600 ms, while the 2D data matrix was constructed from 64  $t_1$  increments and 128 complex points during acquisition. Each increment involved 16 averages. The data are presented in absolute value mode. Because of chemical exchange between PCr and  $\gamma$ -ATP two cross peaks are visible.

a cross peak arises at  $(\omega_B, \omega_A)$  for coherences that have been transferred from B to A. The  $\tau_m$  dependence of the diagonal and cross peak signal intensities depends on the intrinsic  $T_1$  relaxation times of spins A and B as well as the exchange rate  $k_{AB}$  between them. Typical signal build-up curves are shown in Figure 8.29A. Figure 8.30 shows the application of 2D exchange NMR to study the chemical exchange between phosphocreatine (PCr) and ATP in rat skeletal muscle *in vivo* as catalyzed by creatine kinase. On the diagonal of the 2D NMR spectrum the common resonances of ATP, PCr and inorganic phosphate  $P_i$  can be recognized, as they would also occur in a regular 1D  $^{31}\text{P}$  NMR spectrum. Off the diagonal, at the intersection between the frequencies for PCr and  $\gamma$ -ATP, two additional resonances can be observed which are due to the chemical exchange of a phosphate group between PCr and  $\gamma$ -ATP. Given the relaxation rates for PCr and ATP, as well as the exchange rate, the cross peak signal intensity reaches a maximum at  $\tau_m = 0.5\text{--}1.0$  s for the PCr/ATP system in skeletal muscle *in vivo*.

Although 2D NOESY experiments are used to study chemical exchange processes, the main application is in the study of through-space dipolar coupling which can reveal the spatial positions of spins relative to each other. This experiment is crucial in high-resolution NMR studies of macromolecules in which one wants to obtain the spatial structure of the compound under investigation. However, since *in vivo* NMR spectroscopy is mainly detecting resonances from small metabolites (with a known spatial structure), this application has limited value.

When coupled resonances are involved in 2D exchange NMR experiments, artifacts may arise from ZQCs present during the  $\tau_m$  period which can not be removed by phase cycling or magnetic field crusher gradients. One solution to reduce these artifacts is to co-add a

number of 2D exchange NMR spectra with slightly different  $\tau_m$  values, such that ZQCs (which are sensitive to chemical shift evolution) are cancelled [100]. Alternatively, one can dephase ZQCs in an inhomogeneous  $B_1$  magnetic field [101].

## 8.11 Exercises

- 8.1** Consider an INEPT sequence with delays optimized for a  $\text{CH}_2$  spin system with  $^1J_{\text{CH}} = 135$  Hz.

- A** Calculate the polarization transfer signal intensity relative to direct detection for a CH spin system with  $^1J_{\text{CH}} = 165$  Hz. (Assume ideal excitation/refocusing pulses.)
- B** Calculate the delays that would give optimal signal intensity for the CH spin system and calculate the resulting signal intensity for the  $\text{CH}_2$  spin system.
- C** Repeat the calculations under (A) and (B) for a refocused INEPT sequence.

- 8.2** Consider the spectral editing sequence of Figure 8.2A with  $\text{TE} = 68$  ms. For simplicity assume AX spin systems for GABA and macromolecules with  $^1J_{\text{AX}} = 14.7$  Hz. Furthermore, assume GABA and macromolecule concentrations of 1.0 and 2.0 mM, respectively.

- A** For a 25 ms Gaussian refocusing pulse the refocusing profile in terms of nutation angle  $\alpha$  (in degrees) at frequency  $\nu$  (in Hz) is given by:

$$\alpha(\nu) = 180^\circ \times \exp(-(\nu - \nu_0)^2/1000)$$

Assuming  $T_2(\text{GABA}) = 150$  ms and  $T_2(\text{macromolecules}) = 50$  ms, calculate the fraction of macromolecules contribution to the total 'edited' GABA resonance at 1.5 T, 4.0 T and 7.0 T using a refocusing strategy that is symmetrical around the total creatine resonance. Assume ideal  $90^\circ$  and  $180^\circ$  non-selective pulses and regular (unperturbed) scalar coupling evolution during RF pulses.

- 8.3** In the absence of  $T_2$  relaxation and chemical shift displacement artifacts, the optimal echo time for lactate (or any other AX spin system) detection by spectral editing is  $1/J$  which equals 144 ms for  $J = 6.9$  Hz.

- A** Derive an analytical expression for the optimal echo-time to detect the edited signal (AX spin system) in the presence of  $T_2$  relaxation.
- B** Calculate the optimal echo time for lactate when the  $T_2$  relaxation is equal to 100 ms.
- C** Calculate the gain in signal obtained with the optimal echo-time when compared with the standard echo time  $\text{TE} = 1/J$ .
- D** In the absence of  $T_2$  relaxation, calculate the loss in maximum lactate signal intensity when the echo time surrounding the first  $180^\circ$  pulse in Figure 8.6B equals 20 ms.
- E** Repeat the calculation of (D) for GABA detection.

- 8.4**  $[3\text{-}^{13}\text{C}]\text{Lactate}$  can be considered a  $\text{I}_3\text{S}$  spin system with  $J_{\text{IS}} = 127.5$  Hz.

- A** When using a refocused INEPT sequence (see Figure 8.14B) for signal detection, derive an analytical expression for the evolution time  $t_2$  between the polarization transfer step and acquisition which gives the highest detectable (in-phase)  $[3\text{-}^{13}\text{C}]\text{lactate}$  signal.

- B** Using the optimal  $t_1$  and  $t_2$  delays, calculate the signal increase of  $[3\text{-}^{13}\text{C}]\text{lactate}$  detection using polarization transfer over direct  $^{13}\text{C}$  detection (without nOe enhancement). Assume ideal  $90^\circ$  and  $180^\circ$  RF pulses and  $\text{TR} = 2000$  ms,  $T_{1\text{H}} = 1500$  ms,  $T_{1\text{C}} = 1200$  ms,  $T_{2\text{H}} = 150$  ms and  $T_{2\text{C}} = 250$  ms.
- 8.5** Derive expressions for the scalar evolution of  $\text{IS}_2$  and  $\text{IS}_3$  spin systems [i.e. derive Equations (8.9)–(8.12)].
- 8.6** Consider the LASER based POCE sequence shown in Figure 8.12B.
- A** Derive expressions for the two delays surrounding the last  $180^\circ$   $^1\text{H}$  RF pulse in terms of  $^1\text{H}$  and  $^{13}\text{C}$  pulse lengths, gradient rise time and heteronuclear scalar coupling.
- B** Discuss the effects on the spectral editing performance of having the  $^1\text{H}$  and  $^{13}\text{C}$   $180^\circ$  pulses on at the same time during the slice selection gradient.
- 8.7** **A** Sketch the 2D J-resolved NMR spectrum (following a  $45^\circ$  tilt correction) for aspartate under the assumption of weak scalar coupling interactions.
- B** Sketch the absolute valued COSY spectrum for aspartate under the same assumption.
- 8.8** **A** A NMR spectroscopist wants to measure the  $T_2$  relaxation time constant of lactate ( $^3J_{\text{HH}} = 6.9$  Hz) in rat skeletal muscle by increasing the total echo-time of the J-difference sequence in Figure 8.2A from 144 ms to 288 ms. A fit of the integrated signal intensity versus echo-time data reveals an unexpectedly short  $T_2$  relaxation time of 35 ms. Explain this result.
- B** Propose at least three methods to determine the real lactate  $T_2$  relaxation time constant.
- 8.9** The RF carrier frequency at 7.05 T is adjusted to 300.142002 MHz to create an on-resonance condition for water. The methyl creatine resonance appears  $-502$  Hz off-resonance.
- A** Calculate the frequency of the selective  $180^\circ$  refocusing pulses in order to selectively edit GABA-H4.
- B** Calculate the frequency of the selective  $180^\circ$  refocusing pulses at the spectral position mirrored from GABA-H3 relative to macromolecular resonance M4.
- 8.10**  $\text{B}_1$ -insensitive spectral editing pulse [BISEP, [102]] is a single-scan spectral editing method for heteronuclear spin systems. While originally executed with adiabatic RF pulses, the sequence can also be described with regular pulses as :  $90^\circ_{+\text{x}}(^1\text{H}) - t - 180^\circ_{-\text{x}}(^1\text{H}/^{13}\text{C}) - t - 90^\circ_{+\text{x}}(^1\text{H})$ , i.e. the  $90^\circ$  pulses are applied on the  $^1\text{H}$  channel only, while the  $180^\circ$  pulse is applied on both channels.
- A** Derive an expression for the coherences of uncoupled and heteronuclear scalar coupled spin systems at the end of a BISEP sequence.
- B** Propose an extension to the sequence that (1) leads to in-phase detection of heteronuclear scalar-coupled spin systems and (2) gives an additional suppression of uncoupled spins.
- 8.11** Prior to spectral editing the J-difference editing sequence as shown in Figure 8.6B is used for shorter echo-time MRS by reducing the pulse length of the editing pulses to zero, such that the echo-time reduces from 144 ms to 84 ms. All other parameters, like the repetition time (3000 ms), magnetic field crusher gradient duration (4 ms) and strength ( $300 \text{ mT m}^{-1}$ , see Figure 6.30A for the exact placement and orientation

of the 'crushers') and volume selection gradients ( $5 \times 5 \times 5$  mm volume) are being kept constant. The creatine methyl resonance is observed with a SNR of 40.

**A** During the spectral editing procedure for lactate (TE = 144 ms, 30 ms editing pulses), the creatine resonance is observed with a SNR of 16. Given that the creatine  $T_2$  time constant is 250 ms, give a likely explanation for the large drop in SNR and give a possible remedy.

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# 9

## Spectral Quantification

### 9.1 Introduction

Spectra obtained by NMR spectroscopy can, in principle, be used to derive absolute concentrations, expressed in  $\text{mmol L}^{-1}$  or  $\mu\text{mol g}^{-1}$  of tissue, in animal and human tissues *in vivo*. This originates from the fact that the thermal equilibrium magnetization  $M_0$  is directly proportional to the number of spins  $n$ , as expressed in Equation (1.27), which is proportional to the molar concentration. However, in a NMR experiment the thermal equilibrium magnetization  $M_0$  is not detected directly, but rather an induced current proportional to the transverse magnetization is observed. The signal from a metabolite  $M$  induced in a receiver coil following a particular NMR sequence is given by:

$$S_M = NS \times RG \times \omega_0 \times [M] \times V \times f_{\text{sequence}} \times f_{\text{coil}} \quad (9.1)$$

where  $NS$  and  $RG$  equal the number of scans and receiver gain setting,  $\omega_0$  is the Larmor frequency,  $[M]$  is the molar concentration,  $V$  is the volume size and  $f_{\text{sequence}}$  and  $f_{\text{coil}}$  are complicated functions describing the signal modulations due to the NMR pulse sequence and RF coil, respectively.  $f_{\text{sequence}}$  will depend on the repetition time  $TR$ , the echo-time  $TE$ , the number and type of RF pulses, as well as the  $T_1$  and  $T_2$  relaxation times.  $f_{\text{coil}}$  contains factors related to the geometry and quality of the RF coil, like the quality factor  $Q$  and the filling factor (see also Chapter 10). Since several of the coil related factors in the  $f_{\text{coil}}$  function are unknown, direct calculation of the metabolite concentration  $[M]$  from the detected signal  $S_M$  is not possible. In practice all quantification methods utilize a calibration or reference compound of known concentration  $[R]$  to which the metabolite signals are referenced such that the metabolite concentration can be calculated according to:

$$[M] = [R] \frac{S_M}{S_R} C_{MR} \quad (9.2)$$

where  $S_R$  is the detected signal from the reference compound and  $C_{MR}$  is a correction factor accounting for differences in relaxation times  $T_1$  and  $T_2$ , diffusion, gyromagnetic ratio, magnetic susceptibility, spatial position relative to the coil and, in general, any other differences between the reference compound and the metabolites.

Since the calculation of a reliable correction factor  $C_{MR}$  can be very time-consuming, many reports on *in vivo* MRS, especially in the clinical field, have used metabolite ratios as an alternative. Since the correction factor  $C_{MR}$  is identical for both metabolites, a ratio will not be sensitive to several of the unknown parameters in Equation (9.1). However, unlike absolute concentrations, metabolite ratios can not give unambiguous information about metabolic changes, as encountered in many disorders and pathologies studied with *in vivo* NMR. For example, a choline-to-creatine ratio change from 1.0 to 2.0 could mean that (1) the choline concentration has increased, (2) the creatine concentration has decreased, (3) both the choline and creatine concentration has changed or (4) relaxation parameters for either or both metabolites have changed. In order to reach conclusive statements about metabolic changes it is therefore crucial that absolute concentrations are obtained. Furthermore, in order to obtain quantitative fluxes through metabolic pathways as detected from  $^{13}\text{C}$ -label turnover studies (see Chapter 3), knowledge of absolute metabolite concentrations is imperative.

Figure 9.1 shows a flow chart for the absolute quantification of metabolites, which can generally be decomposed into four separate steps. During the data acquisition step, the longitudinal magnetization in the volume of interest is converted to transverse magnetization, which generates a free induction decay (FID) in a receiver coil. While data acquisition strategies are discussed throughout the book, Section 9.2 summarizes the most critical considerations for quantification purposes. Before the NMR resonances can be quantified, the acquired FID signal is often pre-processed (Section 9.3) during which several improvements can be made, typically related to the resonance line shapes and baseline. In order to reduce processing time, as well as to eliminate ambiguous quantification results, data exclusion is an essential step of data pre-processing. Section 9.2 summarizes several criteria that can be used for automated data exclusion. Acceptable time- or frequency-domain data are subsequently quantified to yield relative metabolite resonance areas, uncorrected for  $T_1$ ,  $T_2$  and pulse sequence dependent modulations. The data processing step, as well as the data calibration step, often needs additional data acquired in a separate experiment. Section 9.4 will give an overview of the most commonly used data processing algorithms. The final step concerns the calculation of the  $C_{MR}$  correction term in Equation (9.2) for the metabolite and reference signals. This includes corrections for  $T_1$ ,  $T_2$ ,  $B_1$  and related parameters and will be summarized in Section 9.5. Finally the metabolite concentration can be calculated according to Equation (9.2), possibly followed by a partial volume correction to account for compartmental metabolism. While Figure 9.1 shows the flow chart for single volume MRS data, a similar flow chart can be constructed for MRSI data.

## 9.2 Data Acquisition

The first and arguably most important step in obtaining reliable metabolite concentrations concerns proper acquisition of the raw time-domain signal. The quality of the acquired signal in terms of sensitivity, resolution, artifacts and general information content directly

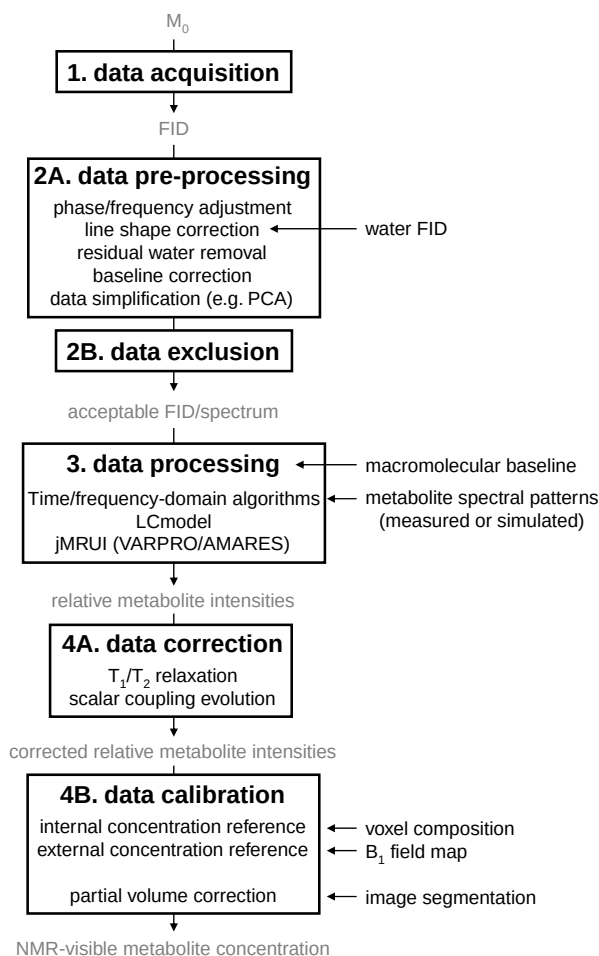


**Figure 9.1** Flow chart for processing and quantification of NMR signals. Following data acquisition of a time-domain signal (e.g. FID), the signal undergoes a pre-processing and exclusion step to obtain signals that are acceptable for further processing. In the data processing step, the relative amplitudes of the metabolite signals are obtained, which are then corrected for a number of effects, including relaxation. The corrected relative metabolite intensities are then compared with a reference signal to arrive at a metabolite concentration as detected by NMR. The necessity of the input signals on the right depend on the exact nature of each step and are discussed in the text.

determines the accuracy and reliability by which metabolite levels can be estimated. While many of these considerations are discussed throughout the other chapters, a short summary of important features concerning optimal data acquisition will be given.

- (1) Magnetic field homogeneity. The most important parameter determining the spectral quality is the magnetic field homogeneity across the region of interest. Inhomogeneity

in the external magnetic field leads to a spread in resonance frequencies which will directly lead to a broadening of spectral lines and thus a decrease in spectral resolution. In order to separate creatine from phosphocreatine in a high-field proton spectrum, the homogeneity needs to be better than 0.01 ppm. Less stringent requirements are encountered for separate detection of glutamate and glutamine ( $\Delta \sim 0.1$  ppm) and even more spread of frequencies can be allowed for the separation of choline from creatine ( $\Delta \sim 0.2$  ppm). However, regardless of the particular resonances, the uncertainty in the amplitude estimation of a broader line is always higher than that for a narrower line. Improving the magnetic field homogeneity and hence the spectral resolution is therefore crucial for the most reliable parameter estimation.

The principle of magnetic field homogeneity optimization through shimming is discussed in Chapter 10. In general it can be stated that optimal magnetic field homogeneity across small single volumes can be adequately optimized with first- and second-order spherical harmonic shims. When acquiring data from 2D slices, the magnetic field homogeneity can be further improved by third-order spherical harmonic shims. With the current state of spherical harmonic shimming technology, perfect magnetic field homogeneity across the human or animal brain can not be expected, as the encountered inhomogeneity can not be approximated with low-order spherical harmonic shims. Chapter 10 will discuss alternative strategies to achieve whole-brain magnetic field homogeneity.

- (2) Spatial localization. The quality of spatial localization is important for several reasons. Firstly, accurate spatial localization eliminates unwanted signals from outside the volume of interest, for example signal from extracranial lipids, as well as signals from areas with an inhomogeneous magnetic field. Secondly, accurate spatial localization allows the determination of the exact voxel content (e.g. fraction of gray matter, white matter and CSF) and allows a reproducible positioning of the voxel. Both considerations are crucial for longitudinal or population-based studies. Chapter 6 described the considerations involved with spatial localization, including the chemical shift displacement artifact, effects of imperfect RF pulses and insufficient magnetic field gradient dephasing.
- (3) Water suppression. The quality of water suppression is often directly related to the magnetic field homogeneity, as well as the quality of spatial localization. Water suppression techniques are discussed in Chapter 6 and can typically achieve suppression factors of  $>10000$ . It should be noted that while many papers on water suppression strive for perfect water suppression, this is in reality not required. Water suppression should achieve a reduction in the water resonance to a degree that (1) water does not limit the dynamic range of the receiver, (2) water does not significantly affect the spectral baseline and (3) vibration-induced sidebands of the water are negligible. More important than the absolute degree of suppression is the shape of the water resonance. A relatively large, but approximately Lorentzian shaped water resonance is readily removed by post-acquisition methods (see Section 9.3.3). However, an incompletely dephased water resonance, spread out over a wide frequency range will lead to a significant baseline distortion which is not readily removed by post-acquisition methods.

- (4) Sensitivity. The sensitivity of the FID or spectrum of course directly determines the accuracy by which parameters can be estimated from the data. Given a certain noise level, parameters like amplitude and frequency can only be estimated to a certain accuracy, independent of the quantification method employed. Therefore, when the minimum SNR to estimate a certain parameter with a given accuracy can not be achieved, only a change in data acquisition can improve the data. For example, a  $32 \times 32$  MRSI dataset with a SNR for NAA of 5 leads to an uncertainty in the NAA amplitude of 20 %. The accuracy of NAA amplitude estimation is most optimally improved by increasing the SNR during acquisition (for example by increasing the number of averages or decreasing the spatial resolution).

While the *in vivo* NMR community currently does not have a consensus on guidelines for acceptable NMR spectra, a number of criteria for rejection of unacceptable spectra can readily be formulated. Unless specific processing methods are followed (e.g. post-acquisition lipid removal from MRSI data) MRS or MRSI spectra (of the brain) should not be considered for further automated data processing when:

- (1) The residual water resonance is not well-defined and dominates the baseline underlying the metabolite resonances.
- (2) The spectrum is characterized by large signals from extracranial lipids.
- (3) The spectrum contains unexplained signals, like spikes, ghosts or other artifacts.
- (4) The NMR resonances are highly asymmetric, even following post-acquisition eddy current correction.
- (5) The metabolite resonances are wider than a certain ppm range. The exact number will strongly depend on the application. For example, for short echo-time proton MRS of neurotransmitter metabolism, the line widths should be smaller than 0.04 ppm in order to reliably separate glutamate from glutamine at high magnetic fields. However, for long echo-time proton MRS, the line width can be as wide as  $\sim 0.1$  ppm before the total choline and total creatine resonances overlap.

NMR spectra containing one or more of the mentioned features should undergo a rigorous manual inspection by an expert NMR spectroscopist.

### 9.3 Data Pre-processing

Following data acquisition the next step in the metabolite quantification process concerns the estimation of resonance areas, as these are ultimately related to the metabolite concentration. However, before spectral quantification algorithms are applied to the data, as will be discussed in Section 9.4, a data pre-processing step is often desired in which several aspects of the acquired data can be improved. Data pre-processing can include phasing and frequency alignment of multiple spectra, eddy current and line shape corrections, removal of the residual water resonance, baseline correction and the exclusion of additional unacceptable data.

### 9.3.1 Phasing and Frequency Alignment

Depending on the application, phasing and frequency alignment of NMR spectra can range from a cosmetic adjustment to a crucial procedure. During extensive data acquisition periods, individual storing and phasing NMR spectra prior to summation can become important in the presence of motion. As discussed in Section 3.4.2 for diffusion measurements (see also Figure 3.18), macroscopic motion can lead to zero-order phase changes. While phase changes do not have to lead to signal loss per se, the addition of spectra with different phases during extensive signal averaging will lead to signal loss due to phase cancellation. Phase correction prior to summation can eliminate this signal loss.

In Chapter 8 it was shown that frequency alignment of NMR spectra is important to (1) obtain the best possible spectral resolution and (2) avoid subtraction artifacts during spectral editing (see also Figure 8.9). For other applications like MRSI, phase correction and frequency alignment are not crucial, but they can improve further data processing steps. While most modeling algorithms (see Section 9.4) can readily account for phase and frequency variations, the algorithms are typically faster and more reliable when the phase and frequency variations can be restricted to a narrow range. Imposing boundaries on phase and frequency ranges is one of several forms of prior knowledge and will be discussed in more detail in Section 9.4.2. The large amount of data associated with MRSI data necessitates the use of automated phasing and frequency alignment algorithms [1, 2].

### 9.3.2 Line Shape Correction

Many resonance quantification algorithms, as discussed in Section 9.4, model or approximate the measured NMR spectrum as the sum of theoretical (Lorentzian, Gaussian, Voigt) line shapes. Unfortunately, NMR resonance lines obtained *in vivo* are typically not well behaved due to residual eddy currents, magnetic field inhomogeneity and multi-exponential relaxation. While some algorithms can impose an arbitrary line shape common to all metabolites (see Section 9.4), the line shape distortions can also be removed or reduced in a pre-processing step. Residual eddy currents lead to a time-varying magnetic field during signal acquisition which distorts the NMR resonances as shown in Figure 9.2A. While pre-emphasis and  $B_0$  compensation offer a hardware-based solution to reducing the time-varying magnetic fields (see Section 10.4.2), residual eddy currents are typically unavoidable. A simple and convenient post-acquisition correction method [3–5] utilizes the fact that the time-varying magnetic fields are the same for all resonances. Therefore, when an unsuppressed on-resonance water signal is acquired, any temporal frequency variations in the water time-domain signal can be attributed to residual eddy currents. Applying the opposite phase modulation to the metabolite spectrum thus automatically cancels phase modulations due to residual eddy currents (Figure 9.2C). Since the water and metabolite spectra are acquired with the same NMR sequence, the eddy current correction automatically performs a first-order phase correction which provides a tool for the automated phasing of large MRSI datasets.

Line shape distortions due to magnetic field inhomogeneity can also be removed by dividing the metabolite FID by the water signal envelope [6], a technique referred to as QUALITY (quantification improvement by converting line shapes to the Lorentzian type).

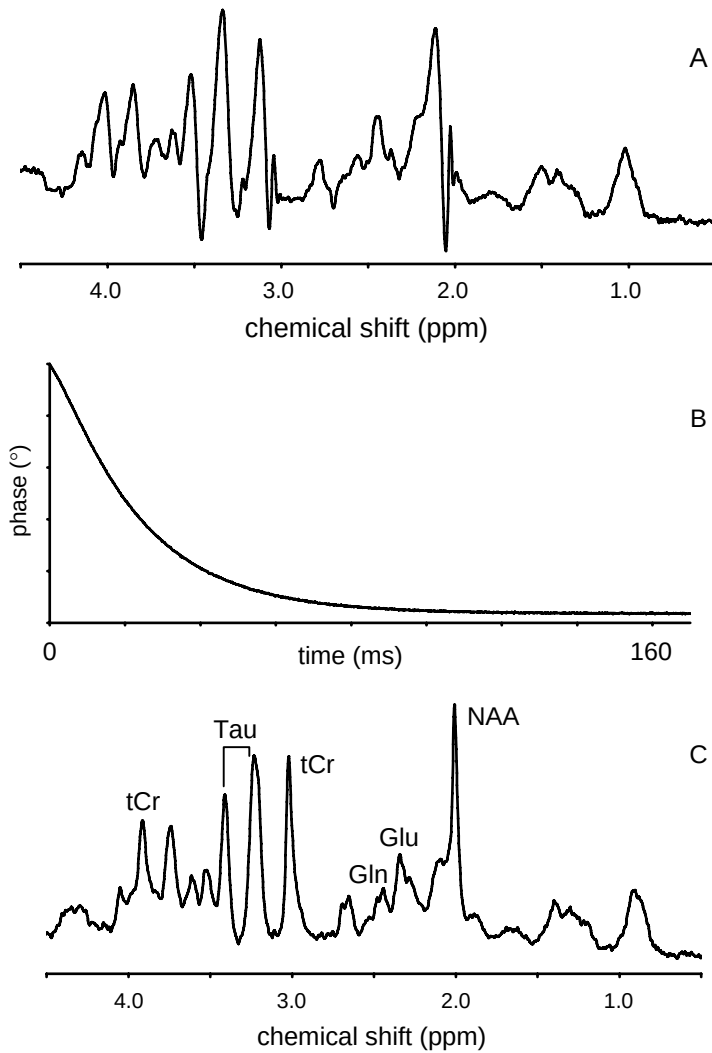


**Figure 9.2** Principle of post-acquisition correction of temporal magnetic field variations. (A)  $^1\text{H}$  NMR spectrum acquired from rat olfactory bulb at 9.4 T in the presence of residual magnetic field variations originating from eddy currents. (B) Phase evolution of the water signal obtained with the same sequence as used to acquire the signal in (A). (C) Phase-corrected signal.

Alternatively, information about local magnetic field homogeneity can be obtained from high-resolution magnetic field maps [7].

### 9.3.3 Removal of Residual Water

With the use of first- and second-order shims, the macroscopic magnetic field homogeneity over a small single voxel in the human brain can, in general, be close to perfect. As a result,

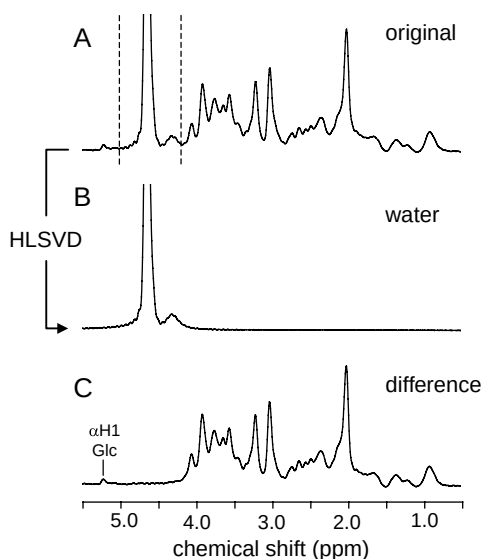


**Figure 9.3** Post-acquisition water removal through the use of a HLSVD algorithm. (A) Short echo-time  $^1\text{H}$  NMR spectrum acquired from human brain at 4.0 T, displaying a relatively small amount of residual water signal. (B) Theoretical water spectrum calculated from the singular values generated by a HLSVD of spectrum (A) of which the frequencies fall within the indicated boundaries. (C) Difference between (A) and (B).

the large water resonance can typically be suppressed to well below the metabolite levels. However, in the case of MRSI or even for single volume MRS in organs outside the brain, the magnetic field homogeneity is typically not perfect, leading to a significant residual water resonance (Figure 9.3A). When only the spectral region upfield from water is analyzed (i.e. 1.0–4.2 ppm), the water does not require any further considerations provided that it does not distort the baseline. However, in cases where the water resonance does distort the baseline or when the complete spectral range is included in the fitting algorithm, the residual water resonance must be removed. While there are several post-acquisition water removal methods available, one of the more reliable techniques that readily allows automation relies on a singular value decomposition (SVD) of the FID signal [8–12]. The largest singular values correspond to the largest resonances in the NMR spectrum. When, following a SVD, the singular values with frequencies corresponding to the water spectral region (indicated in Figure 9.3A) are selected, the corresponding reconstructed NMR spectrum largely resembles the water resonance. Subtraction of the reconstructed water (Figure 9.3B) from the original spectrum yields a water-suppressed metabolite spectrum as shown in Figure 9.3C. As SVD requires little to no user input, it is often referred to as a ‘black-box’ method. And while ‘black-box’ methods do typically not allow the enforcement of prior knowledge information (see Section 9.4.2), they do lend themselves perfectly for automated signal removal [8, 9], especially when executed as the fast Hankel–Lanczos (HLSVD) variant [11, 13].



### 9.3.4 Baseline Correction

NMR spectra from tissue *in vivo* often display sharp resonances from metabolites superimposed on a ‘baseline’ of broader resonances. While the baseline is often approximated and removed by polynomial or cubic-spline based fitting, it is important to realize that most baselines have physical or physiological origins. Targeting the baseline origins, either through measurement or sequence design, often leads to a more robust data acquisition and quantification method. In unlocalized NMR spectroscopy (i.e. pulse-acquire methods), a significant part of the baseline can often be attributed to solids (e.g. plastics) in the RF coil assembly [14]. In unlocalized  $^{31}\text{P}$  NMR, the solids in the human and animal skull lead to a very broad and intense baseline, especially when acquired with surface coil reception. In these cases, the baseline can be significantly reduced by full 3D localization or delayed acquisition. In  $^1\text{H}$  NMR spectra, the metabolites are superimposed on a baseline of macromolecular compounds, as discussed in Section 2.2.25. Delayed acquisition (e.g.  $\text{TE} > 80$  ms) removes the macromolecules due to their shorter  $T_2$  relaxation times ( $\sim 30$  ms), at the expense of loss of information of many scalar-coupled resonances. Macromolecular resonances can also be reduced by utilizing the difference in  $T_1$  relaxation between metabolites and macromolecules [15]. However, rather than eliminating the baseline, the macromolecules hold valuable information in their own right, having demonstrated altered spectral patterns in stroke [16] and tumors [17]. Instead of suppressing the macromolecules through  $T_1$  differences, they can also be enhanced by the same mechanism (see Figure 2.21). As will be demonstrated in Section 9.4.3, once the macromolecular resonances are accounted for, the actual baseline in short echo-time proton NMR spectra is insignificant.

However, in some cases, like unlocalized  $^{31}\text{P}$  NMR spectroscopy of the brain (Figure 9.4A) a large, undesired baseline is unavoidable. In these cases, the baseline can be removed in a pre-processing step by approximating and subtracting the baseline with a polynomial fit (Figure 9.4B). Alternatively, a so-called convolution difference method can be applied in which the original spectrum is line-broadened by 150–300 Hz. This will broaden the metabolite signals to the point that they merge with the broad baseline. The broadened spectrum can then be subtracted from the original spectrum to form a baseline-suppressed spectrum. However, convolution-difference methods do not perform as well as polynomial fitting routines, as the metabolite signals are only broadened, but not completely removed. Furthermore, the baseline is typically also affected by the broadening leading to an incomplete subtraction.

## 9.4 Data Quantification

The third step in the estimation of metabolite levels from *in vivo* NMR spectra concerns the calculation of the relative resonance areas, as these are directly proportional to the number of spins and hence the concentration. The quantification of NMR resonances covers a wide range of methods including integration, noniterative ‘black-box’ methods [8, 18] and iterative, user-dependent model fitting algorithms imposing various amounts of prior knowledge [18–21]. Before these methods are discussed in more detail, a brief summary of the spectral parameters in the frequency- and time-domain will be given.

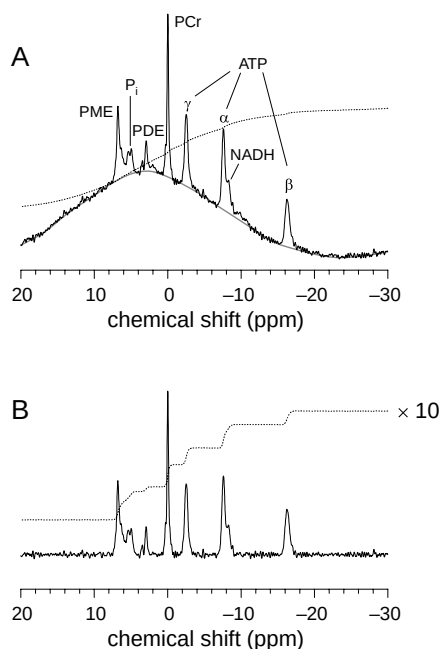


**Figure 9.4** Integration of unlocalized  $^{31}\text{P}$  NMR spectra of new born piglet brain in vivo at 4.7 T. (A) Without additional pre-processing, direct integration will result in an overestimation of the metabolite resonances since the integral signal is dominated by a broad, underlying resonance. (B) Baseline correction based on cubic spline interpolation results in an acceptable baseline, making integration of resonances feasible. Note that partially overlapping resonances can not be accurately separated by integration (e.g. PME and  $\text{P}_i$  or  $\alpha$ -ATP and NADH).

### 9.4.1 Time- and Frequency-domain Parameters

A decision encountered early on in the quantification of NMR resonances is whether the analysis should be performed in the time- or frequency-domain. However, with the Fourier transformation being a linear operation, it should be realized that theoretically the two domains are equivalent and that all parameters can be equally well estimated in either domain [22]. Nevertheless, experimental imperfections, proper visualization and computational considerations may favor one domain over the other. For example, removal of distorted data points at the beginning of the time-domain signal does not significantly affect time domain analysis as the removed data points can be recovered through data extrapolation. However, missing time-domain data points can lead to complicated modulations in the frequency-domain. Furthermore, time-domain calculations are computationally less demanding (i.e. multiplication) than equivalent calculations in the frequency-domain (i.e. convolution). However, regardless of the analysis domain, the visualization of the data and quantification results is always performed in the frequency-domain.

Figure 9.5 and Table 9.1 summarize the relations between the time- and frequency-domain parameters for Lorentzian and Gaussian line shapes. The imaginary part of the Gaussian frequency-domain function is a rather complicated function, but could be

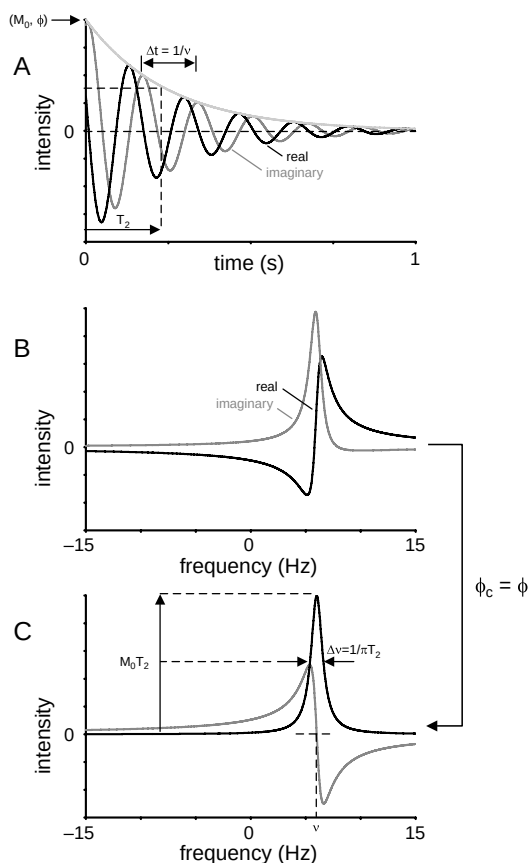


**Figure 9.5** Relations between time- and frequency-domain parameters. (A) An exponentially damped sinusoidal signal is characterized by four parameters, namely the amplitude  $M_0$ , the frequency  $\nu$ , the relaxation time constant  $T_2$  and the phase  $\phi$ .  $M_0$  and  $\phi$  can both be obtained from the first data point. The  $T_2$  relaxation time constant is equal to the time in which the signal decays to 36.8 % of the initial value, while the frequency is equal to the reciprocal of the signal maxima. (B) A nonzero phase leads to a mixture of absorptive and dispersive components in the NMR spectrum, thereby obscuring the other three parameters. (C) Following a phase correction  $\phi_c$ , the resonance frequency  $\nu$  equals the frequency where the real NMR resonance is maximum. The relaxation time constant  $T_2$  is proportional to the reciprocal of the full width at half maximum (FWHM) and  $M_0$  is equal to the resonance area, which can be obtained by integration. Note that the resonance height for a Lorentzian line is equal to  $M_0 T_2$ .

calculated through a Hilbert transformation of the real component. It follows that all four relevant parameters have equivalent representations in both domains. Based on the limited capabilities of pure Lorentzian and Gaussian line shapes to approximate *in vivo* spectral lines, the Voigt line shape, i.e. a combination of Lorentzian and Gaussian shapes, has been introduced. It is calculated by a frequency-domain convolution (or alternatively a time-domain multiplication) of Lorentzian and Gaussian functions.

**Table 9.1** Relationships between time- and frequency-domain parameters for Lorentzian and Gaussian spectral line shapes

| Time-domain (FID)                                                 | Frequency-domain (spectrum)                                                                                                |
|-------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|
| $M_0 e^{i\Delta\omega t} e^{i\phi_0} e^{-t/T_2}$ <sup>a</sup>     | $\frac{M_0 T_2}{1 + \Delta\omega^2 T_2^2} \cos \phi + i \frac{M_0 \Delta\omega T_2^2}{1 + \Delta\omega^2 T_2^2} \sin \phi$ |
| $M_0 e^{i\Delta\omega t} e^{i\phi_0} e^{-t^2/T_2^2}$ <sup>b</sup> | $M_0 T_2 \sqrt{\frac{\pi}{4}} e^{-\left(\frac{\Delta\omega T_2}{2}\right)^2} \cos \phi$                                    |
| Amplitude $M_0$ (first point)                                     | Total integrated area of $A(\omega)$ <sup>c</sup>                                                                          |
| Frequency $\omega$ ( $=2\pi\nu$ )                                 | Frequency $\omega$ ( $=2\pi\nu$ )                                                                                          |
| Phase $\phi$                                                      | Phase $\phi$                                                                                                               |
| $T_2$ relaxation time ( $=1/R_2$ )                                | Linewidth (i.e. FWHM) <sup>d</sup>                                                                                         |

<sup>a</sup>Time-domain signal which gives rise to a Lorentzian spectral line shape.

<sup>b</sup>Time-domain signal which gives rise to a Gaussian spectral line shape. Only the real component in the frequency-domain is shown.

<sup>c</sup>Peak heights for Lorentzian and Gaussian lines equal  $M_0 T_2$  and  $M_0 T_2 \sqrt{(\pi/4)}$ .

<sup>d</sup>See Exercise 1.9 for the exact relationship.

For relatively simple NMR spectra, containing only one or a few nonoverlapping resonances, the resonance areas can be obtained by numerical integration (e.g. see Figure 9.4B). The only required user interaction consists of indicating the frequency boundaries of the spectral region over which numerical integration is to be performed. However, even for single resonances this procedure can lead to significant underestimation of the resonance area due to truncation of the long ‘tails’ for Lorentzian lines. Furthermore, the procedure relies heavily on proper baseline correction, making it unreliable for most *in vivo* NMR applications. For spectra with multiple overlapping resonances, integration does not provide a reliable estimate of the individual resonance areas and more sophisticated methods are required. The majority of spectral fitting algorithms can roughly be divided into noniterative ‘black-box’ or iterative methods. The primary difference is that iterative methods typically allow the incorporation of constraints and prior knowledge on the fitted parameters, whereas noniterative methods do not. Figure 9.6 demonstrates the importance of imposing relevant prior knowledge. Following extensive  $^{13}\text{C}$  labeling during an intravenous infusion of  $[1\text{-}^{13}\text{C}]\text{glucose}$ , the  $[2\text{-}^{13}\text{C}]\text{glutamate}$  and  $[2\text{-}^{13}\text{C}]\text{glutamine}$  resonances around 55 ppm are split into a number of isotopomers, given rise to the multiplet pattern shown in Figure 9.6A. Fitting the spectrum with a noniterative, ‘black-box’ method (SVD in this case) gives a mathematically adequate fit, as can be judged from the small residuals (Figure 9.6B). However, the fit is biophysically useless as the fitted resonances vary widely in phase and width, i.e. rather than corresponding to a single compound, the fitted resonances hold information on multiple compounds. Iterative methods allow the inclusion of prior knowledge, which in the case of Figure 9.6 may include equal phase for all resonances, scalar couplings and multiplet patterns. When the fit is performed with an iterative method imposing prior knowledge (VARPRO in this case), the fit is mathematically adequate (Figure 9.6C) as well as biophysically relevant. Each of the fitted signals in Figure 9.6C directly corresponds to an isotopomer resonance (Figure 9.6D), thereby allowing isotopomer analysis (see also Chapter 3). In summary, the inclusion of prior knowledge is the primary means to impose biophysically relevant information in a spectral fitting algorithm. Furthermore, as will be discussed in Section 9.4.4, the inclusion of prior

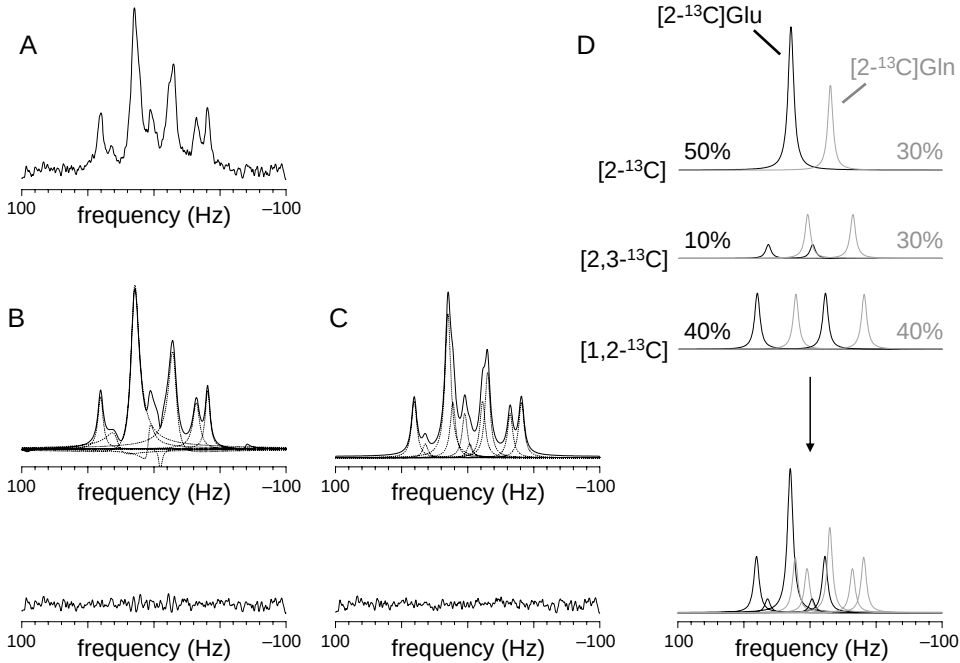


**Figure 9.6** (A) Simulated  $^{13}\text{C}$  NMR spectrum of a mixture of single and double  $^{13}\text{C}$ -labeled glutamate and glutamine. Time-domain curve fitting was either performed with (B) SVD or (C) VARPRO. No large differences can be observed from the residuals, indicating a good mathematical fit in both cases. However, when the individual resonances are inspected, it can be concluded that SVD produces a biophysically meaningless fit. The extensive prior knowledge on  $^{13}\text{C}$ - $^{13}\text{C}$  splitting patterns as imposed by the VARPRO algorithm produces the correct ratio of single and double labeled glutamate and glutamine.

knowledge is the only method of improving the accuracy of parameter estimation for a given noise level.

### 9.4.2 Prior Knowledge

As shown in Figure 9.6 the incorporation of biophysical prior knowledge in a spectral fitting algorithm is of crucial importance to improve the performance. However, there are a number of considerations involved with obtaining and incorporating prior knowledge. First and foremost it should be realized that the incorporation of incorrect prior knowledge will generally lead to systematic bias. Secondly, prior knowledge can be imposed as ‘hard’ or ‘soft’ constraints. Here the decisions involved with prior knowledge will be demonstrated for adenosine triphosphate (ATP) in  $^{31}\text{P}$  NMR. Figure 9.7 shows the theoretical ATP  $^{31}\text{P}$  NMR spectrum. The spectrum is dominated by scalar coupling between the three phosphate groups, leading to doublet resonances for  $\gamma$ - and  $\alpha$ -ATP and a triplet resonance for  $\beta$ -ATP (or more accurately a doublet of doublets). In an unconstrained fit without prior knowledge a total of 28 parameters are involved, namely seven amplitudes, seven frequencies, seven

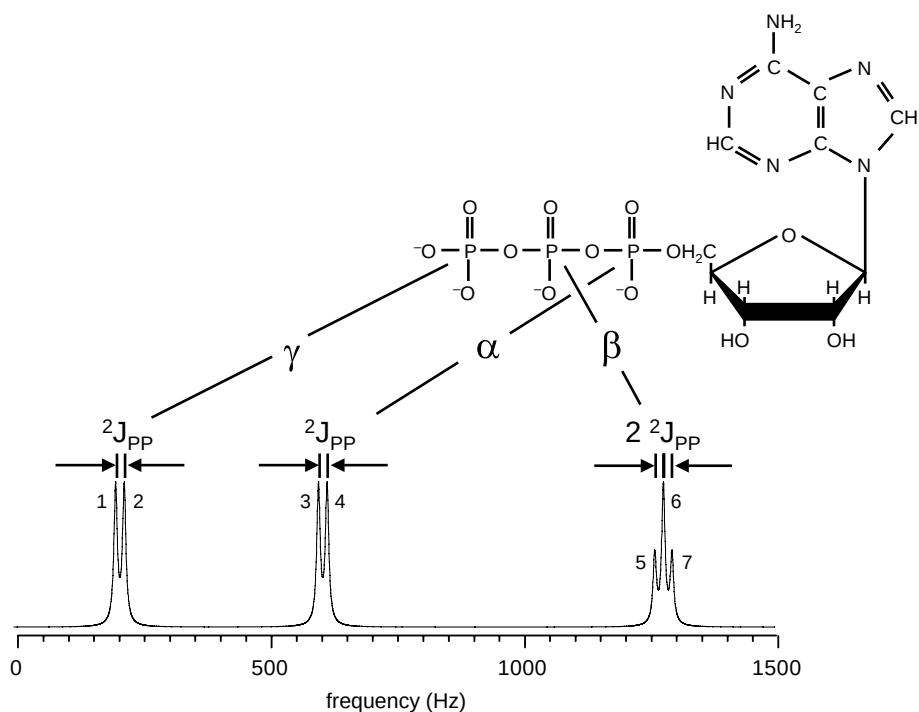


**Figure 9.7** Theoretical (high magnetic field)  $^{31}\text{P}$  NMR spectrum of ATP. The three phosphate nuclei give rise to three separate resonances, which are further divided into seven resonance lines (two doublets and a triplet), as indicated. However, many of the parameters describing the individual resonances, like line width and amplitude, can be made equal for all resonances. This prior knowledge can reduce the numbers of free parameters for ATP from 28 to 6. A NMR spectrum of ATP calculated for a lower magnetic field strength would be more complex, since second-order scalar coupling effects become more pronounced (see Section 1.10). See text for more details.

phases and seven line widths (or  $T_2^*$  relaxation time constants). To a first approximation the following prior knowledge can be obtained:

- (1) The overall, integrated intensities of the  $\alpha$ -,  $\beta$ - and  $\gamma$ -ATP resonances are identical, since they all originate from one phosphorus nucleus. In terms of model parameters this translates to:

$$M_{0,1} + M_{0,2} = M_{0,3} + M_{0,4} = M_{0,5} + M_{0,6} + M_{0,7} = M_0 \quad (9.3)$$

- (2) The intensity ratios between the individual resonance lines within a multiplet are coupled to each other. In a doublet, the individual resonance lines should be of equal intensity, while a triplet exhibits a 1:2:1 ratio between the resonance lines. This leads to the following prior knowledge in terms of model parameters:

$$2M_{0,1} = 2M_{0,2} = 2M_{0,3} = 2M_{0,4} = 2M_{0,6} = M_0 \quad \text{and} \quad 4M_{0,5} = 4M_{0,7} = M_0 \quad (9.4)$$

- (3) The transverse relaxation rates between the  $\alpha$ -,  $\beta$ - and  $\gamma$ -ATP resonances slightly differ. However, since the observed line width *in vivo* is largely dominated by magnetic field inhomogeneity it is reasonable to assume identical line width for all resonances, i.e.:

$$R_{2,1}^* = R_{2,2}^* = R_{2,3}^* = R_{2,4}^* = R_{2,5}^* = R_{2,6}^* = R_{2,7}^* = R_2^* \quad (9.5)$$

- (4) The spin-spin or scalar coupling is constant irrespective of the magnetic field strength or magnetic field homogeneity. Therefore, the frequencies of the resonance lines within individual multiplets are linked according to:

$$|\nu_1 - \nu_2| = |\nu_3 - \nu_4| = |\nu_5 - \nu_6| = |\nu_6 - \nu_7| = \Delta\nu \quad (9.6)$$

where  $\Delta\nu = {}^2J_{pp}$ .

- (5) When the experiment is adequately performed it can be argued that the phases of all resonances should be equal, leading to:

$$\phi_1 = \phi_2 = \phi_3 = \phi_4 = \phi_5 = \phi_6 = \phi_7 = \phi \quad (9.7)$$

Therefore, with the incorporating of prior knowledge the number of free fitting parameters is reduced from 28 to 6 (one amplitude, one relaxation rate, three frequencies and one phase).

However, it must be stressed that each stated point of prior knowledge is accompanied by one or more assumptions, which must be rigorously validated for a given application. For the prior knowledge involved with ATP these assumptions include:

- (1) The 1:1:1 ratio for the ATP multiplet resonances only holds when the FID is acquired with  $TR \gg T_1$  and  $TE = 0$ . If this is not the case, differences in  $T_1$  and  $T_2$  relaxation constants may influence the ratio. Furthermore, in a spin-echo experiment the observed ratio will be influenced by scalar coupling evolution, which differs between the three multiplets. In addition, the frequency profile of the excitation and refocusing pulses may distort the signal amplitudes in a frequency-dependent manner. When the sequence is acquired in the presence of proton saturation, the ratio may be different as the nuclear Overhauser enhancement is not the same for all three resonances per se.
- (2) Second-order quantum mechanical effects (i.e. ‘strong coupling’) will disturb the resonance ratio within a multiplet. This effect can be significant on low-field commercial MR systems, but can easily be accounted for.
- (3) The assumption of equal transverse relaxation rates does not hold when magnetic field inhomogeneity is not the dominating factor determining the observed line width. This is because the  $T_2$  relaxation for  $\beta$ -ATP differs from that of  $\alpha$ - and  $\gamma$ -ATP.
- (4) As indicated previously, the  $\beta$ -ATP resonance is not a true triplet, but is more accurately described by a doublet of doublets. This is because  $J_{\alpha\beta} \neq J_{\beta\gamma}$ . Furthermore,  $J_{\alpha\beta}$  and  $J_{\beta\gamma}$  may vary during an experiment due to physiological changes (pH, temperature, magnesium levels). It is therefore often better to treat  $J_{\alpha\beta}$  and  $J_{\beta\gamma}$  as separate model parameters. Of course the  $\beta$ -ATP multiplet is described by a linear combination of  $J_{\alpha\beta}$  and  $J_{\beta\gamma}$ .

The constraints given by Equations (9.3)–(9.7) can be referred to as ‘hard’ or ‘absolute’ constraints. While hard constraints can make the largest improvement to a given fitting algorithm, the assumptions underlying the constraint are rarely true under all conditions. Often a given parameter is approximately known, e.g.  $J_{\alpha\beta} \sim 16$  Hz and a reasonable range can be estimated, e.g.  $15 \leq J_{\alpha\beta} \leq 17$  Hz. Therefore, instead of using hard constraints ( $J_{\alpha\beta} = 16$  Hz), most fitting algorithms work with so-called ‘soft’ constraints in which a parameter is allowed to vary over a restricted range (e.g.  $15 \leq J_{\alpha\beta} \leq 17$  Hz). Soft constraints can still greatly improve the performance of a fitting algorithm, while at the same time allowing for variations in parameters due to, for example, physiology.

### 9.4.3 Spectral Fitting Algorithms

In the last two decades *in vivo* NMR signal processing has advanced from simple integration and line fitting to advanced fitting algorithms incorporating prior knowledge. Popular fitting algorithms include the jMRUI package [23, 24], which includes the VARPRO [19] and AMARES [20] programs, LCModel [21, 25] and others [26]. Rather than discussing all available fitting algorithms, the principles are discussed using methods that are based on modeling linear combinations of metabolite spectra [21, 25].

*LC modeling.* As expressed by numerous equations throughout the book the total NMR signal obtained from a mixture of compounds can be seen as a linear combination of the NMR signals from the pure or isolated compounds. This forms the basis for so-called linear combination (LC) modeling algorithms as first described by de Graaf and Bovee [25] and popularized by Provencher through the LCModel program [21]. With LC modeling algorithms the measured NMR spectrum (typically  $^1\text{H}$ , but extensions have been made to  $^{13}\text{C}$  [27]) is approximated as a linear combination of metabolite NMR spectra, which are commonly referred to as a “basis set”. Figure 9.8 shows a typical metabolite basis set for the fitting of short TE  $^1\text{H}$  NMR spectra. Depending on the application, the basis set may have to be extended with additional metabolites, like  $\beta$ -hydroxybutyrate (BHB) which can be observed during periods of fasting. Most important is that the basis set is complete, i.e. all metabolites that are present in the *in vivo* NMR spectrum should be included in the basis set. When a metabolite is omitted from the basis set it will typically lead to a systematic bias in the estimated parameters and in particular the amplitudes of the other metabolites [28]. This is because metabolite amplitudes are typically correlated depending on the amount of spectral overlap. Section 9.4.4 discusses the construction of parameter correlation matrices to evaluate the mutual dependence of metabolites. The inclusion of ‘basis set’ metabolites that are not present in the *in vivo* NMR spectrum does typically not lead to error or bias, because the amplitude of any nonexistent metabolite can simply be set to zero. However, the inclusion of nonexistent metabolites does lead to longer calculation times and a slower convergence.

One of the most important aspects of LC modeling algorithms is the creation of an accurate metabolite basis set. There are in general two methods of obtaining a basis set, namely by measurement or through simulation. In the measurement approach, high-concentration solutions (e.g. 100 mM) of each individual metabolite in water are measured with the exact same sequence as used for the *in vivo* NMR measurement. To approximate *in vivo* conditions, the measurements should be made at physiological temperature (310 K) and pH. Furthermore, the SNR of the resulting metabolite spectra should be high.



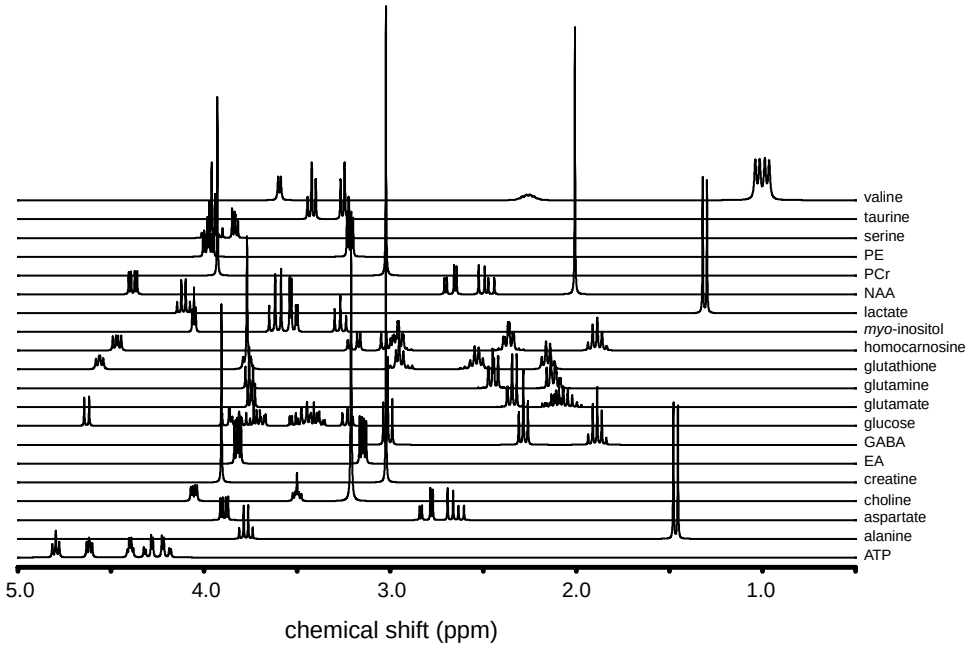


**Figure 9.8** Simulated  $^1\text{H}$  NMR spectrum of 20 metabolites that form a typical basis set for spectral quantification of *in vivo* short echo-time  $^1\text{H}$  NMR spectra by LC modeling algorithms. The spectra are simulated using the density matrix formalism for a 3D LASER sequence ( $TE = 10\text{ ms}$ ) at 7.0 T. The basis set is often extended with an experimentally determined macromolecular baseline.

The main advantage of the method is that all aspects of the NMR sequence, like eddy currents and spatial profiles, are identical between the *in vivo* and *in vitro* measurements. Obvious disadvantages of the measurement approach are that the measurement of 15–20 metabolite solutions can easily take one to several days and has to be repeated for each NMR sequence. Furthermore, obtaining a uniform, raised temperature across a large phantom is nontrivial. Finally, some of the metabolites can be quite costly when purchased at the amounts required for a high-concentration *in vitro* phantom suitable for human MR systems.

As an alternative to *in vitro* measurements, the metabolite NMR spectra can be simulated with the aid of density matrix calculations. Rather than calculating the transverse magnetization directly, density matrices are involved with energy levels and energy level populations. Given an initial density matrix  $\rho(0)$  describing the statistical state of the spin system, the density matrix  $\rho(t)$  generated by RF pulses, delays and magnetic field gradients can be calculated according to:

$$\rho(t) = e^{-iHt}\rho(0)e^{+iHt} \quad (9.8)$$

where  $H$  represents the Hamiltonian or energy operator, which essentially describes the effects of RF pulses, delays and magnetic field gradients on the density matrix. Following the evolution of  $\rho(t)$  under the influence of different Hamiltonian operators allows the

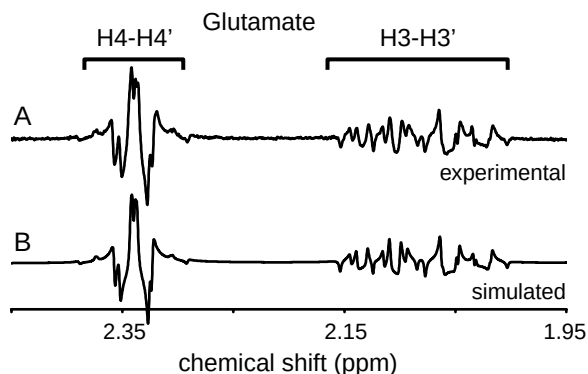


**Figure 9.9** (A) Experimentally measured and (B) simulated  $^1\text{H}$  NMR spectra of glutamate at 11.74 T acquired with a 3D LASER sequence ( $TE = 45$  ms). The close agreement between experiment and simulation can only be achieved when all details of the sequence, like shaped RF pulses and slice selection gradients, are included in the simulation.

calculation of a spin-system response to any NMR pulse sequence. At the end of the sequence, the transverse coherences are readily extracted from the final density matrix.

Figure 9.9 shows the close agreement between an experimentally measured glutamate  $^1\text{H}$  NMR spectrum and one obtained through theoretical density matrix calculations. However, it should be realized that a calculated spectrum is only as good as the prior knowledge used as input. For *in vivo* NMR it has been shown [29] that accurate results are obtained only when the full NMR pulse sequence is taken into account, including shaped RF pulses and spatial localization. While the calculation of 15–20 NMR spectra can also take hours to days, it can be performed on an off-line computer without using valuable MR system time. Furthermore, the simulations are always noiseless, artifact-free (e.g. absence of water) and are readily modified to accommodate different NMR pulse sequences. Obvious disadvantages can be traced back to the use of incomplete or incorrect prior knowledge. However, the rather tedious and time-consuming preparation and measurement of *in vitro* samples (which can also contain incorrect prior knowledge) has fuelled the switch to the flexible density matrix simulation approach. Currently a number of programs like GAMMA [30–32] and QUEST [33,34] are available for the simulation of NMR spectra.

LC modeling algorithms essentially adjust the amplitudes, frequencies, line widths and phases of the metabolite basis set to match the *in vivo* NMR spectrum as close as possible. In most cases, the algorithm imposes many soft constraints on the fitting parameters in order to achieve faster convergence. Unfortunately, short echo-time  $^1\text{H}$  NMR spectra are typically characterized by a significant macromolecular baseline in addition to the linear combination of metabolite signals (Figure 9.10A). In order to achieve a meaningful spectral fitting result, the macromolecular resonances have to be taken into account. In the original LCModel paper by Provencher [21], the macromolecular baseline was approximated with a cubic spline function. However, this introduces a large number of additional fitting parameters that are not based on biophysical parameters. A more robust method is to measure the macromolecular resonances by utilizing differences in  $T_1$  relaxation (Figure 9.10D). When the macromolecular baseline is taken into account it can be

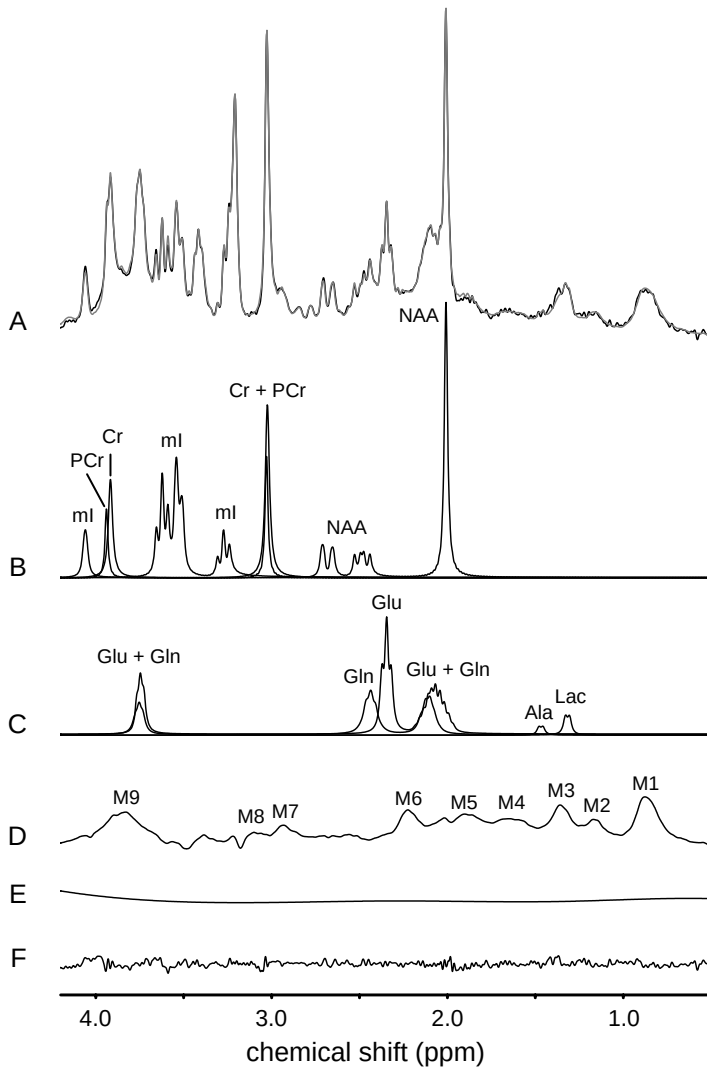


**Figure 9.10** Spectral quantification of  $^1\text{H}$  NMR spectra with a LC modeling algorithm. (A) Experimental  $^1\text{H}$  NMR spectrum acquired from rat brain in vivo at 9.4 T (100  $\mu\text{l}$ , STEAM localization,  $\text{TR}/\text{TE}/\text{TM} = 4000/8/25$  ms,  $\text{NEX} = 128$ ) overlaid with the fitted NMR spectrum calculated by the LC modeling algorithm. (B, C) NMR spectra of individual components as extracted from the LC modeling fit shown in (A) for (B) myo-inositol (ml), phosphocreatine (PCr), creatine (Cr) and N-acetyl aspartate (NAA) and (C) glutamate (Glu), glutamine (Gln), alanine (Ala) and lactate (Lac). (D) Experimentally measured macromolecule baseline (double inversion recovery with  $\text{TR}/\text{TI1}/\text{TI2} = 6000/1950/550$  ms) as included in the basis set of the LC modeling algorithm. (E) Additional cubic spline spectral baseline. (F) Residual difference between the modeled and measured  $^1\text{H}$  NMR spectra.

seen that the residual background or baseline signal is very small and can be readily modeled with a low-order function (e.g. second-order polynomial, Figure 9.10E). The difference between the fitted and measured NMR spectra (Figure 9.10F) can be a good indication for any problems during the spectral fit. Exclusion of metabolites typically leads to a coherent difference at well-defined frequencies, whereas a dispersive component throughout the difference can indicate frequency differences between the fitted and measured spectra. However, as was already demonstrated in Figure 9.6 a small difference spectrum is no guarantee for an accurate fit. Only the opposite is true in that a large difference spectrum indicates a poor spectral fit. In order to assess the accuracy of the fit it is important to find a measure for the error on the estimated parameters, as will be discussed next.

#### 9.4.4 Error Estimation

In the absence of error estimates one can not have confidence in the estimated parameters. Therefore, error estimation is an essential part of metabolite quantification. For a given noise level the lowest possible estimator-independent errors are given by the so-called Cramer–Rao lower bounds (CRLBs) [35–37]. For a proper evaluation of the CRLBs the experimental data must be modeled with an exactly known model function. For real data, the exact model functions are not known by definition, and as such the calculated CRLBs are only approximate. This is especially problematic for data with a large nonparametric component, such as macromolecular resonances in short echo-time  $^1\text{H}$  NMR spectra. The CRLBs are independent of the fitting algorithm so that they equally well apply to the time- and frequency-domains.

Evaluation of the CRLBs requires inversion of the Fisher information matrix  $\mathbf{F}$  whose size is equal to the number of real-valued parameters to be estimated. The Fisher matrix can be calculated as:

$$\mathbf{F} = \frac{1}{\sigma^2} (\mathbf{P}^T \mathbf{D}^H \mathbf{D} \mathbf{P}) \quad (9.9)$$

where  $\sigma$  is the standard deviation of the measurement noise and T and H denote transposition and Hermitian conjugation, respectively. The matrix  $\mathbf{D}$  holds the partial derivatives of the model function  $x_i$  with respect to the parameters  $p_j$ , whereas the matrix  $\mathbf{P}$  is known as the prior knowledge matrix as it holds the derivatives of one parameter  $p_m$  with respect to another parameter  $p_n$ , i.e.:

$$\mathbf{D}_{ij} = \left( \frac{\partial x_i}{\partial p_j} \right) \quad \text{and} \quad \mathbf{P}_{mn} = \left( \frac{\partial p_m}{\partial p_n} \right) \quad (9.10)$$

For a given model function (e.g. exponentially damped sinusoids), Equation (9.10) and the Fisher matrix of Equation (9.9) are readily calculated. The CRLBs are obtained from the covariance matrix, which in turn is obtained from inverting the Fisher matrix according to:

$$\sigma_{p_i} \geq \text{CRLB}_{p_i} = \sqrt{\mathbf{F}_{ii}^{-1}} \quad (9.11)$$

As a rule of thumb it is generally accepted that metabolite concentrations with  $\text{CRLB} < 10\%$  are measured with sufficient precision. Metabolites determined with  $\text{CRLB} < 20\text{--}30\%$  should be considered with caution, whereas those obtained with  $\text{CRLB} > 20\text{--}30\%$  are not reliable. However, it should be realized that a small CRLB is not a guarantee for an

accurate metabolite concentration. For example, in the case of an incorrect or incomplete metabolite basis set, some metabolite concentrations may be biased even though the CRLB can be very low. From the Fisher matrix in Equation (9.9) it follows that the CRLBs increase with increasing noise level, but also with increased spectral overlap (as expressed through the matrix **D**). Therefore, metabolites with very similar chemical structures and thus similar NMR spectra, like creatine and phosphocreatine or glutamate and glutamine, may have high CRLBs for the individual compounds, but a very low CRLB for the sum (e.g. tCr = PCr + Cr). For this reason, it is often advisable to report combined metabolic pools like total choline, total creatine, total NAA (NAA + NAAG) and total lactate (lactate + threonine), especially at lower magnetic fields where the separation between individual metabolite NMR spectra can be very small.

The correlation between parameters in a NMR spectrum can be quantitatively described by the so-called parameter correlation matrix that describes the correlation  $\rho_{mn}$  between parameters  $p_m$  and  $p_n$  according to:

$$\rho_{mn} = \frac{\mathbf{F}_{mn}^{-1}}{\sqrt{\mathbf{F}_{mm}^{-1}\mathbf{F}_{nn}^{-1}}} \quad (9.12)$$

Figure 9.11 gives a typical correlation matrix for metabolites from short echo-time  $^1\text{H}$  NMR spectroscopy data at 2.1 T. One interpretation of the correlation matrix is that in the presence

|      | Asp   | tCho  | Cr    | GABA  | Glu   | Gln   | ml    | Lac   | NAA   | PCr   | Tau   |
|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Asp  | 1.00  | -0.11 | 0.02  | -0.13 | -0.48 | 0.31  | -0.09 | -0.09 | -0.04 | -0.01 | 0.05  |
| tCho | -0.11 | 1.00  | -0.05 | -0.18 | 0.04  | 0.02  | 0.05  | -0.03 | 0.02  | -0.05 | -0.53 |
| Cr   | 0.02  | -0.05 | 1.00  | 0.02  | 0.00  | 0.01  | 0.14  | 0.06  | 0.02  | -0.95 | 0.12  |
| GABA | -0.13 | -0.18 | 0.02  | 1.00  | 0.18  | -0.42 | 0.02  | 0.10  | -0.21 | -0.06 | -0.27 |
| Glu  | -0.48 | 0.04  | 0.00  | 0.18  | 1.00  | -0.90 | -0.08 | 0.03  | -0.44 | 0.03  | -0.09 |
| Gln  | 0.31  | 0.02  | 0.01  | -0.42 | -0.90 | 1.00  | -0.03 | -0.01 | 0.32  | 0.01  | -0.11 |
| ml   | -0.09 | 0.05  | 0.14  | 0.02  | -0.08 | -0.03 | 1.00  | 0.02  | 0.12  | -0.12 | 0.17  |
| Lac  | -0.09 | -0.03 | 0.06  | 0.10  | 0.03  | -0.01 | 0.02  | 1.00  | 0.04  | -0.02 | 0.00  |
| NAA  | -0.04 | 0.02  | 0.02  | -0.21 | -0.44 | 0.32  | 0.12  | 0.04  | 1.00  | -0.04 | 0.07  |
| PCr  | -0.01 | -0.05 | -0.95 | -0.06 | 0.03  | 0.01  | -0.12 | -0.02 | -0.04 | 1.00  | 0.03  |
| Tau  | 0.05  | -0.53 | 0.12  | -0.27 | -0.09 | -0.11 | 0.17  | 0.00  | 0.07  | 0.03  | 1.00  |

**Figure 9.11** Amplitude correlation matrix for a number of cerebral metabolites that are part of a LC modeling basis set for short echo-time  $^1\text{H}$  NMR spectra at 2.1 T. In the presence of noise, the metabolites that have a strong correlation (shaded boxes) can not be separately quantified. However, the sum of those metabolites (e.g. Cr and PCr) is highly reliable. The increased frequency separation that can be achieved at high magnetic fields with optimal shimming will lead to a decrease in the parameter correlations. For example, at 9.4 T glutamate and glutamine can be reliably separated.

of noise, strongly correlated parameters, like creatine and phosphocreatine, can not be accurately retrieved by any quantification algorithm. In those cases it is better to report the combined pool, e.g. total creatine, which is typically highly reliable and accurate.

It should be realized that the macromolecular baseline can normally not be described by an analytical model function, and as such can not be included in the determination of the Fisher matrix. However, by introduction of so-called nuisance parameters, Ratiney *et al.* [33, 37] were able to show that the macromolecular baseline has strong correlations with the majority of metabolites. When the baseline is completely unknown, it will lead to bias in the estimated parameters. Inclusion of a predetermined macromolecular baseline can significantly reduce the metabolite CRLBs.

## 9.5 Data Calibration

The fourth and final step in metabolite quantification is the conversion of relative resonance areas, as detailed in Section 9.3, to absolute concentrations expressed in  $\text{mmol L}^{-1}$  ('molar' concentration) or  $\mu\text{mol g}^{-1}$  of tissue ('molal' concentration). The molar and molal concentration can be converted if the brain water density is known [38]. There are two general approaches to calibrate or reference the relative numbers to absolute concentrations [38–46]. The first group utilizes a so-called external concentration reference, in which a compound of known concentration is positioned outside the object under investigation but within the sensitive volume of the coil. The other group uses an internal concentration reference, which can be a stable metabolite naturally occurring in the tissue (endogenous) or an appropriate compound which can be introduced into the organ being studied (exogenous). Exogenous concentration references are not frequently used for safety reasons and the lack of appropriate (nontoxic, stable, selective for one organ) compounds. Figure 9.12 schematically shows the use of concentration references for the quantification of brain metabolites, but the methods are equally applicable for a wide range of organs. Figure 9.12A shows the strategy of internal concentration referencing in which a spectrum (localized or nonlocalized) is acquired from the region of interest. When the resonance of the internal concentration reference compound is present in this spectrum (e.g. NAA or total creatine for  $^1\text{H}$  MRS or ATP for  $^{31}\text{P}$  MRS) the concentration of the other metabolites can be directly calculated by comparison of resonance areas (after correction for factors like relaxation, as will be discussed next). If this is not the case, a second spectrum containing the resonance of the calibration compound (e.g. unsuppressed water) from the same VOI has to be acquired. Figure 9.12B and C show two possible calibration strategies utilizing an external concentration reference. In Figure 9.12B the external concentration reference compound is placed in the coil together with the object under investigation. Acquisition of two spectra as indicated by the different MRS ROIs (gray boxes) in Figure 9.12B makes quantification possible. The strategy of Figure 9.12C differs from B in that the two spectra are acquired from the exact same spatial position. The specific advantages, disadvantages and correction factors of the different calibration strategies will be discussed in Section 9.5.1–9.5.3. First, the general factors affecting the acquired signals during a calibration procedure will be described.

The resonance area of a metabolite resonance is, in principle, proportional to the concentration, which makes the application of a reference compound with known concentration

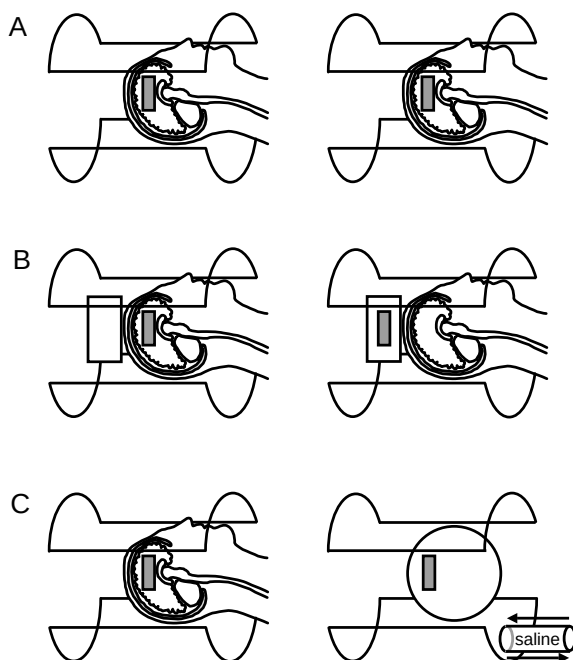


**Figure 9.12** Calibration strategies for the quantification of cerebral metabolite concentrations. (A) Internal concentration reference. (B) External concentration reference. (C) Phantom-replacement concentration reference. The left figures indicate the acquisition of the metabolite spectra, while the right figures indicate the acquisition of the reference compound spectra. In (C, right) a small saline-filled bottle is inserted in (or retracted from) the coil, in order to equalize the in vivo and in vitro coil loads. Alternatively, correction of coil loading can be obtained through the principle of reciprocity, as explained in the text.

a convenient method of quantification. However, any difference between the metabolite and the reference compound needs to be taken into account in order to obtain reliable concentration values. Here the factors which could differ between the two compounds will be summarized:

- (1) Partial saturation. When the repetition time  $TR$  of a pulse sequence is shorter than four to five times the longitudinal relaxation time  $T_1$ , the magnetization can not completely recover before the following excitation, leading a reduction of the steady-state longitudinal magnetization given by:

$$M_z(TR) = M_0 \frac{1 - e^{-TR/T_1}}{1 - \cos \theta e^{-TR/T_1}} \quad (9.13)$$

where  $\theta$  is the nutation angle. With different  $T_1$  relaxation times between metabolites and reference compound, the acquired signal intensity of each resonance must be corrected for partial saturation. This can simply be achieved by using Equation (9.13) as a correction factor, but it requires knowledge of the  $T_1$  relaxation time. Note that

Equation (9.13) only holds for a simple pulse-acquire experiment. For more complicated experiments involving spin-echo delays, Equation (9.13) needs to be modified to account for the additional RF pulses, especially for short  $T_1$  relaxation times and long echo-times TE. With surface coils correcting for partial saturation is further complicated since the nutation angle  $\theta$ , and consequently the saturation factor, depends on the position relative to the coil. This problem can be alleviated by executing the entire pulse sequence with adiabatic RF pulses. The correction for partial saturation can be omitted completely if the experiments are performed with  $TR > 4T_{1\max}$  ( $T_{1\max}$  being the longest  $T_1$  relaxation time present), such that  $M_z \sim M_0$  for all resonances. Even though this increases the experimental duration, the use of long repetition times is highly recommended since it eliminates systematic errors caused by application of an empirically determined  $T_1$  saturation factor.

- (2) Nuclear Overhauser effects. Related to longitudinal  $T_1$  relaxation is the nuclear Overhauser effect nOe (see Section 3.2.2). In homo- or heteronuclear double resonance spectroscopy experiments, the signal intensities depend on the nOe as described in Chapter 3. By determining, for a given pulse sequence, the nOe factor for each resonance, this effect can be corrected. However, for the sake of simplicity (and accuracy) it is recommended to design pulse sequences in such a manner that nOes are completely eliminated (for instance by gated decoupling).
- (3) Transverse relaxation. Any experiment utilizing spin-echo delays induces signal losses due to  $T_2$  relaxation [see Equation (1.75)]. Many authors have minimized this effect by using short echo-times ( $TE < 20$  ms). However, for compounds such as ATP or macromolecules even the shortest echo-time leads to significant signal reduction. On the other hand, many groups have used long echo-times ( $TE > 100$  ms) in order to reduce baseline oscillations, simplify the appearance of spectra and improve water suppression. In all cases a proper correction can only be made if the transverse relaxation time  $T_2$  is known for each resonance.
- (4) Diffusion. Pulse sequences utilizing strong magnetic field crusher gradients to achieve accurate spatial localization or experiments with long echo-times are certainly affected by diffusion. This effect can be pronounced when large molecules like ATP (with a low diffusion constant  $D \sim 0.2 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$ ) are being calibrated against a low molecular weight compound like water ( $D \sim 0.7 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$ ). In analogy to  $T_2$  relaxation, this effect can be minimized by using shorter echo-times (and minimal magnetic field crusher gradients), but in many cases the effect can only be corrected for when the (apparent) diffusion constant is quantitatively known. It should be realized that these four parameters and especially relaxation and diffusion, may change over time due to development of pathology or changes in temperature. For instance, in stroke the apparent diffusion coefficient of water decreases almost immediately after the onset of ischemia. In the more chronic phase of the ischemic lesion, the  $T_2$  relaxation time of water significantly increases.
- (5) Scalar coupling. As was described in Chapter 6 for the STEAM and PRESS localization techniques and demonstrated for several major metabolites in Chapter 2, the signal amplitude of coupled resonances is a complicated function of echo-time, scalar coupling constant, chemical shift (for strongly coupled spins), nutation angle and multiple quantum evolution delay (such as the TM period in STEAM). Several approaches can be followed to eliminate or compensate this effect. The use of short echo-times



(TE < 20 ms) reduces the amplitude and phase modulation for some coupled metabolites. However, even a very short echo-time can lead to significant modulation when the scalar coupling constant is large (e.g. inositol, glucose). Another approach which can be followed is to choose TE as a multiple of  $1/J$ , such that the metabolite of interest is completely refocused. Unfortunately, unless the  $J$  coupling constants are similar, this approach only works for one metabolite (or one resonance of a metabolite). The amplitude and phase distortions caused by  $J$ -modulation can also be determined experimentally in model solutions. The knowledge obtained from the solutions can be used to correct the observed signal *in vivo*. Furthermore, knowledge of the exact chemical shifts and  $J$ -coupling constants can be used as prior knowledge in nonlinear least-square fitting techniques of the time-domain signal [20, 21, 26].

- (6) Localization. Since the absolute concentration is directly proportional to the localized volume, it is clear that the spatial localization needs to be accurate and identical for metabolites and reference compound. Especially for heteronuclear internal calibration (e.g. calibration of  $^{31}\text{P}$  metabolites with the water signal) that is a challenging task. To reduce the duration of the experiment,  $^1\text{H}$  MRS would preferably be performed with a single-scan technique such as STEAM or PRESS. Due to the short  $T_2$  relaxation times,  $^{31}\text{P}$  MRS is most often executed with ISIS localization. As was demonstrated in Chapter 6, the actual localized volume of various localization techniques can substantially differ. Even though these differences could be compensated, it is more convenient (and probably more accurate) to use the same localization technique for both experiments.

Another artifact associated with ISIS localization is the distorted inversion profile in the presence of short  $T_2$  components. The  $T_2$  relaxation times between water ( $T_2 \sim 80$  ms) and for instance ATP ( $T_2 \sim 30$  ms) substantially differ, leading to different localized volumes and erroneous quantification.

Even if the localized volumes were of identical shape, the difference in resonance frequency between metabolites will lead to a chemical shift displacement artifact. When measurements are made in heterogeneous tissues, like cerebral gray and white matter, the voxel composition between the reference compound and the metabolite of interest may differ, leading to quantification errors.

- (7) Frequency-dependent amplitude and phase distortions. This effect is most pronounced when using binomial pulses for water suppression in  $^1\text{H}$  MRS. The spectrum is amplitude modulated according to  $\sin^n \omega$  ( $n = 1, 2, 3, \dots$ ) where the higher-order binomial pulses also exhibit nonlinear phase distortions. By performing simulations based on the Bloch equations these effects can be completely compensated. Other areas where amplitude distortions can play a role are in  $^{31}\text{P}$  and  $^{13}\text{C}$  MRS, where the effective chemical shift range is much larger than for  $^1\text{H}$  MRS. When the RF amplitude is small with respect to this chemical shift dispersion, the nutation angle becomes frequency dependent. This can result in substantial errors when for instance the  $\beta$ -ATP resonance (which is normally on the edge of the *in vivo*  $^{31}\text{P}$  chemical shift range) is used for quantification. The use of smaller nutation angles will reduce these effects.

The application of adiabatic half passage pulses is very popular in (nonlocalized)  $^{31}\text{P}$  MRS (and to a lesser degree in  $^{13}\text{C}$  MRS). But also for adiabatic pulses, the RF amplitude should be large enough to excite the entire chemical shift range uniformly. When the (off-resonance) adiabatic condition is not satisfied, substantial errors will

arise if these effects are not taken into account. Again, the effects of RF pulses can be exactly calculated from the Bloch equations such that complete compensation is possible.

- (8) NMR visibility. The linewidth of resonances is inversely proportional to the  $T_2^*$  relaxation time, which is related to the rotational mobility of the metabolite. Metabolites with low mobility (e.g. bound to macromolecular structures) give rise to very short  $T_2$  relaxation times and hence broad resonances which can be unobservable in conventional NMR spectra. This will in turn lead to an underestimation of the concentration. Furthermore, if a water suppression technique like presaturation is used, the narrow resonance line arising from the mobile component of the metabolite under investigation may decrease due to magnetization transfer effects (see Chapter 3) leading to a further underestimation of the true concentration.

Considering the above described factors, a general applicable formula can be constructed for the concentration of a metabolite:

$$S_m = S_{mm} C_{T_1,m} C_{T_2,m} C_{nOe,m} C_{ADC,m} C_{J,m} C_{loc,m} C_{RF,m} \quad (9.14)$$

$$S_r = S_{rm} C_{T_1,r} C_{T_2,r} C_{nOe,r} C_{ADC,r} C_{J,r} C_{loc,r} C_{RF,r} \quad (9.15)$$

$$[m] = \left( \frac{S_m}{S_r} \right) [r] C_n C_{av} C_{gain} \quad (9.16)$$

where  $S_{mm}$  is the measured metabolite signal,  $S_m$  is the corrected metabolite signal,  $S_{rm}$  is the measured reference signal,  $S_r$  is the corrected reference signal,  $C_{T_1}$  is the correction factor for partial saturation due to incomplete signal recovery due to finite  $T_1$  relaxation [Equation (9.13)],  $C_{T_2}$  is the correction factor for  $T_2$  relaxation [Equation (1.75)],  $C_{nOe}$  is the correction factor for the nOe [Equation (3.20)],  $C_{ADC}$  is the correction factor for diffusion [Equations (3.60) and (3.63)–(3.65)],  $C_J$  is the correction factor for amplitude and phase modulations due to scalar-coupling evolution (this factor can include the effects of frequency selective RF pulses on scalar-coupled spins [47]),  $C_{loc}$  is the correction factor for deviations from the ideal localization profile,  $C_{RF}$  is the correction factor for amplitude and phase distortions due to specific RF pulse combinations like binomial RF pulses,  $[m]$  is the concentration of the metabolite under investigation,  $[r]$  is the concentration of the reference compound,  $C_n$  is the correction for the number of equivalent nuclei for each resonance,  $C_{av}$  is the correction for the number of averages, and  $C_{gain}$  is the correction for the receiver gain setting.

The factor of partial NMR invisibility is difficult to correct for and can only be established by comparing the *in vivo* MRS data with invasive *in vitro* results like chemical analysis of biopsies or high-resolution NMR of tissue extracts. When some factors do not affect the measured signal, the corresponding correction factor equals one. Each individual calibration technique as shown in Figure 9.12 requires, in addition to the general applicable factors in Equations (9.14)–(9.16), specific correction factors which will be described next.

### 9.5.1 Internal Concentration Reference

The strategy of an internal concentration reference is straightforward. The (corrected) resonance areas in the acquired spectrum are compared with that of a stable endogenous

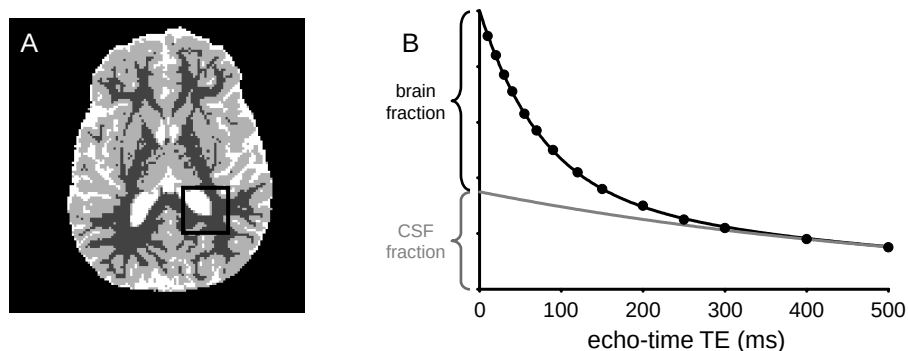


**Figure 9.13** Calculation of partial volume effects from (A) segmented MR images or (B) multi-exponential  $T_2$  relaxation. The CSF contribution to a spectroscopic volume can be directly calculated in (A), whereas the CSF fraction in (B) is given by the long  $T_2$  component.

reference compound. For  $^1\text{H}$  MRS, water, total creatine and NAA have been proposed as endogenous concentration references. However, it should always be kept in mind that the concentration of endogenous concentration references can change during the development of pathologies. NAA shows a substantial decrease in a wide range of neurodegenerative pathologies ranging from ischemia to Alzheimer's disease. Total creatine and water are relatively stable metabolites, although changes in their rotational mobility may change their NMR visibility. Furthermore, the concentration is often a function of spatial position. Especially important is the discrimination between water from tissue and CSF, which may differ by 30–40% in water content. This discrimination can be achieved on the basis of a double-exponential decay of the water ([42], see Figure 9.13). Alternatively, the compartments can be retrieved from high-resolution MR images with appropriate contrast (e.g. Figure 7.20). Despite the potential difficulties, water has been used by several NMR groups with some degree of success, judging by the favorable comparison with concentrations obtained using other techniques. For  $^{31}\text{P}$  MRS, ATP and water have been used as endogenous concentration markers. ATP is only a suitable reference compound in those applications where the system under investigation is only mildly challenged. With severe pathologies like ischemia, anaerobic glycolysis rapidly consumes the available PCr pool (through the creatine kinase equilibrium) after which the ATP resonances start to decline.

In applications where the peak ratios are used, the total phosphate pool, defined as:

$$[\text{P}_{\text{tot}}] = [\text{PME}] + [\text{P}_i] + [\text{PDE}] + [\text{PCr}] + 3[\text{NTP}] + 2[\text{NAD} + \text{NADH}] \quad (9.17)$$

can be used. The total phosphate pool should in principle be constant (assuming that none of the metabolites becomes NMR invisible and none of the metabolites are transported out of the cells), such that changes in peak ratio (e.g.  $[\text{PCr}]/[\text{P}_{\text{tot}}]$ ) can be attributed to a single metabolite (i.e.  $[\text{PCr}]$ ).

The water reference is in practice equally suitable for  $^1\text{H}$  and  $^{31}\text{P}$  MRS. For internal water referencing, Equation (9.16) should be modified and extended to:

$$[\text{m}] = \left( \frac{S_{\text{m}}}{S_{\text{water}}} \right) [\text{water}] C_{\text{n}} C_{\text{av}} C_{\text{wc}} C_{\text{HX}} \quad (9.18)$$

where  $S_{\text{water}}$  is the corrected water signal,  $[\text{water}]$  is the water concentration (55.14 mmol  $\text{L}^{-1}$  at 310 K),  $C_{\text{wc}}$  is the correction for the water content in the VOI ( $C_{\text{wc}} = \sim 0.82$  for gray matter,  $\sim 0.73$  for white matter,  $> 0.98$  for CSF and  $\sim 0.78$  for skeletal muscle) and  $C_{\text{HX}}$  is the correction factor for the relative sensitivities between the proton and X channels ( $X = ^{31}\text{P}$  or  $^{13}\text{C}$ ).

The mentioned factors  $C_{\text{wc}}$  for different tissue types are from biochemical measurements. It should be realized that this factor will be reduced if NMR invisible pools are present. The factor  $C_{\text{HP}}$  (i.e.  $X = ^{31}\text{P}$ ) can be assessed by performing a phantom experiment with a phosphorus metabolite of known concentration in water.  $C_{\text{HP}}$  is then calculated as the ratio of the  $^1\text{H}$  signal per mM of protons over the  $^{31}\text{P}$  signal per mM of phosphorus. Since  $C_{\text{HP}}$  depends on a number of factors, including coil load, it is advisable to mimic the *in vivo* conditions as close as possible. For the homonuclear calibration strategy  $C_{\text{HH}}$  equals  $C_{\text{gain}}$ .

## 9.5.2 External Concentration Reference

One of the methods that utilizes an external concentration reference is executed as shown in Figure 9.12B. After the collection of the desired *in vivo* spectrum, a reference spectrum from a calibration sample is obtained. To minimize the effects of  $B_1$  magnetic field inhomogeneity, the two voxels are chosen symmetrically about the center of the coil. Alternatively, the  $B_1$  magnetic field inhomogeneity can be accounted for by measuring the  $B_1$  distribution of the particular RF coil used. The metabolite concentration can be calculated according to:

$$[\text{m}] = \left( \frac{S_{\text{m}}}{S_{\text{r}}} \right) [\text{r}] C_{\text{n}} C_{\text{av}} \quad (9.19)$$

where  $S_{\text{r}}$  is the corrected reference signal and  $[\text{r}]$  is the concentration of external reference.

Equation (9.19) immediately shows the main distinction with the method of internal water calibration, in that no assumption needs to be made for the internal water concentration (which may vary with pathology, age, and voxel composition). Furthermore, for heteronuclear experiments no calibration factor for the relative  $^1\text{H}$  and  $^{31}\text{P}$  (or  $^{13}\text{C}$ ) sensitivities is required.

The method of external concentration referencing is relatively simple since the experimental set-up or the position of the patient need not be changed. Furthermore, it is a robust method, mainly being hampered by a dependency on the  $B_1$  field distribution (which can be minimized by symmetrical placement of the sample with respect to the coil). The method can also be very time-efficient if the two localized volumes are acquired simultaneously with Hadamard or two-volume ISIS (or STEAM/PRESS) localization.

### 9.5.3 Phantom Replacement External Concentration Reference

Another method of quantification is aimed at simulating (human) tissue as close as possible with a (spherical) phantom of known composition. Because almost all systematic errors like  $B_1$  inhomogeneity (at low magnetic field) and localization affect the tissue and phantom in identical ways, this calibration method is in principle very robust. The method is only complicated by differences in coil loading between the tissue and the phantom. Two methods are available to compensate for differences in coil loading, i.e. load adjustment and load correction through the principle of reciprocity. For load adjustment, the electrical conductivity of the solution in the phantom is slightly lower than that of human tissue ( $\sigma \sim 0.64 \text{ S m}^{-1}$ ) such that it allows for fine adjustment of the coil load with a second (smaller) phantom containing, for example, saline. During the procedure of load adjustment, the matching capacitance of the RF coil is left unchanged at the end of the *in vivo* experiment. After removal of the patient and accurate positioning of the phantom, the matching is optimized by slowly inserting the saline bottle (i.e. increasing the coil load). When the *in vitro* matching equals the previous *in vivo* matching, the *in vivo* and *in vitro* coil loads are identical. The concentration can then simply be calculated with Equation (9.19).

The method of load correction involves the addition of an external capillary which is measured nonselectively (nonlocalized) during the *in vivo* and during the *in vitro* experiments. Most conveniently a compound is used which falls outside the spectral region of interest [e.g. tetramethylsilane (TMS) for  $^1\text{H}$  or  $^{13}\text{C}$  MRS and phenylphosphonic acid (PPA) or hexamethylphosphorotriamide (HMPT) for  $^{31}\text{P}$  MRS]. The correction factor for the difference in coil loading is then calculated from the capillary signals obtained from the *in vivo* and *in vitro* experiments according to:

$$C_{\text{load}} = \frac{S_{\text{in vitro}}}{S_{\text{in vivo}}} \quad (9.20)$$

after which the concentration can be calculated as:

$$[m] = \left( \frac{S_m}{S_r} \right) [r] C_n C_{\text{av}} C_{\text{load}} \quad (9.21)$$

Alternatively, a load correction term can be obtained by determining the power/voltage to obtain a  $90^\circ$  nutation angle *in vivo* and *in vitro*. Through the principle of reciprocity the difference in  $B_1$  magnetic field strength is then directly proportional to the difference in acquired signal strength.

While there is no single calibration strategy that is optimal under all experimental conditions, the method of internal concentration referencing using the water signal appears to be the most convenient method with a reasonable accuracy. Especially at higher magnetic fields, the phantom replacement method has serious drawbacks concerning differences between *in vivo* and *in vitro*  $B_1$  magnetic field distributions.

## 9.6 Exercises

**9.1** A proton NMR spectrum is acquired from a 12 ml volume positioned in the human occipital lobe. A surface coil is used for RF pulse transmission as well as signal reception. The spectrum is acquired with the double spin-echo localization method PRESS with  $\text{TR} = 2000 \text{ ms}$ ,  $\text{TE} = 100 \text{ ms}$  and number of averages = 64. The integrals

of NAA ( $\text{CH}_3$ ), creatine ( $\text{CH}_3$ ) and choline ( $\text{N}(\text{CH}_3)_3$ ) are 100, 70 and 60, respectively. To obtain the absolute concentrations, water will be used as an internal concentration reference. The water spectrum is acquired with a single average from the same volume with the same sequence and has an integral of 5000.

The relaxation parameters are given by:

NAA  $T_1 = 2000$  ms,  $T_2 = 150$  ms;  
Creatine  $T_1 = 2500$  ms,  $T_2 = 200$  ms;  
Choline  $T_1 = 2250$  ms,  $T_2 = 150$  ms;  
Water  $T_1 = 1650$  ms,  $T_2 = 75$  ms.

From quantitative  $T_1$  images it can be deduced that the voxel contains 40 % CSF, 50 % gray matter (GM) and 10 % white matter (WM). The water content of CSF, GM and WM is 100 %, 87 % and 83 % of pure water, respectively. The water content of pure water is  $55.6 \text{ mol L}^{-1}$ .

**A** Calculate the metabolite and water integrals corrected for  $T_1$  and  $T_2$  relaxation losses.

**B** Calculate the water concentration in the selected volume.

**C** Calculate the average metabolite concentrations inside the volume.

**D** Calculate the average metabolite concentrations inside the brain.

**9.2** Consider a CHESS sequence with six 10 ms Gaussian excitation pulses ( $R = 2.7$ ).

**A** Assuming a Gaussian shaped excitation profile for individual RF pulses, calculate the signal loss for  $\alpha\text{H1-glucose}$  at 4.7 T and 9.4 T. Further assume that the chemical shift of water is 4.7 ppm.

**B** Under the same assumptions, calculate the relative signal intensities for  $\alpha\text{H1-glucose}$  and the two (nondecoupled)  $^{13}\text{C-}^1\text{H}$  satellites of  $[1-^{13}\text{C}]\alpha\text{H1-glucose}$  for a 50 % fractional enrichment at 4.7 T and 9.4 T.

**C** Calculate the experimental fractional enrichment ( $^{13}\text{C}/(^{12}\text{C}+^{13}\text{C})$ ) when the RF-induced distortions are not taken into account.

**9.3** Consider the pulse-acquire  $^1\text{H}$  NMR spectrum of aspartic acid (see Figure 2.7 and Table 2.1).

**A** Determine the number of free parameters when the spectrum is fitted unconstrained with a sum of single Lorentzian lines.

**B** Determine the number of free parameters when the spectrum is fitted with full prior knowledge of the NMR characteristics and chemical structure of aspartic acid. Discuss the underlying assumptions.

**C** Repeat the calculation under (B) when the  $^{13}\text{C}$  fraction of aspartate in the POCE difference spectrum of rat brain *in vivo* is fitted after 40 min of intravenous infusion of  $[1-^{13}\text{C}]\text{glucose}$ .

**9.4** The presence of IMCL and EMCL lipids in rat skeletal muscle necessitates the use of spectral editing to unambiguously detect lactic acid. Furthermore, it is known that lactate displays bi-exponential  $T_2$  relaxation with 70 % and 30 % of the lactate having  $T_2$ s of 50 ms and 150 ms, respectively. The lactate  $T_1$  equals 1500 ms, whereas water  $T_1$  and  $T_2$  relaxation time constants are 1200 ms and 30 ms, respectively. RF magnetic field inhomogeneity reduces the lactate editing efficiency from 100 to 82%.

**A** During a spectral editing experiment ( $\text{TR} = 2500$  ms,  $\text{TE} = 144$  ms), lactate is observed with an intensity of 45 in 128 scans. When water is observed with a relative intensity of 620 in 16 scans, calculate the absolute lactate concentration.

- B** Determine the echo-time that gives the optimal detection of lactate during a J-difference editing method. Assume single-exponential  $T_2$  relaxation with  $T_2 = 100$  ms.
- 9.5** During a  $^1\text{H}$  MRSI study of the lactate distribution detection in cerebral tumors, the spectroscopist suspects that the tumor water content is different from that of gray matter. Describe an imaging experiment by which the tumor water content can be estimated.
- 9.6** Name at least three methods for the measurement of the  $T_2$  of glutamate and three methods for the  $T_2$  of lactate at 11.74 T. Explain why increasing the echo time of a PRESS sequence is likely to give an incorrect estimate of  $T_2$ .
- 9.7 A** In the presence of a  $\pm 10^\circ$  transmitter phase instability, calculate the maximum integrated signal loss for a FID averaged over 128 transients.
- B** In the presence of a linear  $+8$  Hz frequency drift over 1 h, calculate the maximum linewidth at half maximum for a FID averaged over 1024 transients (assume  $\text{TR} = 1000$  ms,  $T_2^* = 100$  ms).
- C** Describe a method to minimize the signal loss and line broadening calculated under (A) and (B).
- 9.8** Consider a spin-echo sequence with a jump-return excitation pulse ( $\text{TR} - \text{JR90} - \text{TE}/2 - \text{JR180} - \text{TE}/2$ ) where the JR intrapulse pulse delay is adjusted to give maximal excitation 500 Hz off-resonance from the water resonance at 4.7 T.
- A** Using the NAA, choline and creatine relaxation intensity and averaging parameters given in Exercise 9.1, calculate the steady-state metabolite signals when  $\text{TR} = 1500$  ms and  $\text{TE} = 50$  ms. Ignore  $T_1$  relaxation during the echo-time.
- B** Due to residual  $B_0$  eddy currents, the first pulse of the JR sequence experiences a  $+20^\circ$  additional phase rotation relative to the second pulse. Recalculate the metabolite signals for this situation.
- C** Continuing with the condition from Exercise 9.8B, the unsuppressed water signal is acquired by placing the JR pulses  $+500$  Hz off-resonance. The water intensity equals 4000. Using the metabolite relaxation and averaging parameters given under Exercise 9.1. Calculate the absolute metabolite concentrations under the assumption of a 40 M water concentration and NNA, tCr and tCho intensities of 90, 50 and 35, respectively.

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# 10

## Hardware

### 10.1 Introduction

The objectives of this chapter are to describe the instrumentation involved in *in vivo* NMR. The complete NMR system can roughly be divided in four categories: (1) a magnet having a bore size that is large enough to accommodate entire living subjects, including humans. For convenience the bore of animal and human systems is normally horizontal. Besides the superconducting coil to produce the main magnet field, a set of superconducting shim coils are supplied to adjust the homogeneity of the 'raw' magnet (i.e. without the presence of a sample); (2) a gradient coil system to create time dependent magnetic field gradients for spatial encoding as used in MRI, localized spectroscopy and many other NMR experiments. Room temperature shim coils to adjust the magnetic field homogeneity on a subject-specific basis are often an integral part of the gradient coil assembly; (3) a radiofrequency (RF) transmitter/receiver (transceiver) system for generating the RF field  $B_1$  and for detecting the NMR signal; (4) a computer system for managing the magnet, shims, gradient and transceiver components of the entire NMR system. Furthermore, a computer is necessary for processing and storing the raw NMR signal (FID or echo) and processing and displaying the final NMR data signal (spectrum or image).

A complete overview of the hardware involved in a NMR experiment would represent an enormous amount of work and could easily fill a number of books. Furthermore, an extensive discussion of, for example, high-field superconducting magnet design would be inappropriate and outside the scope of a book on NMR techniques and principles. Therefore, the choice was made to focus on the general aspects of a complete MR system, like the magnet, magnet field gradients and RF coils. Furthermore, some specific components of the NMR system which require attention during experiments, like tuning and matching of RF coils, optimizing the magnetic field homogeneity and the effects of time-varying magnetic fields (eddy currents), will be discussed.

## 10.2 Magnets

The main magnetic field is an most essential component of a complete NMR system. The magnetic field strength determines the intrinsic NMR sensitivity, while the magnet design (i.e. bore size, orientation) largely determines its applications [1–5].

There are essentially three types of magnet designs which are suitable for NMR, that is, the magnet should have a relatively intense (0.1–10 T), homogeneous and stable field. The oldest magnet design is a permanent magnet, which is constructed of ferromagnetic materials such as iron, nickel, cobalt and alloys thereof. Once the polycrystalline, ferromagnetic material is aligned in a suitable external magnetic field, the material will be permanently magnetized. However, the generation of a suitable homogeneous magnetic field requires large amounts of ferromagnetic material, easily in excess of 10 tons. Although this problem can be somewhat reduced by using rare earth alloys, it nevertheless limits the magnetic field strength to  $\sim 0.2$  T. An advantage of low field permanent magnetic fields is that they can be constructed in a variety of configurations, some of them with an easy patient access and an open structure which eliminates an occasional claustrophobic reaction induced by the small bore size of high field MR systems.

A resistive magnet is an electromagnet in which the magnetic field is generated by the passage of current through a wire that is a good electrical conductor (but with a finite electrical resistance). Typically, resistive whole body magnets are constructed in a four-coil Helmholtz configuration with the outer coils smaller than the inner as to approximate a spherical geometry. At normal temperature, the finite resistance of the coils to passage of electrical current places high power requirements on the system to produce sufficient electrical current for the generation of the desired magnetic field. Typically for a 0.15 T magnet, the required power is  $\sim 50$  kW. The power to magnetize a resistive magnet is dissipated as heat in the coils, which must be removed by passing cooled water along the coils. Although power and cooling requirements are easily met for low field strengths, they limit the field strength since the power increases with the square of the magnetic field strength. The stability (expressed in ppm  $\text{h}^{-1}$ ) of resistive magnets is not nearly as good as superconducting magnets, which will be described next. Just as permanent magnets, resistive magnets can be designed in a number of open, patient-friendly configurations.

Almost all modern MRI systems are based on a superconducting magnet design. Superconductivity is a phenomenon occurring in certain materials at low temperatures, characterized by zero electrical resistance and the exclusion of internal magnetic fields (the Meissner effect). Figure 10.1A shows a graphical depiction of the resistance of a superconductor and a copper conductor as a function of temperature. At a material-specific critical temperature  $T_c$ , the finite resistance of the superconductor becomes zero. The resistance of a copper conductor also decreases with decreasing temperature, but never reaches zero, thereby preventing superconductivity. As long as the temperature of the superconductor remains below the critical temperature, current can continue to flow without heat dissipation. This in turn can maintain a constant magnetic field indefinitely. However, the critical temperature of a material is lowered when placed in a strong external magnetic field. Figure 10.1B shows a superconductor boundary surface for the variables temperature  $T$ , magnetic field  $B$  and current  $I$ . Only behind the surface does the material maintain superconductive properties. This means that at a given temperature (e.g. 4.2 K, liquid helium at atmospheric pressure)

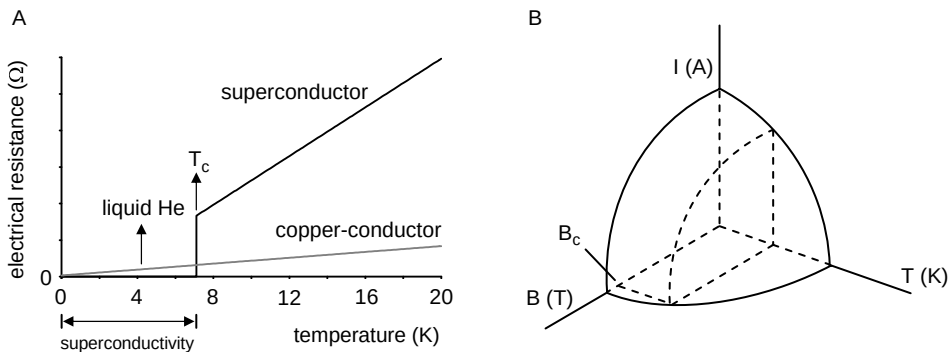


**Figure 10.1** (A) Electrical resistance as a function of absolute temperature for a typical superconductor (black line) and a copper conductor (gray line). While the copper conductor retains a finite electrical resistance at any temperature, the resistance for a superconductor drops to zero below a critical temperature  $T_c$ . (B) The critical temperature for a given material reduces with increasing magnetic field strength  $B$  and increasing current density  $I$ , such that superconductivity can only be maintained behind the 3D surface.  $B_c$ , critical magnetic field.

and current, there is a maximum magnetic field strength that can be achieved before the material becomes resistive. The magnetic field can only be further increased by lowering the temperature or using a different material.

Common materials for superconducting wire are niobium-titanium (NbTi) and niobium-tin ( $Nb_3Sn$ ) alloys. NbTi filaments are normally placed within a copper matrix (Figure 10.2A) which aids in electrically and mechanically stabilizing the superconductor. Friction or mechanical motion makes superconductors susceptible to local heating, which could potentially lead to a local loss of superconductivity. The local effect may in turn propagate throughout the entire magnet, leading to a full blown quench, i.e. a total and abrupt

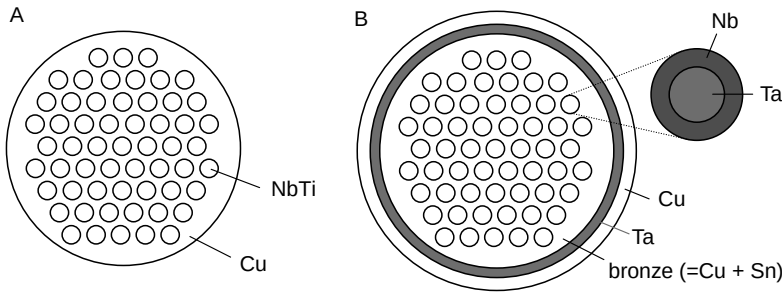


**Figure 10.2** Cross-sectional views of (A) niobium-titanium (NbTi) and (B) niobium-tin ( $Nb_3Sn$ ) superconducting wires. The  $Nb_3Sn$  wire is shown in an unreacted state. During the high-temperature bronze diffusion processes, the tin diffuses to the niobium and forms the superconducting  $Nb_3Sn$  crystals. The copper acts as a thermal and structural stabilizer. See text for more details.

discharge of the coil. However, the surrounding copper can take over the current flowing in the superconductor and allows the superconductor to recover and return to its initial (superconducting) condition. The superconducting properties of NbTi stretch to magnetic fields up to 9–10 T. For higher magnetic fields, the Nb<sub>3</sub>Sn alloy offers more favorable properties. However, because Nb<sub>3</sub>Sn is brittle rather than ductile, it must be formed at its final shape and position. In an unreacted state (Figure 10.2B) a Nb-Sn wire is flexible enough to be pulled into thin wires to be used for winding of the main magnet coils. The superconductive properties of the pure Nb in the unreactive state are poor. The superconductive Nb<sub>3</sub>Sn alloy is formed by exposing the wire to temperatures in excess of 700 °C during which the tin in the bronze matrix diffuses to the niobium where it forms superconducting Nb<sub>3</sub>Sn crystals. This process is referred to as the bronze diffusion technique. The tantalum acts as a barrier to prevent tin from diffusing into the copper layer, which would greatly reduce its stabilizing effect.

Following transportation and installation, the magnet needs to be energized to its final magnetic field. Typically, the first step is to cool the magnet coils with liquid nitrogen, followed by liquid helium, a process that may take several days. Next, a power supply is connected to the magnet through the use of a superconducting switch. A small portion of the superconducting wire inside the switch is made resistive by an adjacent heater. Since the magnet has no resistance, all current from the power supply runs through the magnet coil. Once the desired current has been established, the heater is turned off and the wire of the switch becomes superconductive, thereby closing the current loop of the main magnet coil. After the driving current has slowly been reduced to zero, the power supply can be disconnected, leaving the magnet in a persistent mode.

The homogeneity of the magnetic field is, besides the strength, the most important parameter characterizing a NMR magnet. It can be shown that a magnet designed as an infinitely long solenoid will produce a homogeneous magnetic field [6]. Short truncated solenoidal coils with correction coils at either end, or optimized multi-coil designs are the practical implementation of this theoretical solution. Figure 10.3 shows a 3D model drawing of a typical MR magnet for animal applications. The position and size of the individual coils are optimized to provide maximum homogeneity over a given diameter spherical volume (DSV). However, the finite length of the magnet, together with unavoidable errors due to fabrication tolerances, lead to a small deviation from the designed homogeneity. The homogeneity of a ‘raw’ or ‘bare’ magnet can be >20 ppm over the DSV (which could be specified as 45 cm on a human system). To improve the homogeneity further, modern magnets are typically equipped with superconductive shims. The principles of magnet shimming are identical to those of subject-specific *in vivo* shimming and will be described in Section 10.3. The most noticeable difference is that superconductive magnet shimming has to be performed only once, during the installation of the magnet. In cases where superconductive shimming can not provide the specified homogeneity, additional higher-order homogeneity improvements can be obtained through passive shimming, in which small pieces of ferromagnetic metal (iron, steel, nickel) are placed in optimized spatial locations in order to cancel the measured inhomogeneity. Passive shim trays with predetermined slots for the metallic pieces are often integrated into the design of the magnet or gradient coil, whether passive shimming is required or not. Following superconductive shimming (and passive shimming) the magnetic field homogeneity over the DSV can be as low as 1–2 ppm. The final magnetic field homogeneity during the *in vivo* measurement will

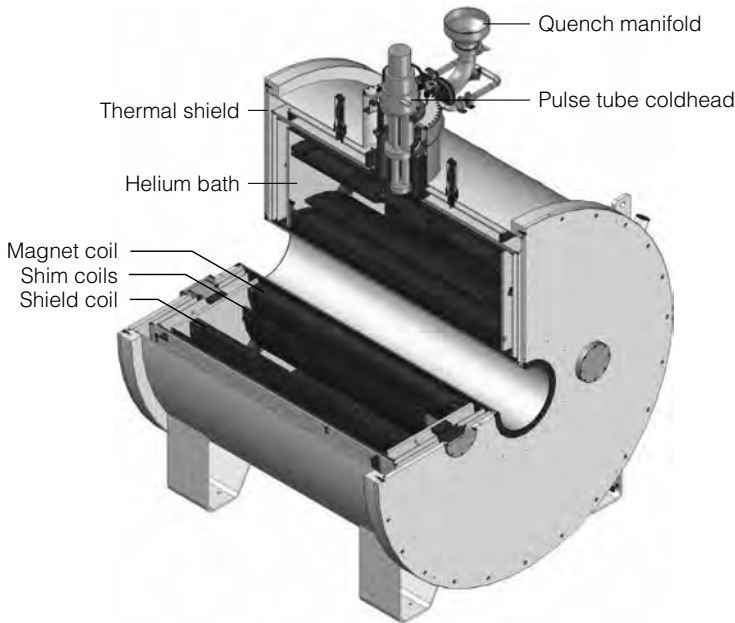


**Figure 10.3** 3D cross-sectional view of a 9.4 T magnet, showing the primary magnetic and secondary shield coils, the superconducting shim coils as well as the thermal shield. The pulse tube coldhead virtually eliminates net helium boil-off. (Courtesy of S. Pittard, Magnex Scientific.)

be optimized on a subject-specific basis with room-temperature shims, as will be discussed in Section 10.3.

Other considerations involved with the magnet include temporal stability of the magnetic field, the spatial extent of the magnetic field outside the magnet, and the boil-off of the cryogenics. The temporal stability of the magnetic field, also referred to as ‘magnet drift’, is an important parameter for longer experiments. Modern superconducting magnets typically have a drift of less than  $-0.1 \text{ ppm h}^{-1}$ . However, larger apparent magnet drifts can, for example, be observed when passive shims are heating up due to insufficient thermal isolation from the gradients. When an experiment requires signal averaging, significant broadening of spectral resonances can result, even when the inherent magnet drift is very low. It is therefore essential to either correct for magnet drifts by magnetic field locks [7], similar to those used in high-resolution NMR, or by post-acquisition frequency alignment as detailed in Chapters 8 and 9.

The spatial extent of the magnetic field outside the magnet, also known as the ‘fringe field’, is important for magnet placement and study-related logistics, like placement of physiology monitoring equipment and computers. The fringe field is often specified as the distance where the magnetic field has dropped to 5 or 10 G. A conventional, nonshielded 1.5 T magnet can have a 5 G fringe field stretching out for 12 and 10 m in the axial and radial directions, respectively. This poses serious limitations for placement of the MRI system

and placement of peripheral equipment. As a result, many modern magnets are of a self-shielded design in which additional shield coils on the outside of the main magnetic coils (see Figure 10.3) are constructed to minimize the magnet fringe field without sacrificing too much of the desired internal magnetic field. The 5 G fringe field of a shielded 1.5 T magnet is reduced to 4 and 3 m in the axial and radial directions, respectively. Since magnet shielding invariably leads to a reduction in the desired magnet field, as a rule of thumb magnetic shielding is not available at the highest magnetic fields. Other methods of reducing the fringe field include surrounding the magnet directly with steel or placing the magnet in a room with steel walls. Magnetic materials like steel have a smaller resistance to magnetic flux than air and as a result magnetic flux tends to focus inside the steel, thereby reducing the fringe field outside the shielding. This method remains effective as long as the steel does not become magnetically saturated.

A final consideration with superconducting magnets concerns the boil-off of the cryogenics and in particular the liquid helium. Due to a number of reasons, most importantly heat leaks of the helium Dewar to the surrounding environment, there will be a constant boil-off of helium. Since the magnet must remain in a superconductive state this necessitates regular filling of the magnet with cryogenics. To relieve the constant need for expensive cryogenics (i.e. helium) modern magnets are equipped with so-called cold heads, which extract excess heat from the magnet through the so-called Joule–Thomson effect (e.g. [8]). While cold heads can reduce the helium boil-off several orders of magnitude, there will be a finite boil-off which necessitates replenishment of liquid helium (typically only once a year). Many manufacturers now also offer ‘zero-boil-off’ magnets, which supposedly have no net helium boil-off. These magnets are equipped with reliquifier units, which essentially work as a ‘cold finger’. As the helium is evaporating at a normal rate, the helium gas recondenses on the cold finger after which it drips back into the liquid helium reservoir. Under ideal conditions this set-up will lead to a close to zero helium boil-off.

## 10.3 Magnetic Field Homogeneity

### 10.3.1 Origins and Effects of Magnetic Field Inhomogeneity *In Vivo*

A current passing through a conductor of a specified geometry creates a magnetic field  $\mathbf{H}$  (also referred to as the auxiliary field) that, in turn establishes a magnetic induction  $\mathbf{B}$ . The actual value of  $\mathbf{B}$  in a given linear substance is related to  $\mathbf{H}$  according to:

$$\mathbf{B} = \mu \mathbf{H} \quad (10.1)$$

where  $\mu$  is the magnetic permeability (in  $\text{H m}^{-1}$ ). In a vacuum the magnetic permeability is given by  $\mu_0 = 4\pi \times 10^{-7} \text{ H m}^{-1}$ .  $\mathbf{H}$  (in  $\text{A m}^{-1}$ ) describes the physical arrangement of the current-carrying wires (i.e. magnet design), while  $\mathbf{B}$  (in T) combines this characteristic with the tendency of the medium in which the field resides to be inductively magnetized. In any medium the interaction of moving charges in atoms and molecules within a magnetic field leads to the induction of a bulk magnetic moment, denoted by the magnetization  $\mathbf{M}$ . The induced magnetization  $\mathbf{M}$  contributes to the magnetic induction  $\mathbf{B}$  experienced by the nuclear moments according to:

$$\mathbf{B} = \mu_0 \mathbf{H} + \mu_0 \mathbf{M} = \mu \mathbf{H} \quad (10.2)$$

Since for most materials  $\mu$  is very close to  $\mu_0$ , it is convenient to introduce the magnetic susceptibility  $\chi = (\mu/\mu_0) - 1$  which describes how the relative permeability ( $\mu/\mu_0$ ) deviates from unity. Equation (10.2) then simplifies to:

$$\mathbf{B} = \mu_0(1 + \chi)\mathbf{H} \quad (10.3)$$

which can be rewritten as:

$$\mathbf{M} \approx \mathbf{M}_0 = \frac{\chi \mathbf{B}_0}{\mu_0(1 + \chi)} \approx \frac{\chi}{\mu_0} \mathbf{B}_0 \quad (10.4)$$

The magnetic susceptibility is zero, negative ( $\chi < 0$ ) or positive ( $\chi > 0$ ) for nonmagnetic, diamagnetic or paramagnetic materials, respectively. For ferromagnetic materials,  $\chi \gg 1$ . However, for all MR compatible materials  $|\chi| \ll 1$ .

Given a magnetic susceptibility distribution, the macroscopic auxiliary field  $\mathbf{H}$  can be calculated using Maxwell's equations. At the microscopic level of the nucleus, the susceptibility effects of adjacent molecules can partially cancel or are taken as part of the chemical shift effect (see Section 1.8). In order to avoid these local susceptibility effects, the nucleus is typically placed in a so-called sphere of Lorentz [9, 10] in which the nucleus is considered to be placed in an imaginary sphere of zero magnetic susceptibility such that any time-varying effects of adjacent molecules can be viewed as that of a homogenous medium. The microscopic field experienced within the sphere of Lorentz can be shown to experience an offset from the macroscopic auxiliary field by a factor of  $-2/3\chi \mathbf{H}$  [10, 11].

For simple geometric objects, like spheres and cylinders, analytical expressions for the auxiliary field  $\mathbf{H}$  can be obtained [6, 11]. Table 10.1 gives these expressions, whereas Figure 10.4 presents a graphical depiction of the fields. It follows that at susceptibility boundaries, significant magnetic field perturbations occur that are geometry and direction dependent.

Figure 10.5A shows an anatomical spin-echo MRI of a sagittal slice through the human head. One of the most significant air-tissue interfaces is found between the nasal sinus cavity and the lower frontal cortex. The magnetic susceptibility difference between air and tissue leads to a perturbation of the magnetic field as can be seen from the quantitative magnetic field map shown in Figure 10.5B. Other areas of decreased magnetic field homogeneity due to susceptibility differences can be found in the temporal cortices and around

**Table 10.1** Magnetic fields of simple geometric objects with different internal ( $\chi_i$ ) and external ( $\chi_e$ ) magnetic susceptibilities<sup>a</sup>

|                                                           | Internal magnetic fields                            | External magnetic field                                                                                  |
|-----------------------------------------------------------|-----------------------------------------------------|----------------------------------------------------------------------------------------------------------|
| Sphere (radius R)                                         | $(1 + \frac{\chi_e}{3}) B_0$                        | $(1 + \frac{\chi_e}{3} + \frac{\Delta\chi}{3} R^3 \frac{2z^2 - x^2 - y^2}{(x^2 + y^2 + z^2)^{5/2}}) B_0$ |
| Cylinder (radius R, perpendicular to $B_0$ ) <sup>b</sup> | $(1 + \frac{\chi_e}{3} - \frac{\Delta\chi}{6}) B_0$ | $(1 + \frac{\chi_e}{3} + \frac{\Delta\chi}{2} R^2 \frac{z^2 - x^2}{(z^2 + x^2)^2}) B_0$                  |
| Cylinder (radius R, parallel to $B_0$ )                   | $(1 + \frac{\chi_e}{3} + \frac{\Delta\chi}{3}) B_0$ | $(1 + \frac{\chi_e}{3}) B_0$                                                                             |

<sup>a</sup>  $\Delta\chi = \chi_e - \chi_i$ .

<sup>b</sup> Cylinder axis oriented in y direction.



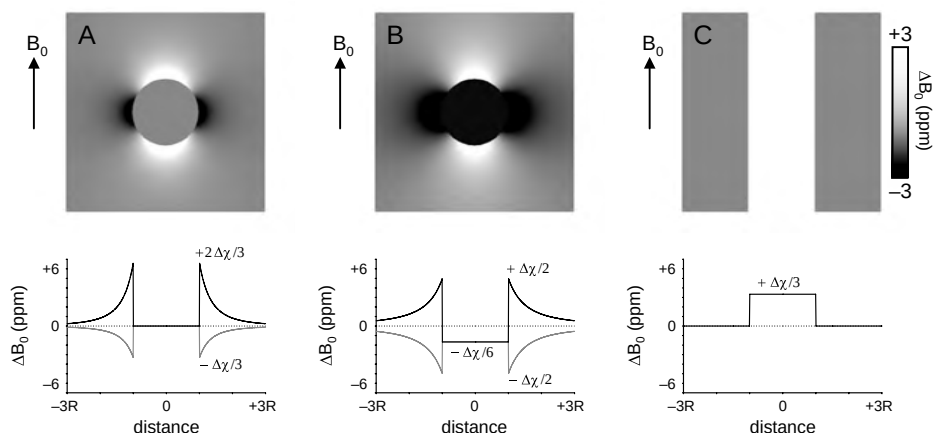


**Figure 10.4** Graphical depiction of the equations given in Table 10.1 for (A) a spherical geometry and for a cylindrical geometry (B) perpendicular and (C) parallel to the main magnetic field. The field distributions shown are for traces in the middle of the object, perpendicular (gray line) and parallel (black line) to the main magnetic field.

the auditory tracts (Figure 10.5C), while smaller effects can be observed around ventricles, close to the skull and between gray and white matter.

The effects of magnetic field inhomogeneity on the NMR signal are best described by dividing a macroscopic sample into a large number of small volumes over which the magnetic field is constant. The NMR signal from the total sample in an inhomogeneous magnetic field can then be described by:

$$M_{xy}(t) = M_0 \sum_{n=1}^N e^{i\omega_n t} e^{-t/T_2} = M_0 e^{i\omega_0 t} e^{-t/T_2} \sum_{n=1}^N e^{i\Delta\omega_n t} \quad (10.5)$$

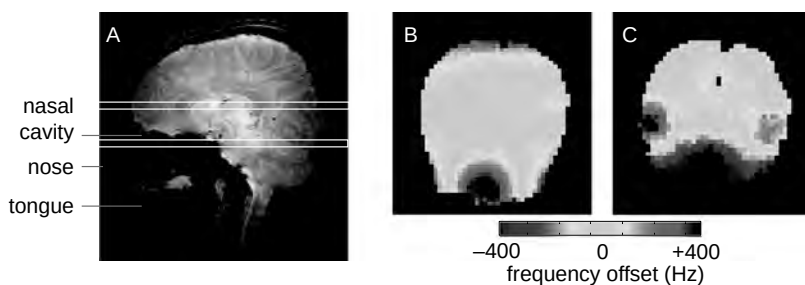


**Figure 10.5** (A) Anatomical spin echo MRI of a human head in the sagittal plane. (B, C) Magnetic field  $B_0$  maps acquired at 4 T from the two axial slices shown in (A) following whole-brain second-order shimming. Significant magnetic field inhomogeneity can be observed (B) above the sinus cavity and (C) around the auditory tracts. (See color plate 6).

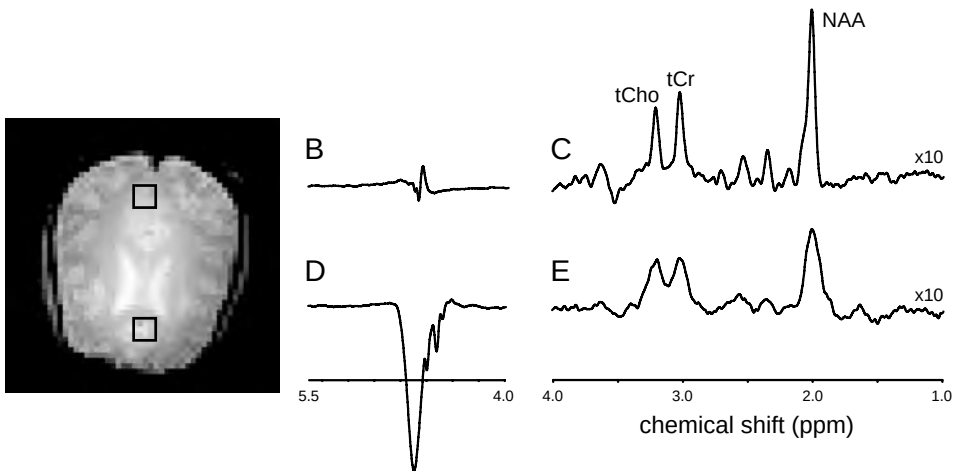


**Figure 10.6** Effects of magnetic field inhomogeneity on MR spectroscopic measurements. (B,D) Localized water and (C,E) water-suppressed metabolite  $^1\text{H}$  NMR spectra acquired at 4.0 T from the occipital (B,C) and frontal (D,E) volumes as indicated on the axial image in (A). The reduced magnetic field homogeneity in the frontal cortex (see Figure 10.5B) leads to a larger residual water signal (D) and a decrease in spectral resolution (E).

where  $N$  equals the total number of small volumes elements and  $\omega_n$  represents the frequency (which is proportional to the local magnetic field) in a volume element  $n$ . For simplicity, the longitudinal equilibrium magnetization  $M_0$  and the inherent  $T_2$  relaxation time constant are assumed constant throughout the sample.  $\Delta\omega_n$  represents the difference between the frequency in volume element  $n$  and the nominal Larmor frequency  $\omega_0$ . The effects of magnetic field inhomogeneity on the appearance of NMR spectra can now easily be deduced from Equation (10.5). In a perfectly homogeneous field,  $\Delta\omega_n = 0$  for all  $N$  volumes, such that the  $N$  Lorentzian lines originating from those volumes are centered at the same frequency  $\omega_0$  and therefore add coherently, resulting in a single Lorentzian spectral line with a FWHM ( $= 1/(\pi T_2)$ ) inversely proportional to the inherent  $T_2$  relaxation time constant. In the presence of magnetic field inhomogeneity the spins in the  $N$  volumes resonate at different Larmor frequencies  $\omega_n$ . As a result the Lorentzian lines are centered at different frequencies  $\omega_n$  and do therefore not add coherently. Instead a broadened, non-Lorentzian resonance line is obtained in which the frequencies are spread over a range dictated by  $\omega_n$ . Alternatively stated, the spread of frequencies leads to a loss of phase coherence for  $t > 0$  in the time-domain, which results in a faster apparent decay of transverse magnetization and hence a broadening of the spectral lines. Figure 10.6 shows two spectra extracted from a MRSI dataset acquired from human brain at 4.0 T. The volume originating from the occipital cortex has a relatively high magnetic field homogeneity, resulting in a high-resolution NMR spectrum. The  $^1\text{H}$  NMR spectrum from the volume in the frontal cortex is characterized by broadening resonances and poor water suppression due to the low magnetic field homogeneity at that spatial position. Spectral quantification of the spectrum in Figure 10.6E will be highly unreliable (see also Chapter 9).

In MRI applications the effects of magnetic field inhomogeneity, though identical in nature, manifest themselves differently. The loss of phase coherence will lead to signal loss in any dimension that is not (sufficiently) spatially resolved (e.g. the slice selection dimension in 2D MRI). Furthermore, as the MRI magnetic field gradients encode spatial positions as different frequencies, a range of frequencies caused by magnetic field inhomogeneity will lead to a spatial shift and hence spatial misregistration, blurring and/or signal loss. Figure 10.7A and B shows spin-echo and gradient-echo MR images of two slices centered above the nasal cavity (top) and above the auditory tracts (bottom). Since spin-echo (or spin-warp) MRI methods are largely immune to magnetic field inhomogeneity, the image has no significant distortions or signal loss. The gradient-echo has no compensation towards magnetic field inhomogeneity, resulting in a position-dependent phase during the 20 ms echo-time. In all dimensions, and especially the slice selection direction, the phase evolution leads to signal cancellation as can be clearly seen in the frontal cortex and around the ear canals. Gradient-echo images do not show any appreciable in-plane image distortion (i.e. pixels shifts), because the (effective) spectral bandwidths are large in both spatial dimensions. Note that even longer echo-times are required for optimal BOLD contrast at 4.0 T, necessarily leading to more signal loss in the indicated regions. Figure 10.7C shows

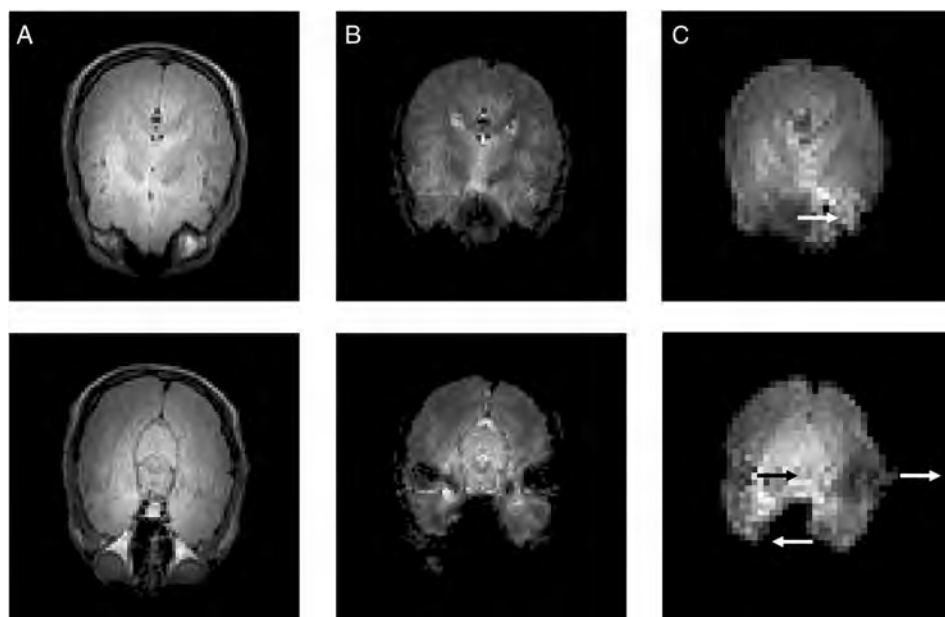


**Figure 10.7** Effects of magnetic field inhomogeneity on MRI measurements. (A) Anatomical spin-echo MR images from the human head acquired at 4.0 T. (B) Gradient-echo images ( $TE = 20$  ms) acquired from the same spatial slices as shown in (A), displaying signal loss in areas of low magnetic field homogeneity. (C) Simulated echo-planar images (bandwidth = 100 kHz,  $64 \times 64$  matrix) displaying geometric image distortion. The arrows indicate the direction of the local pixel shifts. Experimental echo-planar images would also exhibit through-slice signal loss as shown in (B) in addition to the image distortion.

simulated, single-shot echo-planar images acquired with an acquisition bandwidth of 100 kHz, which is typical for EPI acquisitions. Since echo-planar images are largely based on gradient echoes, the areas with decreased magnetic field homogeneity are characterized by severe geometric distortions (i.e. pixel shifts) in the phase-encoding direction. (See Chapter 4 for more details.) For many applications that require accurate knowledge of spatial positions, like diffusion tensor imaging (DTI) and functional magnetic resonance imaging (fMRI), EPI may not be the best choice. However, the superior temporal resolution of EPI makes it an ideal candidate for DTI and fMRI. The conflicting features of EPI has led to the development of a wide range of alternative fast MRI methods, each with restrictions towards temporal resolution, RF power deposition and sensitivity towards magnetic field inhomogeneity (e.g. [12]).

The minimum magnetic field homogeneity required is strongly dependent on the application. Consider for example an imaging experiment with imaging gradients of  $1.0 \text{ G cm}^{-1}$  ( $= 4257 \text{ Hz cm}^{-1}$  for  $^1\text{H}$ ). For an in-plane resolution of  $1.0 \times 1.0 \text{ mm}^2$ , the field homogeneity over the voxel needs to be better than  $0.1 \text{ cm} \times 4257 \text{ Hz cm}^{-1} = 425.7 \text{ Hz}$ . If this criterion is not met, the voxels will be misregistered and the image will appear blurred. On 1.5 T and 4.7 T, a minimum homogeneity of 425.7 Hz corresponds to 6.67 and 2.13 ppm, respectively. Thus homogeneity requirements (expressed in ppm) become more stringent at higher field strengths. For MRS studies the requirements become even more stringent. For example, a clear discrimination between choline and total creatine resonances in  $^1\text{H}$  MRS requires a minimum magnetic field homogeneity of 0.1–0.2 ppm. Even though the minimum homogeneity requirement for MRI and MRS experiments may differ by an order of magnitude, they will both benefit (in terms of resolution and S/N) from a high magnetic field homogeneity. A high magnetic field homogeneity on a subject-specific basis can be achieved by active shimming.

### 10.3.2 Active Shimming

The magnetic field distribution  $\mathbf{B}_0$  inside the bore of a superconducting magnet can be described by Laplace's equation:

$$\nabla^2 \mathbf{B}_0 = 0 \quad \text{or} \quad \left( \frac{d^2}{dx^2} + \frac{d^2}{dy^2} + \frac{d^2}{dz^2} \right) \mathbf{B}_0 = 0 \quad (10.6)$$

Using spherical polar coordinates  $(r, \theta, \phi)$  the solution of Equation (10.6), i.e. the magnetic field  $\mathbf{B}_0$ , can be described as a linear expansion of spherical harmonic functions  $Y_{n,m}(\theta, \phi)$  of order  $n$  and degree  $m$ :

$$\mathbf{B}_0(r, \theta, \phi) = \sum_{n=0}^{\infty} \sum_{m=0}^n C_{n,m} r^n Y_{n,m}(\theta, \phi) \quad (10.7)$$

in which  $C_{n,m}$  is a constant coefficient and the spherical harmonic function  $Y_{n,m}(\theta, \phi)$  is given by:

$$Y_{n,m}(\theta, \phi) = \sqrt{\frac{2n+1}{4\pi} \frac{(n-m)!}{(n+m)!}} P_{n,m}(\cos \theta) e^{im\phi} \quad (10.8)$$

$n$  and  $m$  are integers for which  $n \geq m \geq 0$ .  $P_{n,m}(\cos\theta)$  is the associated Legendre function of  $\cos\theta$  and is defined as ( $x = \cos\theta$ ):

$$P_{n,m}(x) = \frac{(-1)^m}{2^n n!} (1-x^2)^{m/2} \frac{d^{n+m}}{dx^{n+m}} (x^2-1)^n \quad (10.9)$$

The first term of Equation (10.7), i.e.  $C_{0,0}$  when  $n = 0$ , represents the constant, nominal magnetic field  $B_{0,\text{nom}}$  and an off-resonance offset,  $B_{0,\text{offset}}$ . The magnetic field inhomogeneity  $\Delta B_0(r, \theta, \phi)$  can then be calculated as  $B_0(r, \theta, \phi) - B_{0,\text{nom}}$  which is given by:

$$\Delta B_0(r, \theta, \phi) = B_{0,\text{offset}} + \sum_{n=1}^{\infty} \sum_{m=0}^n r^n P_{n,m}(\cos\theta) (A_{n,m} \cos(m\phi) + B_{n,m} \sin(m\phi)) \quad (10.10)$$

On many systems the different shims are referred to by their Cartesian functions. The spherical coordinate base ( $r, \theta, \phi$ ) of Equation (10.10) is readily converted to a Cartesian coordinate base ( $x, y, z$ ) by using  $x = r \sin\theta \cos\phi$ ,  $y = r \sin\theta \sin\phi$  and  $z = r \cos\theta$ , giving  $\Delta B_0(x, y, z)$  as:

$$\Delta B_0(x, y, z) = B_{0,\text{offset}} + \sum_{n=1}^{\infty} \sum_{m=0}^n C_{n,m} F_{n,m}(x, y, z) \quad (10.11)$$

The expansion of  $\Delta B_0$  up to fourth order in spherical and Cartesian coordinates is summarized in Table 10.2. The spatial dependence of the terms in Equation (10.11) are graphically depicted in Figure 10.8. Note that all noninteger constants in front of the functions in the

**Table 10.2** Polar and Cartesian representation of low-order ( $n \leq 4$ ) spherical harmonic functions<sup>a</sup>

| Order<br>$n$ | Degree<br>$m$ | Polar function                              | Cartesian function                         | Common name                 |
|--------------|---------------|---------------------------------------------|--------------------------------------------|-----------------------------|
| 0            | 0             | 1                                           | 1                                          | $Z^0$                       |
| 1            | 0             | $r \cos\theta$                              | $z$                                        | $Z$                         |
| 1            | 1             | $r \sin\theta \cos\phi$                     | $x$                                        | $X$                         |
| 1            | 1'            | $r \sin\theta \sin\phi$                     | $y$                                        | $Y$                         |
| 2            | 0             | $r^2(3\cos^2\theta - 1)$                    | $2z^2 - (x^2 + y^2)$                       | $Z^2$                       |
| 2            | 1             | $r^2 \sin\theta \cos\theta \sin\phi$        | $zx$                                       | $ZX$                        |
| 2            | 1'            | $r^2 \sin\theta \cos\theta \cos\phi$        | $zy$                                       | $ZY$                        |
| 2            | 2             | $r^2 \sin^2\theta \cos 2\phi$               | $x^2 - y^2$                                | $X^2 - Y^2$ (or $C^2$ )     |
| 2            | 2'            | $r^2 \sin^2\theta \sin 2\phi$               | $2xy$                                      | $XY$ (or $S^2$ )            |
| 3            | 0             | $r^3(5\cos^3\theta - 3\cos\theta)$          | $2z^3 - 3z(x^2 + y^2)$                     | $Z^3$                       |
| 3            | 1             | $r^3 \sin\theta(5\cos^2\theta - 1)\cos\phi$ | $4z^2x - x(x^2 + y^2)$                     | $Z^2X$                      |
| 3            | 1'            | $r^3 \sin\theta(5\cos^2\theta - 1)\sin\phi$ | $4z^2y - y(x^2 + y^2)$                     | $Z^2Y$                      |
| 3            | 2             | $r^3 \sin^2\theta \cos\theta \cos 2\phi$    | $z(x^2 - y^2)$                             | $Z(X^2 - Y^2)$ (or $ZC^2$ ) |
| 3            | 2'            | $r^3 \sin^2\theta \cos\theta \sin 2\phi$    | $2zxxy$                                    | $ZXY$ (or $ZS^2$ )          |
| 3            | 3             | $r^3 \sin^3\theta \cos 3\phi$               | $x(x^2 - 3y^2)$                            | $X^3$                       |
| 3            | 3'            | $r^3 \sin^3\theta \sin 3\phi$               | $y(3x^2 - y^2)$                            | $Y^3$                       |
| 4            | 0             | $r^4(35\cos^4\theta - 30\cos^2\theta + 3)$  | $8z^4 - 24z^2(x^2 + y^2) + 3(x^2 + y^2)^2$ | $Z^4$                       |

<sup>a</sup>Spherical harmonic functions are defined here to have no constants or nonintegers in the polar form.

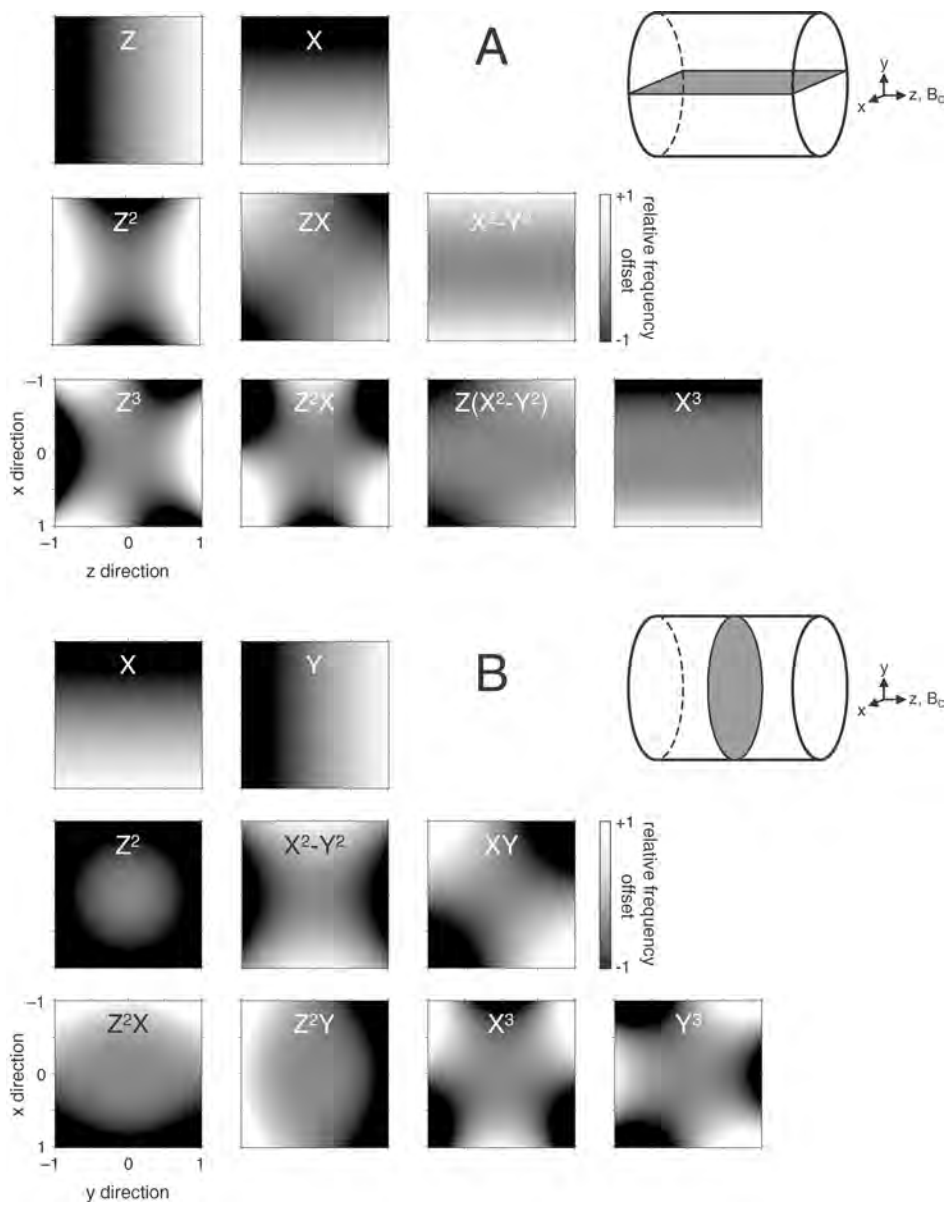


**Figure 10.8** 2D representations of spherical harmonic fields in (A) coronal (xz) and (B) axial (xy) slices positioned in the magnet isocenter. Some of the spherical harmonic fields are not shown since they have zero intensity throughout the displayed slices [e.g. Y, XY and YZ shims in the coronal ( $y = 0$ ) orientation].

spherical basis have been included in the  $C_{n,m}$  coefficients. This is inconsequential for shim optimization, as the  $C_{n,m}$  coefficients are normally calibrated on a system-specific basis. However, when comparing absolute (calibrated) shim strengths between systems, it is crucial to confirm that identical expressions for the spherical harmonic functions are used.

The process of shimming then simply reduces to a minimization procedure in which one attempts to cancel the measured magnetic field  $\Delta B_{0,\text{measured}}$  with the available spherical harmonics shims as denoted in Equation (10.11). The minimization procedure can be performed manually by iterative adjustment of shims or by fully automated, noniterative methods based on quantitative magnetic field maps. The process of shimming will be detailed in Sections 10.3.4–10.3.8, following a description of the required hardware.

### 10.3.3 Shimming Hardware

Shim coils are normally composed of several current loops connected in series and placed geometrically so that the magnetic field generated by them across a DSV corresponds to a specific spherical harmonic. The current flowing in the shim coil, as defined by  $A_{nm}$  and  $B_{nm}$  in the spherical coordinate basis or  $C_{nm}$  in the Cartesian coordinate basis, governs the strength of the magnetic shim field. On most *in vivo* NMR systems, only shim coils corresponding to lower order spherical harmonics are available and are typically referred to in the Cartesian coordinate basis. For example, the first-order shim coils are typically referred to as Z, X and Y shims, and second-order shim coils as  $Z^2$ , ZX, ZY,  $(X^2 - Y^2)$ , and XY shims. Note that this nomenclature is somewhat misleading, as a  $Z^2$  shim coil does not produce a magnetic field that varies spatially as  $z^2$ . Instead the magnetic field is proportional to  $z^2 - 1/2(x^2 + y^2)$ . As a rule of thumb, the short-hand Cartesian notation of shim coils does not accurately reflect the spatial dependence of the shim magnetic field of higher-order shims. All quantitative calculations involving spherical harmonic shims should therefore use Equation (10.10) or the full expressions summarized in Table 10.2.

The first-order shim fields are generated using the standard gradient amplifiers and are applied as an offset into the gradient coils (see Section 10.4). All other, higher-order shim fields are generated by dedicated shim amplifiers and shim coils. Figure 10.9A shows schematic representations of all zero, first and second spherical harmonic shim coils.

Since the magnetic field distribution in animal and human brain is characterized by localized higher-order inhomogeneity, *in vivo* shimming can greatly benefit from higher order shim fields. However, the number of available shim coils is typically limited to second- or third-order spherical harmonics, primarily due to magnet bore space limitations. Furthermore, the shim strength of the available shim coils can be limited by the maximum current provided by the shim power supply, which is often restricted to circa 2–5 A and 4–20 A on animal and human MR systems, respectively. In addition, limitations on the combined currents from all shims are required in order to prevent excessive heating of the shim coils. These inherent limitations have led to incomplete compensation of magnetic field inhomogeneity in human and animal brain, which in turn has fuelled the development of alternative strategies for *in vivo* shimming, as will be discussed in Sections 10.3.7 and 10.3.8.

### 10.3.4 Manual Shimming

The oldest and, on the surface the simplest method to improve the magnetic field homogeneity is to manually adjust the currents in the individual shim coils until the encountered

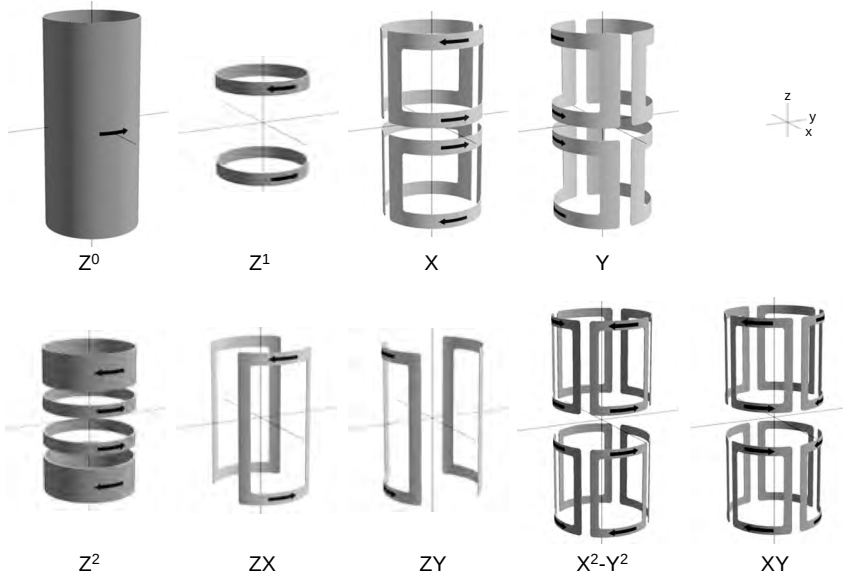


**Figure 10.9** 3D representations of the wire positions and current directions (black arrows) for shim coils that produce zero-, first- and second-order spherical harmonic fields. (Courtesy of D. Green and S. Pittard, Magnex Scientific.)

magnetic field distribution has been homogenized. While this was and continues to be a routine procedure on many high-resolution NMR magnets, it is bound to fail frequently on *in vivo* NMR magnets involving humans or animals. The complications of manual *in vivo* shimming arise from spatial variations in spin density and  $B_1$  homogeneity, higher-to-lower order shim interactions, off-center sample positioning and limited sensitivity. The complication of off-center shimming was pointed out by Hoult [13] and is graphically depicted in Figure 10.10. Consider a 1D sample positioned some distance  $z$  from the magnet isocenter  $z_0$ . The magnetic field distribution across the sample in the  $z$  direction is given by:

$$\Delta B_0 = a + bz^2 \quad (10.12)$$

When during manual shimming a  $-z^2$  shim field is applied, the situation shown in Figure 10.10B arises. Even though the correct shim value (and sign) was set to cancel the quadratic magnetic field distribution, the overall homogeneity considerably worsened since, out of the isocenter, a  $z^2$  shim also produces low-order terms according to:

$$\Delta B_0 = a + bz^2 - b(z_0 + z)^2 = a - bz_0^2 - 2bz_0z \quad (10.13)$$

The drastic decrease in homogeneity due to the increased linear inhomogeneity ( $-2bz_0z$ ) may persuade the spectroscopist to reverse the  $z^2$  shim value, which indeed improves the homogeneity (Figure 10.10C). However, this minimum is not the global minimum which would have been obtained after the inhomogeneous, but correctly adjusted situation of Figure 10.10B was improved by adjusting the linear  $z$  shim to give the optimal homogeneity (Figure 10.10D). Note that both the  $z^2$  and  $z$  shims generate magnetic field offsets,  $-bz_0^2$  and  $+2bz_0z$ , respectively. However, the on-resonance condition can be obtained by adjusting



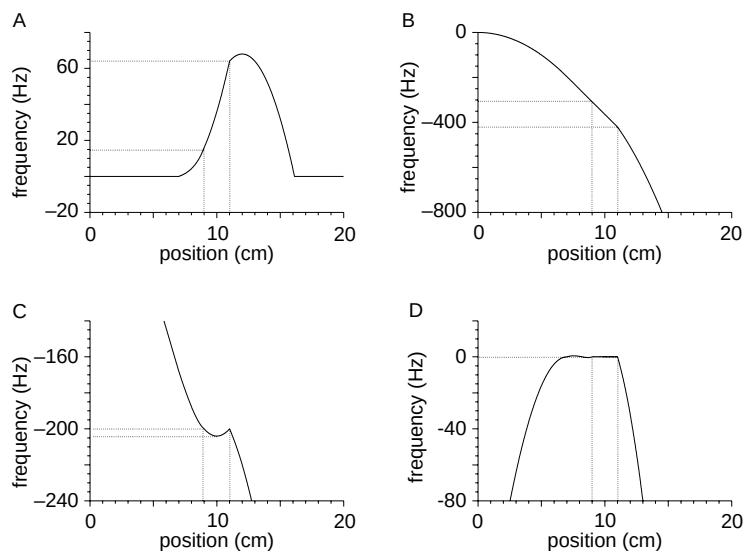


**Figure 10.10** The effects of sample displacement during manual second-order shimming. (A) The region of interest within a sample is placed  $10 \pm 1$  cm out of the magnet isocenter in the  $z$  direction. The  $B_0$  magnetic field inhomogeneity, which is directly proportional to the spread in resonance frequencies, is given by  $a + bz^2$ , where  $z$  is the spatial position. (B) The correct application of a  $-z^2$  shim gradient leads to a significant reduction in the magnetic field homogeneity because the  $z^2$  shim also generates a first-order magnetic field gradient out of the magnet isocenter [i.e. Equation (10.13)]. (C) Because the homogeneity decreased in (B), the spectroscopist may be persuaded to reverse the  $z^2$  shim and only use the first-order  $z$ -shim, which indeed improves the homogeneity. (D) However, if the operator would have proceeded with the correct situation of (B) by adding a  $+z$  shim, the global optimum of  $B_0$  magnetic field homogeneity would have been obtained, instead of a local optimum as shown in (C). Note that the  $B_0$  magnetic field homogeneity outside the region of interest is considerably worse.

the carrier frequency, or alternatively by an appropriate current in the room temperature  $B_0$  coil.

Even though most *in vivo* MR systems only have a limited number of shim coils (first, second and sometimes third order), it is obvious that manual shimming on an inhomogeneous sample out of the magnet isocenter in the presence of shim coil interactions is a complex task that requires considerable skill and training. As a result, almost all *in vivo* shimming is currently achieved with automated algorithms that quantitatively measure magnetic field inhomogeneity and analytically determine the required shim currents.

### 10.3.5 Magnetic Field Map Based Shimming

The basic NMR sequences for magnetic field mapping have been discussed in Chapter 4. Here attention will be focused on some of the practical aspects of magnetic field map based shimming, some of which are equally valid for projection-based shimming (see Section 10.3.6). In particular, the effects of shim degeneracy and shim interactions will be discussed, as well as the effects of spatial displacements and shim amplitude limitations.

The spherical harmonic shim fields as described by Equation (10.10) are orthogonal and thus independent when considered across three spatial dimensions. However, when considered in two dimensions, spherical harmonic functions may become identical. This situation can occur when the magnetic field homogeneity is analyzed and optimized across a slice or thin slab (see also Section 10.3.7). The spherical harmonic shims with identical spatial dependencies across the slice are therefore no longer independent, i.e. the shims are degenerate. When shim degeneracy is not taken into account, it can lead to incorrectly set shim corrections.

In-plane shim degeneracies for infinitesimally thin, nonoblique slices are readily determined by visual inspection. For example, for axial slices (i.e. slice selection in  $z$  direction) the only nondegenerate shims are  $X$ ,  $Y$ ,  $Z^2$ ,  $X^2 - Y^2$ ,  $XY$ ,  $Z^2X$ ,  $Z^2Y$ ,  $X^3$  and  $Y^3$  for the first-through third-order spherical harmonic functions. The other shims can be written as linear combinations of the shims in the full nondegenerate set. For example, the  $ZX$  shim becomes a  $X$  shim when  $z$  is constant, whereas a  $Z^3$  shim degenerates to a  $Z^2$  shim (and a frequency offset). Degeneracy analysis is readily extended to oblique slices [14, 15], albeit the calculations are more tedious and error prone. Alternatively, the problem of shim degeneracy can be solved through regularization algorithms [16].

The next step for *in vivo* shimming using magnetic field maps is to determine the coefficients  $C_{n,m}$  of Equation (10.11). Following the definition of a region of interest (e.g. the entire human brain) the optimal spherical harmonic shim combinations are determined through least-squares minimization by finding the coefficients  $C_{n,m}$  that minimizes:

$$\left[ B_{0,\text{offset}} + \sum_{n=1}^{\infty} \sum_{m=0}^n C_{n,m} F_{n,m}(x, y, z) - \Delta B_{0,\text{measured}}(x, y, z) \right]^2 \quad (10.14)$$

Figure 10.11 shows the principle of shimming using a least-squares minimization as described by Equation (10.14), as well as the effect on a water  $^1\text{H}$  NMR spectrum. It follows that as higher-order shims are included, the magnetic field homogeneity improves, leading to a narrower water resonance that approximates the theoretical Lorentzian line shape. Figure 10.12 shows a typical example of least-squares minimization on human brain *in vivo*. Despite the convergence of all first-, second- and third-order spherical harmonic shims, significant magnetic field inhomogeneity remains. This is because the magnetic field distribution inside the human brain contains, besides first- and second-order inhomogeneity, many local, higher-order magnetic field gradients that can not be compensated with low-order spherical harmonic shims. The inability of low-order spherical harmonic shims to homogenize the entire human brain continues to fuel the development of alternative shimming strategies, as will be described in Sections 10.3.7 and 10.3.8. When the required shims exceed the limits of the shim power supply, the minimization algorithm can be modified to impose current constraints [17].

When the spatial origin used for the shim determination does not coincide with the shim coil isocenter, it becomes important to take spatial displacement effects into account, as was already detailed in the simple example shown in Figure 10.10. Consider for example the second-order ( $X^2 - Y^2$ ) shim in Cartesian coordinates. At a position  $(x', y', z')$  relative to the shim isocenter  $(x_0, y_0, z_0)$  the expression can be rewritten as:

$$\begin{aligned} (x' + x_0)^2 - (y' + y_0)^2 &= x'^2 + 2x'x_0 + x_0^2 - y'^2 - 2y'y_0 - y_0^2 \\ &= (x_0^2 - y_0^2) + 2x'x_0 - 2y'y_0 + (x'^2 - y'^2) \end{aligned} \quad (10.15)$$

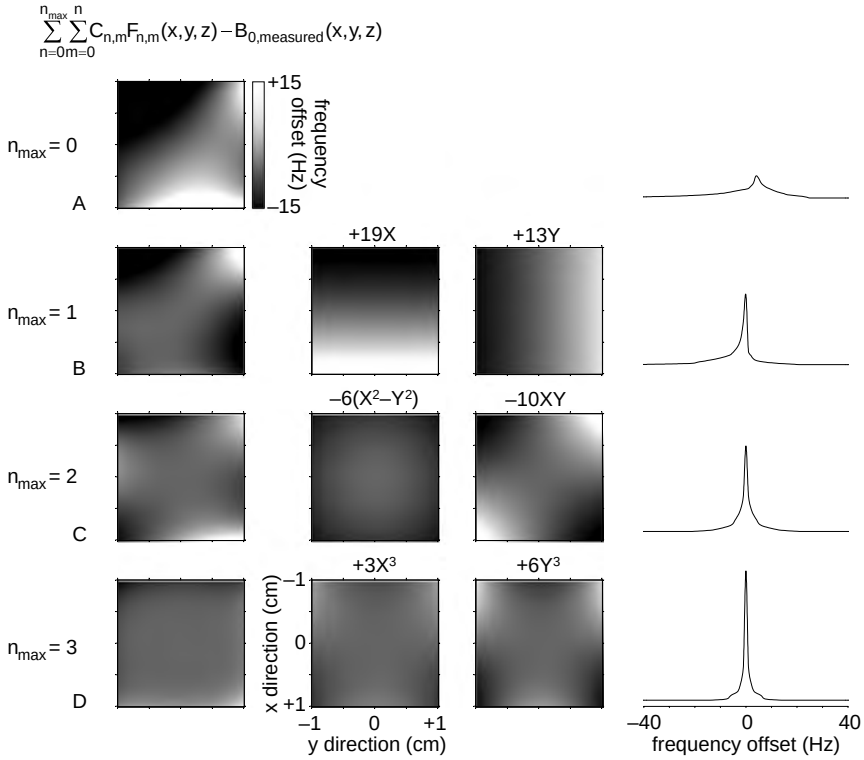


**Figure 10.11** Effect of shimming with increasing order of compensation on a simulated phantom. (A) 2D distribution in the axial x-y plane of the magnetic field inhomogeneity without additional shim fields and the obtained spectral line (right). (B) Removal of the first-order magnetic field inhomogeneity by the application of optimized X and Y shim fields, leads to an improvement in magnetic field homogeneity and a reduction in spectral linewidth. Removing (C) second- and (D) third-order contributions continues to improve the magnetic field homogeneity and spectral line shape.

The field produced by the  $(X^2 - Y^2)$  shim coil at position  $(x', y', z')$  is given by Equation (10.15), multiplied by  $+C_{2,2}$ , and contains besides the desired second order  $(x'^2 - y'^2)$  shim also two first-order shims ( $x'$  and  $y'$ ) as well as a  $B_{0,\text{offset}}(x_0^2 - y_0^2)$ . Therefore the linear X and Y shims must be corrected by  $-2C_{2,2}x_0$  and  $+2C_{2,2}y_0$ , respectively. The corrected first-order shim terms are then given by:

$$C_{1,0,\text{corr}} = C_{1,0} - 2C_{2,0}z_0 - C_{2,1}x_0 - C_{2,1'}y_0 \quad (10.16a)$$

$$C_{1,1,\text{corr}} = C_{1,1} + C_{2,0}x_0 - C_{2,1}z_0 - 2C_{2,2}x_0 - 2C_{2,2'}y_0 \quad (10.16b)$$

$$C_{1,1',\text{corr}} = C_{1,1'} + C_{2,0}y_0 - C_{2,1'}z_0 + 2C_{2,2}y_0 - 2C_{2,2'}x_0 \quad (10.16c)$$

Including higher-order shims ( $n > 2$ ) will require displacement correction terms for all lower-order shims. While this effect can be quantitatively accounted for, it should be kept in mind that with increasing shim order and increasing distance from the shim

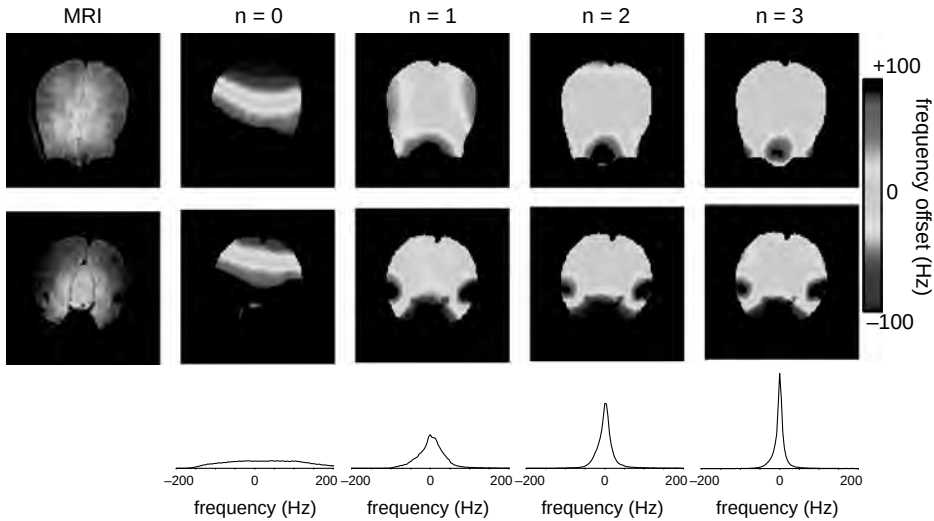


**Figure 10.12** Effect of shimming with increasing order of compensation on human brain in vivo at 4.0 T. The magnetic field inhomogeneity caused by the magnet, and to some degree by the human torso ( $n = 0$ ), can be largely eliminated by first ( $n = 1$ )- and second ( $n = 2$ )-order shimming. The residual magnetic field inhomogeneity following second-order shimming is largely caused by air–tissue magnetic susceptibility boundaries in the human head. The strength of third ( $n = 3$ )- and higher-order shims available on current MR systems is insufficient to completely homogenize the magnetic field inside the human head. (See color plate 7)

isocenter, the correction terms can become the dominant factor determining the magnetic field homogeneity.

In order to apply the correct current changes in the shim amplifiers, the magnetic field generated by each shim coil needs to be calibrated in terms of  $\Delta C_{n,m}/\Delta I_{n,m}$ , where  $I_{n,m}$  is the shim current for the shim coil of order  $n$  and degree  $m$ . This can be performed by successively changing the shim current  $I_{n,m}$  in one shim coil and measuring  $C_{n,m}$ . The slope of the linear relation between  $C_{n,m}$  and  $I_{n,m}$  gives the desired calibration constant. Under ideal circumstances (i.e. when all shim coils are perfectly orthogonal), a current  $I_{n,m}$  in shim coil  $n,m$  only produces the spherical harmonic  $Y_{n,m}(\theta, \phi)$  with corresponding amplitude  $C_{n,m}$ . However, slight imperfections in shim coil design may cause the production of first-order spherical harmonics when a second-order shim coil is used. These imperfections can be measured during the calibration of the shim currents and be accounted for in further calculations. Figure 10.13 shows an example of the calibration of a second-order shim coil.

### 10.3.6 Projection Based Shimming

Linear projection methods like FASTMAP (fast automatic shimming technique by mapping along projections) [18] and its derivatives [19–21] are based on the argument that measurements of the magnetic field along a limited number of linear projections is sufficient to accurately characterize the magnetic field in terms of low-order spherical harmonics.

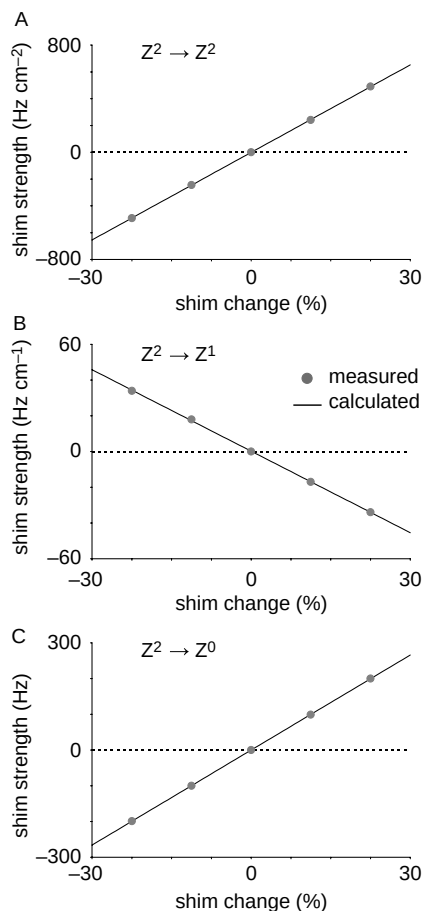


**Figure 10.13** Calibration of the  $Z^2$  shim strength. Changing the current in the  $Z^2$  shim coil and measuring the change in corresponding magnetic field distribution in terms of spherical harmonic functions allows the calibration of the (A)  $Z^2$  shim strength and (B) the linear  $Z^1$  and (C) constant  $Z^0$  magnetic fields that are generated by the  $Z^2$  shim.

From Equation (10.8) it can be seen that the spherical harmonic function  $Y_{n,m}(\theta, \phi)$  is a function of  $\theta$  and  $\phi$  only. Therefore, along a straight line  $i$  that runs through the magnet isocenter,  $Y_{n,m}^i(\theta, \phi)$  is constant and the field distribution  $\Delta B_0$  is given by a polynomial of distance  $r$ , according to:

$$\Delta B_0^i(r) = B_{0,\text{offset}} + \sum_{n=1}^{\infty} r^n \sum_{m=0}^n C_{n,m} Y_{n,m}^i \quad (10.17)$$

The measured magnetic field distribution  $\Delta B_0^i(r)$  along projection  $i$  can be fitted using polynomial regression to yield the polynomial coefficients  $a_n^i$  according to:

$$\Delta B_0^i(r) = a_0^i + \sum_{n=1}^{\infty} a_n^i r^n \quad (10.18)$$

When the orientation of the projections is chosen such that  $Y_{n,m}^i$  has orthogonal columns, the coefficients  $C_{n,m}$  can be calculated according to:

$$C_{n,m} = \frac{\sum_i a_n^i Y_{n,m}^i(\theta, \phi)}{\sum_i Y_{n,m}^i(\theta, \phi)^2} \quad (10.19)$$

Three projections along the x ( $\theta = 90^\circ$ ,  $\phi = 0^\circ$ ), y ( $\theta = 90^\circ$ ,  $\phi = 90^\circ$ ) and z ( $\theta = 0^\circ$ ) axes are required to determine all three first-order shims. To determine all eight first- and second-order shims requires six orthogonal projections, xy ( $\theta = 90^\circ$ ,  $\phi = 45^\circ$ ), yx ( $\theta = 90^\circ$ ,  $\phi = 135^\circ$ ), xz ( $\theta = 45^\circ$ ,  $\phi = 0^\circ$ ), zx ( $\theta = 135^\circ$ ,  $\phi = 0^\circ$ ), yz ( $\theta = 45^\circ$ ,  $\phi = 90^\circ$ ) and zy ( $\theta = 135^\circ$ ,  $\phi = 90^\circ$ ). Table 10.3 summarizes the relative contributions  $Y_{n,m}(\theta, \phi)$  of individual shims for projections along different columns ( $\theta, \phi$ ). For example, the projection along a xy column contains contributions from X, Y,  $Z^2$  and XY spherical harmonics. While the X and Y shims can be separated from the  $Z^2$  and XY shims based on their spatial dependencies along the projection (linear and quadratic, respectively), shims of the same order can not be separated. However, by using six projections along columns in different directions all eight first- and second-order shims can be determined. For example, the  $Z^2$  shim contribution is given by:

$$C_{2,0} = (-2a_2^{xy} - 2a_2^{yx} + a_2^{xz} + a_2^{zx} + a_2^{yz} + a_2^{zy})/6 \quad (10.20)$$

When the volume of interest (VOI) is positioned out of the magnet/shim isocenter, the low-order coefficients need to be compensated according to Equation (10.16) for terms introduced by the higher-order shims.

FASTMAP is a time-efficient, user-friendly shimming routine for single volumes that are adequately approximated by a spherical geometry. The technique is readily extended to shim elliptical areas in 2D slices [14,20]. When the VOI is more complex, the multidimensional magnetic field mapping routines described in Section 10.3.5 offer more flexibility.

### 10.3.7 Dynamic Shim Updating (DSU)

The magnetic field homogeneity encountered on *in vivo* samples, like the human head, is characterized by localized, higher-order ( $n > 3$ ) spherical harmonics. Examples can be found in the frontal and auditory cortices, where the magnetic field distribution is dictated by strong magnetic induction fields from nearby air–tissue boundaries. Space and current restrictions typically limit shim coils on *in vivo* MR systems to the correction of low-order spherical harmonics ( $n \leq 3$ ). As a result, the higher-order *in vivo* magnetic field distribution is not, or only partially compensated (see Figure 10.12) leading to line-broadening in MRS and MRSI and artifacts and signal loss in gradient-echo-based MRI.

Magnetic field inhomogeneity becomes in general more manageable across smaller volumes, as the higher-order terms can be approximated by lower-order spherical harmonics. This forms the basis of dynamic shim updating (DSU) for multislice or multivoxel experiments [15,22–24]. In all the previous discussions, shimming was presented as a static technique in which a single shim setting is optimized over the entire sample. However, for many *in vivo* experiments signal is not acquired from the entire sample, but from multiple slices or voxels. The optimal shim setting in a slice generally differs from the global setting and can also differ from slice-to-slice such that a single global shim can not be optimal for all slices. For dynamic shimming applications the optimal slice shim settings are established prior to the experiment. During the multislice experiment, the optimal slice



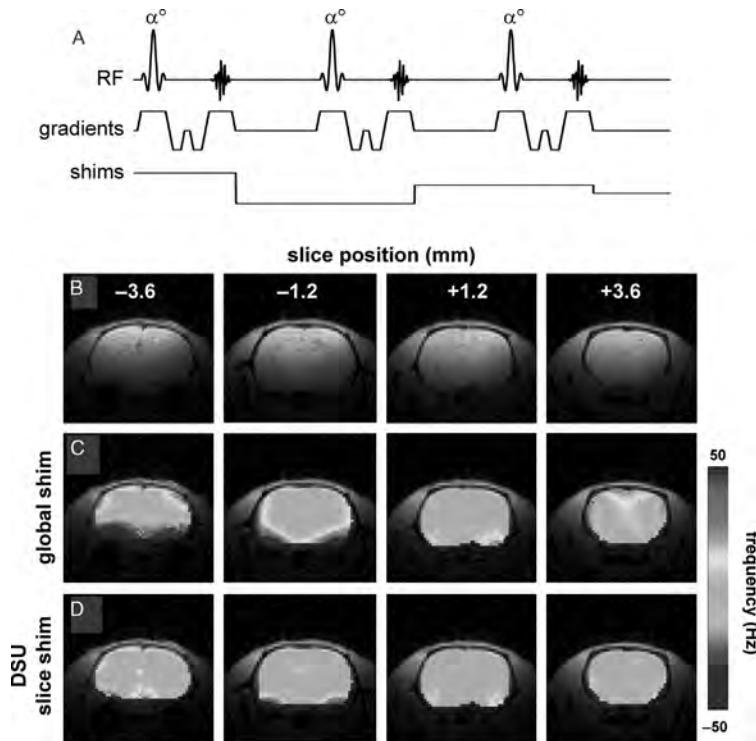


**Figure 10.14** (A) Principle of DSU. Instead of a constant, global shim setting, DSU allows dynamic changes in the shim coil currents to achieve an optimal shim setting for each slice in a multislice MRI protocol. (B–D) Typical DSU results on rat brain at 4.0 T. (B) Anatomical spin-echo MR images and (C) corresponding magnetic field maps using a global shim setting. (D) Magnetic field maps with an optimal shim setting for each slice. Clear improvements can be observed in all slices. (See color plate 8)

shims are temporally updated, thereby providing optimal magnetic field homogeneity for all slices.

Figure 10.14A shows a typical multislice MRI sequence implementation utilizing DSU. Optimal slice-specific shim settings are established prior to the experiment. During the multislice acquisition, the shims are updated in real-time to provide improved homogeneity for the specific slice being excited and acquired. Figure 10.14B–D shows typical results for DSU on rat brain at 4.0 T. With a single, global shim setting the magnetic field maps are characterized by large areas of low magnetic field homogeneity, especially at the outer edges of the brain (Figure 10.14C). With DSU the inhomogeneous areas are greatly reduced (Figure 10.14D), because optimization of the shims on a slice-specific basis can approximate the magnetic field inhomogeneity better with low-order spherical harmonic fields.

The challenges of dynamic shimming includes the development of rapid  $B_0$  mapping techniques, rapid calculation of optimal, nondegenerate shim settings and the minimization of eddy currents originating from the abrupt shim changes [15,24]. It should be realized that the performance of dynamic shimming is ultimately limited by the restrictions posed



by the available spherical harmonic shims. Even though the performance of an optimal slice shim is improved relative to an optimal global shim (Figure 10.14), the magnetic field inhomogeneity within a slice still contains contributions of high-order spherical harmonics that simply can not be compensated with conventional, low-order spherical harmonic shims.

### 10.3.8 Passive Shimming

Passive shimming historically refers to the process of optimizing the magnetic field homogeneity of the raw magnet by strategic placement of small pieces of ferromagnetic material, like iron, steel or nickel [4]. Passive in this context therefore refers to the fact that the optimization is performed once, during the commission of the magnet, and does not require (or allow) any subsequent, active adjustments. For raw magnets passive shimming is perfectly acceptable, as the magnet configuration and surroundings and hence the raw magnet homogeneity do not change during the lifespan of the system. However, *in vivo* shimming requires active adjustment of room temperature shims as described in the previous sections, since the magnetic field inhomogeneity is subject-dependent.

Recently, the principle of passive shimming has been extended to subject-specific *in vivo* shimming through the use of ferromagnetic [25], strong diamagnetic [26] or diamagnetic and paramagnetic materials [27]. Juchem *et al.* [25] approximated the second-order spherical harmonic shim fields with strategical placement of a limited number of strips of a ferromagnetic Ni-Fe alloy. With this approach they were able to achieve reproducible and subject-specific improvements in the magnetic field homogeneity in the macaque visual cortex. Wilson *et al.* [26] utilized the diamagnetic properties of highly oriented pyrolytic graphite (HOPG,  $\chi = -450$  ppm) to improve the magnetic field homogeneity in the frontal cortex of the human brain. As demonstrated in Figure 10.5 the magnetic field inhomogeneity in the frontal cortex is largely caused by the paramagnetic air in the nasal cavity. By placing HOPG in the mouth, directly underneath the nasal cavity and frontal cortex, the HOPG-induced magnetic field distortion largely cancels the magnetic field distortions generated by air in the nasal cavity. While HOPG-based mouth shims do improve the magnetic field homogeneity significantly, the single-material approach allows little flexibility for optimization. Furthermore, intra-oral shims may lead to subject compliance concerns. As an alternative to passive local shims, Hsu and Glover [28] explored the possibility of shimming with an active local shim coil placed inside the mouth. A more flexible passive shimming approach was suggested by Koch *et al.* [27] which used optimized combinations of diamagnetic bismuth (Bi,  $\chi = -164$  ppm) and paramagnetic zirconium (Zr,  $\chi = +109$  ppm) to greatly increase the magnetic field homogeneity across large volumes. Figure 10.15 shows a typical result for passive shimming on mouse brain at 9.4 T. From a measured 3D magnetic field map, the optimal placement and amounts of dia- and paramagnetic material is calculated through a noniterative matrix inversion. With this approach, near-perfect magnetic field homogeneity across the entire brain is possible, especially when the materials can be placed closer to the head (Figure 10.15F).

## 10.4 Magnetic Field Gradients

Magnetic field gradients are linearly varying magnetic fields that are applied in addition to the main magnetic field  $B_0$  to achieve spatial encoding. The principle of magnetic field

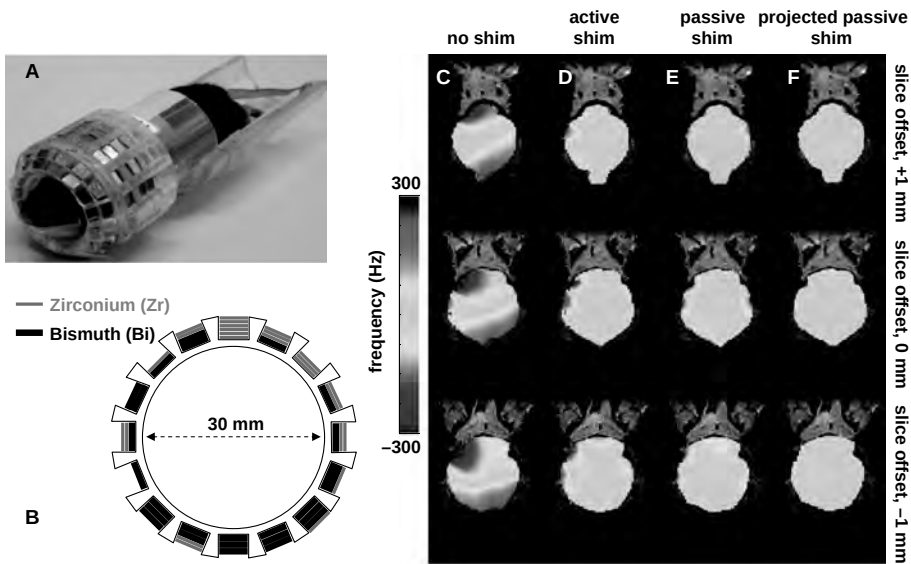


**Figure 10.15** Passive shimming on mouse brain at 9.4 T. (A) Photograph of a mouse surrounded by a passive shim assembly. (B) Cross-sectional drawing of one ring through the eight-ring passive shim assembly in which 16 slots are available for the placement of paramagnetic zirconium and diamagnetic bismuth pieces. (C–F) Magnetic field maps acquired (C) without any (passive or active) shim, (D) with optimal first- and second-order active shims and (E) with an optimal passive shim setting. When the diameter of the passive shim assembly shown in (A, B) is reduced by 5 mm, the (simulated) results in (F) can be obtained. (See color plate 9)

gradients has been discussed in detail in Chapter 4. Slice selection, frequency and phase encoding in MRI are all performed with the aid of magnetic field gradients, just as single voxel localization, spectroscopic imaging and many types of water suppression. Magnetic field gradients are further used to select specific coherence transfer pathways in spectral editing and 2D NMR, thereby often providing a single-scan alternative to phase cycling. For these reasons all clinical and most research MR systems are equipped with a set of three orthogonal X, Y, and Z magnetic field gradients.

The basic gradient designs are still based on the coils that were initially developed as first-order spherical harmonic shim coils for the homogenization of magnetic fields [29]. A linear magnetic field gradient in the z direction, collinear with the main magnetic field is generated with a so-called Maxwell pair (Figure 10.9). Essentially it consists of two parallel coils perpendicular to the main magnetic field direction in which the DC currents flow in opposite directions. As a consequence the direction of the generated magnetic field is different for the two coils leading to complete cancellation of the magnetic field at the center between the coils. This point corresponds to the gradient (and normally also magnet and shim coil) isocenter. On one side of the gradient isocenter the magnetic field of one coil dominates, making the magnetic field direction for instance positive and the amplitude dependent on the distance from the isocenter. On the other side of the gradient isocenter, the reverse is true, i.e. the magnetic field direction is negative. In practice more than two

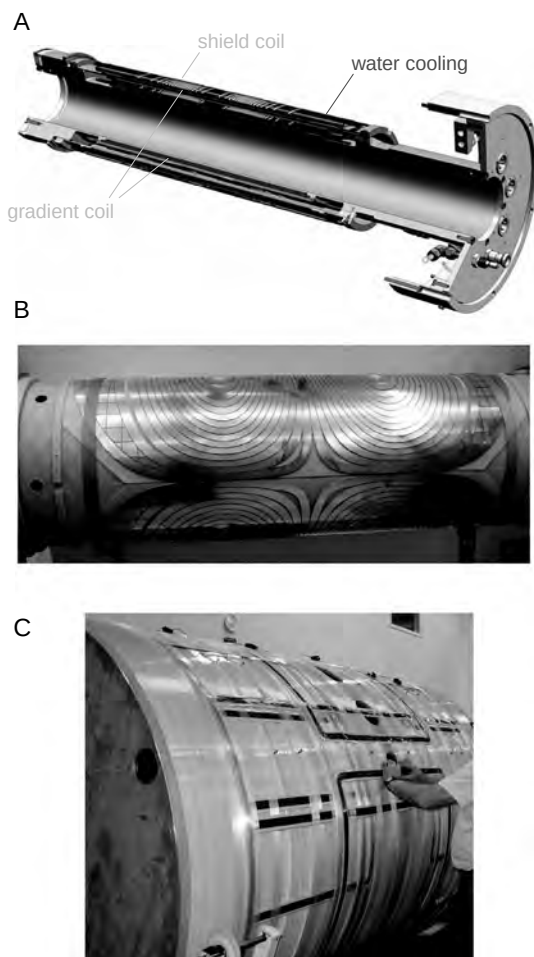


**Figure 10.16** (A) Cross-sectional 3D drawing of an actively shielded, water-cooled magnetic field gradient set. (B) Photograph of a Y magnetic field gradient. (C) Photograph of higher-order shim coils being placed on a human-sized MR magnet. (Courtesy of D. Green and S. Pittard, Magnex Scientific.)

coils are used to improve the linearity of the magnetic field gradient. The design for X and Y magnetic field gradients is more complicated than for Z gradients. Most commonly used X and Y magnetic field gradients are based on the Golay gradient coil design [29] and are shown in Figure 10.9. The principle is essentially the same as for the Maxwell pair in that opposite currents in the four different saddle-shaped coils produce magnetic fields which partially cancel each other in order to generate a linear magnetic field gradient. The linearity of the magnetic gradient fields is improved by using more loops. In reality, gradient coils are not made from wire, but are designed as a continuous pattern of copper sheets as optimized by computer simulations. Figure 10.16A shows a schematic of the integrated design of magnetic field gradient coils and water cooling, whereas Figure 10.16B shows a realistic example of a Y gradient coil.

Magnetic field gradients are characterized by several parameters. The amplitude, expressed in  $\text{G cm}^{-1}$  or  $\text{mT m}^{-1}$  ( $10,000 \text{ G} = 1 \text{ T}$ ), is probably the most important, since it largely determines the minimum voxel sizes and acquisition time in MRI and voxel sizes and dephasing capabilities in MRS. On most clinical systems, the gradient amplitude is limited to  $4\text{--}5 \text{ G cm}^{-1}$ , while on animal research systems gradients can be as high as  $20\text{--}100 \text{ G cm}^{-1}$ . At a fundamental level the amplitude of a magnetic field or magnetic field gradient is governed by the Biot–Savart law for electromagnetism, which states that for a simple current loop the generated magnetic field is inversely proportional to the square of the radius of the loop and linearly proportional to the current. Therefore, for the same current, gradient coils designed for human applications necessarily generate smaller magnetic fields than gradient coils designed for animals. To counteract the reduced efficiency due to the increased coil radius, human MR systems often are equipped with powerful high-voltage and high-current amplifiers. However, increasing the current density is limited by heat removal considerations. As can be seen in Figure 10.16B, magnetic gradient coils are typically made of copper sheets. Since copper has a finite resistance, the increased current density will lead to increased power deposition in the form of heat dissipation. The excess heat is removed by an extensive water cooling network built in between the coil windings (Figure 10.16A). However, the heat removal capacity of the cooling water is limited. When the heat dissipation exceeds the heat removal, the temperature of the gradient coil will rise, which in the extreme case may damage the gradient coil. While the gradient amplitude is the parameter of interest for the user, it is the gradient coil efficiency that ultimately determines the performance of the gradient set. Gradient coil efficiency  $\eta$  is defined as the gradient strength  $G$  produced by a current  $I$ , i.e.  $\eta = G/I$ . The efficiency  $\eta$  should be as large as possible, but is inherently linked to two fundamental parameters in gradient coil design, namely the inductance  $L$  and the resistance  $R$ .

Another crucial characteristic of magnetic field gradients is their linearity (i.e. homogeneity). If a magnetic field gradient varies nonlinearly across the field of view (FOV), the spatial information is not encoded linearly by the imaging gradients and consequently the image will appear distorted. With gradient systems presently available, nonlinearities have a negligible effect on image resolution. Distortions may be visible when the FOV approximates the dimensions of the gradient coil as in the case of whole body scanning. In these extreme cases, distortions may be corrected by experimental or theoretical methods. Note that it is especially important that the magnetic field gradient amplitude does not tend towards zero within the sensitive volume of the RF coil, as this can lead to image artifacts that can not be corrected by post-processing. Especially head gradient coils are sensitive to this phenomenon, during which signal from the chest region can fold back on top of signal from the head.

The final characteristic of magnetic field gradient coils is their rise time, i.e. the time it takes to switch a gradient from zero to full amplitude. Ideally, the gradient switching time is as short as possible, since this minimizes echo-times, improves the performance of fast MRI methods, like EPI, and allows for more efficient diffusion-weighting as required in DTI. However, for human applications in particular, there are physiological limitations on the rate at which the magnetic field can change (i.e.  $\text{dB/dt}$ ). A time-varying magnetic field generates an electric field in human and animal subjects placed within the magnet. At sufficiently high amplitudes the electric field, which is proportional to  $\text{dB/dt}$ , can stimulate peripheral nerves and muscles (i.e. twitching). At higher levels, painful nerve stimulation has been observed. At extremely high levels cardiac stimulation or even ventricular fibrillation are of

concern. However, these extreme cases are not encountered in human MRI systems due to the previously mentioned hardware restrictions. These observations and potential concerns have led to safety standards for magnetic field gradient switching rates. A commonly used safety standard was developed by the International Electrotechnical Commission (IEC) and differentiates between two operating modes. In the normal mode, the upper dB/dt limit is specified as  $20 \text{ T s}^{-1}$  for gradient ramp times,  $t_{\text{ramp}}$ , exceeding  $120 \mu\text{s}$ . For ramp times between  $12 \mu\text{s}$  and  $120 \mu\text{s}$ , the dB/dt limit is given by  $(2400/t_{\text{ramp}}) \text{ T s}^{-1}$ . In the normal mode, patient discomfort is minimal and no peripheral nerve stimulation is expected. In the first controlled mode, the dB/dt limit is raised to  $(60\,000/t_{\text{ramp}}) \text{ T s}^{-1}$ . In this mode, the operator should maintain contact with the subject at all times, as patient discomfort and peripheral nerve stimulation may be observed. The dB/dt limit in the first operating mode is primarily designed to prevent cardiac stimulation.

Besides restrictions dictated by physiological considerations, the minimum gradient ramp time is also limited by the hardware, i.e. gradient coil and amplifier. The ramp time,  $t_{\text{ramp}}$ , of a gradient coil is governed by the voltage  $V$  and current  $I$  supplied by the gradient amplifier, as well as the inductance  $L$  and efficiency  $\eta$  of the coil according to:

$$t_{\text{ramp}} = \frac{GL}{\eta V} = \frac{IL}{V} \quad (10.21)$$

Equation (10.21) indicates that short ramp times can be achieved with low inductance gradient coils in combination with high-voltage amplifiers. The inductance of a gradient coil can be lowered by using a small number of turns,  $n$ , in the gradient coil since  $L$  is proportional to  $n^2$ . However, the gradient coil efficiency  $\eta$  is proportional to  $n$ , such that a small number of turns compromises the maximum achievable gradient strength. A useful parameter to characterize the gradient coil performance is the ratio  $\eta^2/L$ , which is independent of  $n$  and indicates the efficiency that can be achieved from a given inductance. The power,  $P_{\text{amplifier}}$ , that a gradient amplifier must generate in order to produce a gradient strength  $G$  within a ramp time  $t_{\text{ramp}}$  is given by:

$$P_{\text{amplifier}} = \frac{G^2}{t_{\text{ramp}}} \frac{L}{\eta^2} \quad (10.22)$$

This indicates the importance of maximizing the ratio  $\eta^2/L$  in order to reduce the power requirements. It can be shown that the ratio  $\eta^2/L$  depends on the gradient coil radius  $r$  according to  $r^{-5}$ , indicating the importance of minimizing the gradient size to achieve optimal performance. Besides the inductance  $L$ , the resistance  $R$  of the gradient coil is also an important design parameter as it is directly related to the amount of power dissipated in the gradient coil (i.e.  $P = I^2R$ ). The resistance can also be lowered by reducing the number of turns,  $n$ , at the cost of reduced coil efficiency. Resistance also depends on the average conductor cross-section in the coil (i.e. the wire thickness), which should be made as large as possible. The interplay of efficiency  $\eta$ , inductance  $L$ , resistance  $R$  and gradient coil uniformity dominates gradient coil design and necessarily always leads to a compromise.

#### 10.4.1 Eddy Currents

Magnetic field gradients are most commonly used as short pulses during which the current in the gradient coil is quickly maximized, maintained for a short delay and quickly reduced

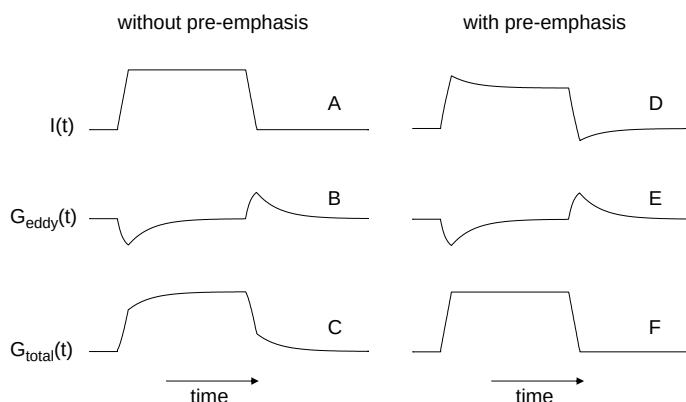


**Figure 10.17** Principle of pre-emphasis. (A) A fast switching current, serving as input to a gradient coil, will generate eddy currents in surrounding conducting structures that oppose the current input, which in turn generates (B) time-varying  $B_0$  and gradient magnetic fields. (C) The total magnetic field gradient is thus a superposition of the desired gradient and the undesired, eddy-current-related gradient. (D) During pre-emphasis the current waveform is distorted, such that the sum of (E) the eddy-current-related gradient and the gradient generated by (D) leads to the desired gradient waveform (F).

to zero amplitude (Figure 10.17A). Ideally the generated magnetic field gradient would follow the current instantaneously, resulting in a desired rectangular magnetic field gradient pulse. However, rapid switching of the gradients induces so-called eddy currents in all nearby conducting structures, such as the cryostat, heat shields, magnet and shim coils. These eddy currents are a manifestation of Faraday's law of induction which states that a time-varying magnetic field will induce a current (and consequently a second time-varying magnetic field) in a nearby conductor that opposes the effect of the applied time-varying magnetic field (Figure 10.17B). High gradient amplitudes and/or fast gradient switching will produce the largest eddy currents. As a result of the additional, time-varying magnetic fields, the desired magnetic field gradient is heavily distorted with a decreased amplitude at the beginning and an additional decaying field following the actual pulse (Figure 10.17C). Unless measures are taken to eliminate or compensate for these effects, they will severely degrade image quality and may preclude MRS experiments altogether (Figure 10.18), due to the much higher magnetic field homogeneity requirements of MRS.

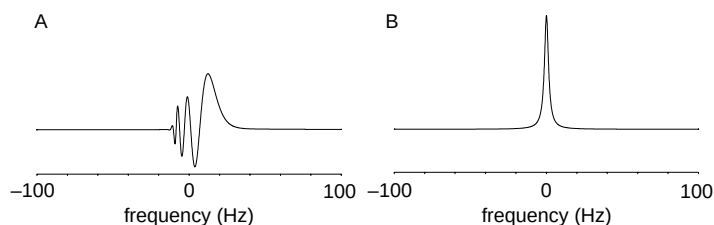


**Figure 10.18** The effect of eddy currents on NMR spectra.  $^1\text{H}$  NMR spectrum of water (A) without and (B) with eddy-current compensation by pre-emphasis and  $B_0$  magnetic field compensation.

The induced time-varying magnetic fields are composed of two main components, a magnetic field gradient  $G(t)$  opposite to the applied gradient and a shift in the main magnetic field  $\Delta B_0(t)$ . The main magnetic field shift  $\Delta B_0(t)$  is identical for all positions, while the induced gradient varies linearly with position. Besides  $G(t)$  and  $\Delta B_0(t)$  the eddy currents may also hold so-called cross-talk terms, e.g. induction of gradients in directions perpendicular to that of the applied gradient.

Several methods to (partially) compensate eddy currents are available of which pre-emphases and active screening (or shielding) are the most robust and universally applicable. In order to understand the principles of these techniques it is informative to describe the time behavior and origin of the induced eddy currents. The formation and decay of eddy currents can be modeled by a series of inductive-resistive (LR) circuits which are inductively coupled to the gradient coil [30]. These LR circuits can include conducting structures within the magnet (like the radiation shield), the gradient/shim coils and the RF coil.  $L$  represents an inductance coil and  $R$  the resistance. More details on LR (and LCR) circuits can be found in Section 10.5. The total magnetic field  $G(t)$  generated in the magnet is a superposition of the gradients generated by the applied and various induced currents:

$$G(t) = aI(t) + \sum_{k=1}^n a_k I_k(t) \quad (10.23)$$

where  $aI(t)$  is the desired gradient field generated by applied current  $I(t)$  and  $a_k I_k(t)$  are the unwanted gradient fields generated by eddy currents  $I_k(t)$ .  $n$  is the number of eddy current loops. The current  $I_k(t)$  in loop  $k$  depends on the applied current  $I(t)$  according to Faraday's law:

$$L_k \frac{dI_k(t)}{dt} + R_k I_k(t) = -M_k \frac{dI(t)}{dt} \quad (10.24)$$

where  $L_k$  and  $R_k$  are the inductance and resistance of loop  $k$  and  $M_k$  is the mutual inductance between LR circuit  $k$  and the gradient coil. Performing a Laplace transformation of Equation (10.24) and summing over all eddy current loops, the time dependence of the induced time-varying magnetic field gradients may be described in terms of  $R_k$ ,  $L_k$  and  $M_k$  according to:

$$G(t) = a \left( 1 - \sum_{k=1}^n c_k e^{-w_k t} \right) \quad (10.25)$$

where

$$c_k = \frac{a_k}{a} \frac{M_k}{L_k} \quad \text{and} \quad w_k = \frac{R_k}{L_k} \quad (10.26)$$

The time-varying eddy currents can therefore be described by a sum of exponentially decaying gradients with amplitudes  $c_k$  and time constants  $1/w_k$ .

### 10.4.2 Pre-Emphasis

The time-varying eddy currents can be significantly reduced by a process referred to a pre-emphasis [30–32], which involves cancellation of the exponentially decaying gradient fields by overdriving the applied current (Figure 10.17D). Most commercial MR systems are equipped with several pre-emphasis circuits (usually up to three per gradient direction)

in which a high-pass RC filter generates an exponentially decaying signal with adjustable amplitude and time constant. Summation of this additional signal to the input current step function gives a current input which will produce the desired rectangular magnetic field gradient. Due to the multi-exponential character of eddy currents [see Equation (10.25)] several pre-emphasis circuits with different amplitudes and time constants are used to obtain an adequate cancellation of eddy currents.

The actual time-varying magnetic field gradients need to be quantitatively measured if a significant reduction of eddy currents is to be achieved. It is important to realize that eddy currents are actually the sum of time-varying magnetic field gradients  $G(r, t)$  and magnetic field shifts  $\Delta B_0(t)$  that need to be compensated separately, leading to twelve or more parameters (i.e. six amplitudes and six time constants) per channel to be optimized. In order to distinguish between  $G(r, t)$  and  $\Delta B_0(t)$ , the total magnetic field  $B(r, t)$  must be measured in, at least, two spatial positions. Several methods have been reported for the measurement of eddy currents, either using NMR signals from separate samples, using NMR signals along a spatially encoded column [33] or with small pickup coils [31].

The MR sequence of a convenient hybrid method to measure eddy currents is shown in Figure 10.19. A water-filled tube is placed in the magnet (and gradient) isocenter, parallel to the direction along which the eddy currents need to be measured. The tube should be narrow in the other two dimensions in order to minimize signal loss due to cross-term eddy currents (e.g. eddy currents generated in the  $y$  direction by a gradient pulsed in the  $x$  direction). A length-to-diameter ratio of  $\geq 10$  is recommended. Reference and test spatial profiles are obtained in the absence and presence of the test gradient, respectively, after a delay  $\tau$  following the end of the test gradient (Figure 10.20A). During the echo time  $TE$  phase accumulation occurs due to eddy currents generated by the imaging and test gradients, magnetic field inhomogeneity and frequency offsets. However, since the accumulated phase due to the imaging gradients, magnetic field inhomogeneity and frequency offsets are identical between the reference and test spatial profiles, the phase

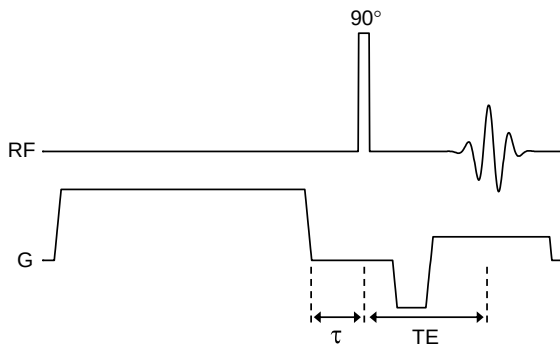


**Figure 10.19** NMR sequence for the quantitative measurement of eddy-current-related  $B_0$  and gradient magnetic field perturbations. The evolution delay  $\tau$  is incremented after which the magnetic field distortions from the magnetic field test gradient are sampled as the phase of a gradient-echo signal. The NMR sequence is used in combination with a tube phantom in which the tube length is parallel with the magnetic field gradient direction for which the eddy-current-related magnetic field perturbations are determined. See text for more details.



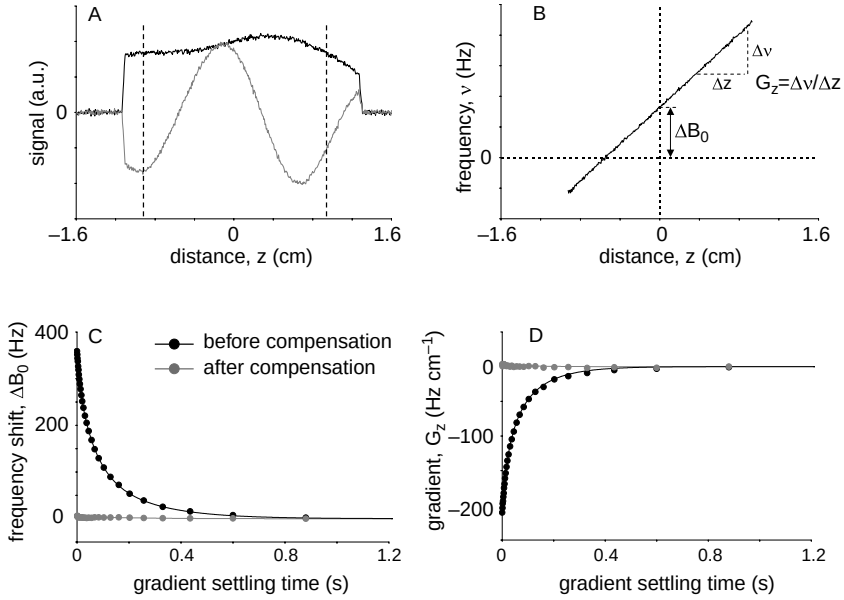


**Figure 10.20** (A) Spatial profiles acquired with the NMR sequence shown in Figure 10.19 in the absence (black line) and presence (gray line) of a magnetic field test gradient at a specific delay  $\tau$  following the end of the test gradient. (B) Frequency distribution along the tube phantom as calculated from the phase difference between the two profiles in (A). The intercept and slope are proportional to the  $B_0$  and gradient perturbations at the given delay  $\tau$ . (C) Frequency shift  $\Delta B_0$  and (D) gradient perturbations as a function of the settling delay  $\tau$  without (black line) and with (gray line) eddy-current compensation by pre-emphasis and  $B_0$  magnetic field compensation.

difference will hold information about the eddy currents generated by the test gradient according to:

$$\Delta\phi(r, \tau) = \gamma \int_{\tau}^{TE+\tau} B(r, t) dt = \gamma \int_{\tau}^{TE+\tau} [G(r, t) + \Delta B_0(t)] dt \quad (10.27)$$

which simplifies to:

$$\Delta\phi(r, \tau) = \gamma [G(r, \tau) + \Delta B_0(\tau)] TE \quad (10.28)$$

when  $B(r, t)$  can be assumed constant over the echo-time  $TE$  (which can be approximated by making  $TE$  very short). Note that the test gradient should ideally be longer than the longest eddy current, such that eddy currents from the up-going ramp can be ignored and only the later down-going ramp has to be considered. Following a simple 1D spatial phase unwrapping procedure a linear fit of the spatially dependent frequency shift  $\Delta\nu(r, \tau) = (\Delta\phi(r, \tau)/TE)$  as a function of position  $r$  gives the eddy current gradient term  $G(r, \tau)$  as the slope and the eddy current frequency shift  $\Delta B_0(\tau)$  as the intercept (Figure 10.20B). The time dependence of  $G(r, \tau)$  and  $\Delta B_0(\tau)$  can be obtained by repeating the measurement for

different gradient settling delays  $\tau$ . Typically around 30 nonlinearly spaced settling delays are adequate to sufficiently characterize the eddy currents.

Since pre-emphasis units typically approximate the measured eddy currents with a sum of decaying exponentials, the next step would be to fit the  $G(r, t) = rG(t)$  and  $\Delta B_0(t)$  curves according to:

$$B_0(t) = \sum_{k=1}^n B_{0,k} e^{-t/TC_{B0,k}} \quad \text{and} \quad G(t) = \sum_{k=1}^n G_k e^{-t/TC_{G,k}} \quad (10.29)$$

where  $B_{0,k}$  and  $G_k$  are the  $B_0$  and gradient pre-emphasis amplitudes, respectively and  $TC_{B0,k}$  and  $TC_{G,k}$  the corresponding time constants (Figure 10.20C and D).

The final step is the adjustment of the pre-emphasis unit to give the desired amplitudes and time constants. However, the real time constants being generated by the unit may be different from the entered time constants, depending on the tolerances and specifications of the individual R and C components used to generate the exponentials. Furthermore, the amplitudes are typically completely unknown as they are entered as a percentage of a maximum (often arbitrarily set) value. While the amplitude and time constant adjustments can be performed iteratively, a more reliable and consistent method is to calibrate the pre-emphasis unit before compensation. Suppose that Equation (10.29) yielded an eddy current gradient of amplitude  $20 \text{ Hz cm}^{-1}$  with a 100 ms time constant. The unit can be quickly calibrated by performing two measurements with pre-emphasis settings  $(G_1, TC_{G1}) = (0\%, 100 \text{ ms})$  and  $(5\%, 100 \text{ ms})$ . While both measurements are affected by the system eddy currents, the difference will reveal a single exponential as generated by the pre-emphasis unit. Fitting of the difference data with a single exponential will give the calibrated amplitude  $G_{1\text{cal}}$  and time constant  $TC_{G1\text{cal}}$  (e.g.  $-10 \text{ Hz cm}^{-1} \%^{-1}$  and 110 ms). Therefore, a time constant of 90.91 ms and amplitude of  $+2\%$  are required to completely cancel the measured eddy current gradient. When the unit has an inherent offset (e.g.  $TC_{G1} = 0 \text{ ms}$  gives  $TC_{G1\text{cal}} > 0 \text{ ms}$ ) the calibration can be extended with a third measurement  $(G_1, TC_{G1}) = (5\%, 200 \text{ ms})$  to characterize the intercept. The outlined calibration can be repeated for other time constants, for  $B_0$  compensation and for other channels. While labor intensive, the outlined calibration is completely quantitative and can set the pre-emphasis and  $B_0$  compensation noniteratively.

Similar to magnetic field homogeneity, the requirements for eddy current compensation differ widely for MRI and MRS applications. Typically, DTI has the most stringent requirements for MRI applications. Multiple images with strong diffusion weighting ( $b > 0 \text{ s mm}^{-2}$ ) in different directions are compared with a base image ( $b = 0 \text{ s mm}^{-2}$ ). Since DTI data are typically acquired with EPI methods, the effects of eddy currents lead to image distortion and signal loss and subsequently errors in the estimation of the diffusion tensor. For MRS applications, the requirements for eddy current compensation are dictated by the spectral resolution. As a general rule of thumb the effects of eddy currents should be minimized to the point that the spectral lines are not significantly broadened or distorted. For a line broadening of less than or equal to 25% of the line width at half maximum,  $\Delta\nu_{1/2}$ , this quantitatively translates into  $|\Delta B_0| \leq (\Delta\nu_{1/2}/4)$  and  $|G(x)| \leq \Delta\nu_{1/2}/(4\Delta x)$ , where  $\Delta x$  represent the voxel dimension in the direction of the eddy current induced gradient field [33]. For  $^1\text{H}$  NMR experiments at 11.75 T, the optimal spectral resolution corresponds to metabolite line widths of 10–14 Hz in a volume of  $3 \times 3 \times 3 \text{ mm} = 27 \mu\text{l}$

[34]. Therefore, the eddy current induced  $B_0$  and gradient fields need to be compensated to better than circa 3 Hz and 10 Hz  $\text{cm}^{-1}$ , respectively. While residual eddy currents will always lead to line broadening and/or signal loss, residual  $B_0$  variations can be taken out during a post-acquisition correction step, as detailed in Section 9.3.

### 10.4.3 Active Shielding

The problem of eddy currents can also be reduced through a completely different approach. Instead of compensating the effects of eddy currents (for example by pre-emphasis), eddy currents could be minimized by so-called active shielding [35–38]. As described in Section 10.4.1, eddy currents are a manifestation of Faraday's law of induction, i.e. a time-varying magnetic field gradient induces a current and consequently a second opposing magnetic field in surrounding conductors like the magnet cryostat. If one could reduce (or even eliminate) the extraneous magnetic fields outside the active volume of the magnetic field gradient coil system, the problem of eddy currents would be eliminated. This is achieved by active screening. Although a detailed description of active screening will be beyond the scope of this book, the principle of active screening will briefly be discussed and is shown in Figure 10.21 for a single loop of current-carrying wire. Without any screening, the magnetic field lines extend far beyond the dimensions of the loop. However, by strategically positioning a discrete wire array which carries an opposite current, the magnetic field lines outside the active volume will be drastically reduced. For the simple example of a single current loop, Figure 10.21C shows the magnetic fields in the absence and presence of magnetic shielding. It follows that active shielding can reduce the magnetic field outside the coil by more than two orders of magnitude for positions  $>60$  cm (coil radius = 25 cm). Note that the homogeneous volume is typically much smaller than the gradient coil dimensions. While active shielding is crucial for a wide range of experiments, in particular EPI and NMR spectroscopy, the methodology comes at the price of a reduced magnetic field inside the coil. This reduction is typically 30–50 % of the amplitude of the unshielded coil geometry. However, for pulse sequences with high gradient demands like EPI, this reduction is often acceptable when the substantial gain in gradient performance is taken into account. The relatively simple principle is readily extended to three dimensions and more complex wire positions as encountered for Maxwell and Golay type magnetic field gradient coils. Figure 10.21D shows a realistic coil design for an actively shielded Y magnetic field gradient.

In practice, active shielding is always combined with pre-emphasis, since active shielding is never perfect. The finite length of gradient coils compromises the shielding performance, as do fabrication errors.

## 10.5 Radiofrequency Coils

Radiofrequency (RF) coils are another essential component of what makes up a complete NMR system. Whereas the magnet creates the spin population difference and hence the longitudinal magnetization, it is the transverse magnetic field oscillating in the RF range that rotates the longitudinal magnetization into the transverse plane where it can be detected as an induced signal in an adjacent receiver coil.

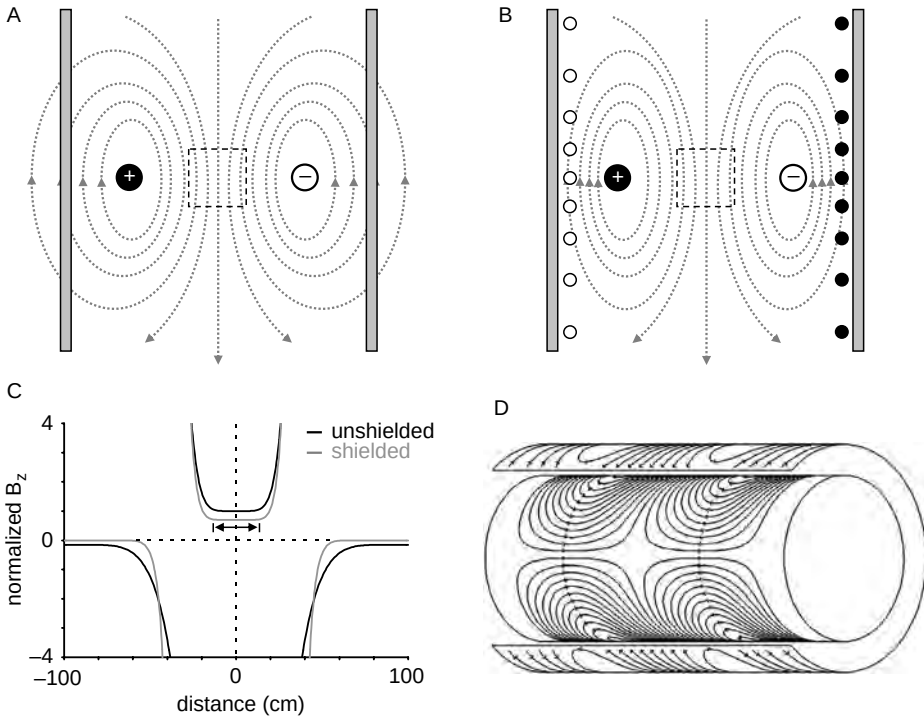


**Figure 10.21** Principle of active shielding. (A) A single loop of current carrying wire produces a magnetic field that can stretch far outside the magnet bore (gray lines), thereby causing eddy currents in conducting structures like the cryostat. (B) By strategically placing a mesh of current-carrying wires on the outside of the single loop, the magnetic field outside the magnet can be greatly reduced. (C) Typical magnetic field profiles for unshielded (black lines) and shielded (gray lines) magnetic field gradients. While the magnetic field gradient amplitude of a shielded design is typically lower inside the magnet than that of an unshielded variant, the greatly reduced magnetic fields and thus eddy currents outside the magnet often outweigh that drawback. (D) Schematic drawing of an actively shielded Y magnetic field gradient. (Courtesy of S. Pittard, Magnex Scientific.)

This section describes the principles of RF coil design in terms of its basic components, namely inductance, capacitance and resistance (L, C and R). Following the description of elementary LCR circuits, the spatial and temporal behavior of RF waves in realistic *in vivo* samples will be considered utilizing the principle of reciprocity.

### 10.5.1 Resonant LCR Circuits

A RF coil can be seen as a LCR circuit, consisting of a pure inductance coil L (i.e. ‘the coil’), a resistance R (from the wires of the coil) and a capacitance C. The circuit is powered by a generator delivering a time alternating voltage [or driving electromotive force (emf)]

$\xi$ ] with angular frequency  $\omega$ , i.e.:

$$\xi = \xi_{\max} \sin \omega t \quad (10.30)$$

The corresponding current is given by:

$$I = I_{\max} \sin(\omega t - \phi) \quad (10.31)$$

The performance of the RF coil can be evaluated when it is known how  $I_{\max}$  and  $\phi$  are related to the components of the circuit, L, R and C. To deduce this relationship, we start with Kirchhoff's rule which states that the applied emf  $\xi$  equals the sum of voltages over the resistor, inductor and capacitor, i.e.:

$$\xi = V_R + V_L + V_C \quad (10.32)$$

The individual voltages in Equation (10.32) can now be described with well-known electromagnetic theory. Consider the series-connected LCR circuit of Figure 10.22A. The voltage over the resistance, which is in phase with the current, is simply given by Ohm's law:

$$V_R = IR = I_{\max} R \sin(\omega t - \phi) \quad (10.33)$$

The voltage over the inductor is a quarter of a wavelength ahead (i.e.  $+90^\circ$ ) relative to the current and is given by:

$$V_L = \omega L I_{\max} \cos(\omega t - \phi) = X_L I_{\max} \cos(\omega t - \phi) \quad (10.34)$$

where L is the inductance (in Henry, H). The inductance of a solenoidal coil is proportional to  $N^2 A \mu l^{-1}$ , where N is the number of turns, A is the cross-sectional area,  $\mu$  is the magnetic permeability of the material inside the coil cavity and l is the length of the coil.  $X_L$  represents the inductive reactance, a measure for the resistance to changes in current flow. The  $90^\circ$  phase difference relative to the resistance is a direct consequence of the fact that energy is stored rather than dissipated in an inductor. The voltage over the capacitor is a quarter of a wavelength behind (i.e.  $-90^\circ$ ) relative to the current and is given by:

$$V_C = -\frac{I_{\max}}{\omega C} \cos(\omega t - \phi) = -X_C I_{\max} \cos(\omega t - \phi) \quad (10.35)$$

where  $X_C$  is the capacitive reactance, a measure for the resistance against changes in voltage. Again, the  $-90^\circ$  phase difference implies that energy is stored and not dissipated. In fact, the only electrical element that dissipates energy (normally as heat) is a resistor. After summation of Equations (10.33)–(10.35) and applying some trigonometric identities, the phase angle  $\phi$  and current  $I_{\max}$  can be calculated:

$$\tan \phi = \frac{(\omega L - \frac{1}{\omega C})}{R} = \frac{(X_L - X_C)}{R} \quad (10.36)$$

and

$$I_{\max} = \frac{\xi_{\max}}{\sqrt{R^2 + (\omega L - \frac{1}{\omega C})^2}} \quad (10.37)$$

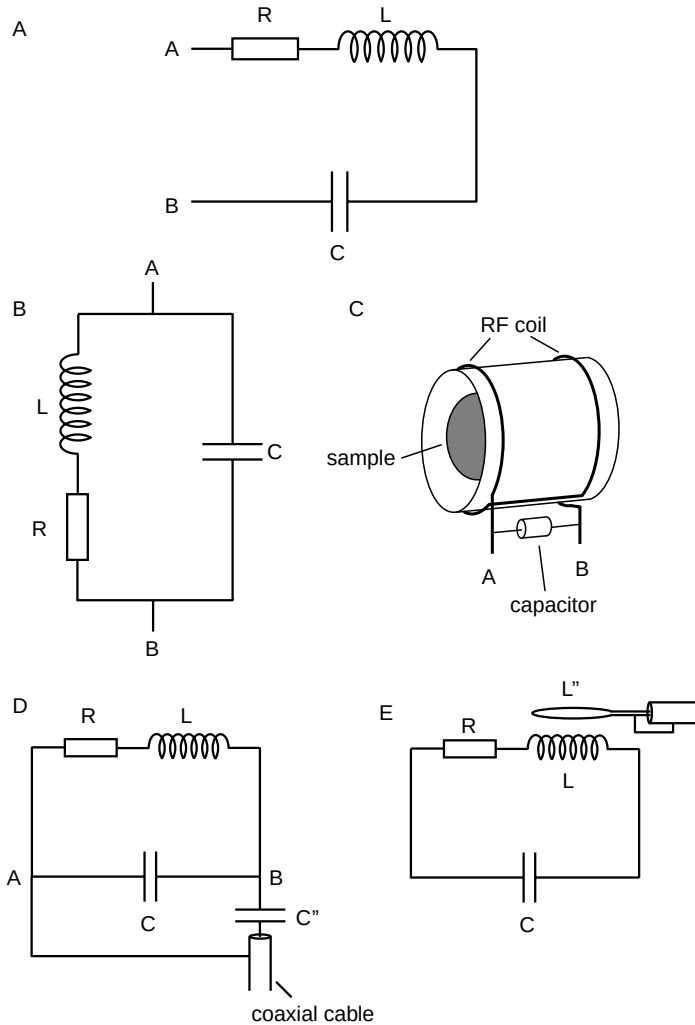


**Figure 10.22** (A) Schematic drawing of a series LCR circuit, where  $R$  is a resistor,  $L$  is an inductor and  $C$  is a capacitor. When the inductive reactance  $X_L$  equals the capacitive reactance  $X_C$  (leaving a small resistance  $R$ ) the system is in resonance at frequency  $\omega_0 = 1/\sqrt{LC}$ . The circuit is powered through the points  $A$  and  $B$ . (B) Parallel LCR circuit as a schematic representation of (C) a real RF coil. Note that the tuning capacitor is parallel to the RF coil. The reactance in a parallel LCR circuit cancels at the same frequency as in a series LCR circuit, but the remaining resistance is increased to  $Q\omega_0 L$ . (D) By using a second matching capacitor, an impedance transformation to  $50\ \Omega$  can be achieved as described in the text. (E) Instead of using capacitance matching to achieve the impedance transformation, one can also use inductance matching.

where the quantity

$$Z = \sqrt{R^2 + \left(\omega L - \frac{1}{\omega C}\right)^2} = \sqrt{R^2 + (X_L - X_C)^2} \quad (10.38)$$

is known as the impedance of the circuit. The impedance of an AC (alternating current) circuit can be regarded as the analog of resistance of a DC (direct current) circuit. Impedance resembles the total opposition of the circuit to current flow. In general the impedance is written in complex notation as  $Z = R + iX$ . Note that resistance  $R$  and reactance  $X$  are both in units of ohms ( $\Omega$ ).

The current within the circuit depends on the frequency  $\omega$  of the applied emf. The current is maximized when the impedance  $Z$  is minimized, which corresponds to:

$$\omega L - \frac{1}{\omega C} = 0, \quad \text{or} \quad \omega = \omega_0 = \frac{1}{\sqrt{LC}} \quad (10.39)$$

where  $\omega_0$  is the natural frequency of the circuit. According to Equation (10.39) the current is maximized when the frequency of the applied emf  $\omega$ , equals the natural frequency  $\omega_0$  of the circuit. In this case a state of resonance is achieved. When a series RF circuit is brought into resonance, the impedance is minimal and equal to  $R$ , the current is maximized and equal to  $\xi_{\max}/R$  and the voltage and current are in phase ( $\phi = 0$ ). For parallel RF circuits, the impedance is maximized and equal to  $1/R$ . A given RF coil can be brought into resonance for a given frequency by adjusting either  $L$  or  $C$ . Because the inductance  $L$  can essentially only be altered by changing the coil geometry, in practice the capacitance  $C$  is varied in a process referred to as ‘tuning’.

However, bringing the LCR circuit in a state of resonance is not sufficient, since the RF coil needs to be connected to the preamplifier and the rest of the receiver system. Generally it is not possible to connect the preamplifier directly to the RF coil. Therefore, standard coaxial cables with  $50 \Omega$  impedance are employed to connect the coil to the preamplifier. In general the impedance of the RF coil (and the sample) differs from this  $50 \Omega$ , resulting in an inefficient power transfer from the amplifier (and coaxial cable) to the RF probe [39, 40]. This would result in a decrease of the attainable current and consequently the  $B_1$  field strength. Furthermore, if the same cable were used to transport the NMR signal from the RF probe to a preamplifier (with a  $50 \Omega$  input impedance) again the power transfer efficiency would be decreased, resulting in a decreased S/N. In order to avoid significant signal losses, the RF coil must therefore be matched to  $50 \Omega$ . The principle of impedance matching can be understood by considering the response of a LCR circuit to an impulse. When a capacitor is charged (by a battery) and then connected to an inductor, an alternating current (AC) is generated which is most intense at the resonant frequency  $\omega_0$ . The capacitor is continuously charged and discharged as the electric energy is transferred between capacitor and inductor. Because some energy will be dissipated as heat in the resistance, the current (and voltage) will decrease as a function of time. A practical measure for the resistance of a coil (i.e. an AC circuit) is the quality factor  $Q$ , which is the ratio of reactance over resistance and equals  $\omega L/R$  and  $(1/\omega CR)$  for pure inductors and capacitors, respectively.  $Q$  is the time-constant describing the exponential decay of voltage and current following an impulse. The Fourier transformation with respect to time will give information on the impedance as a function of frequency (Figure 10.23).

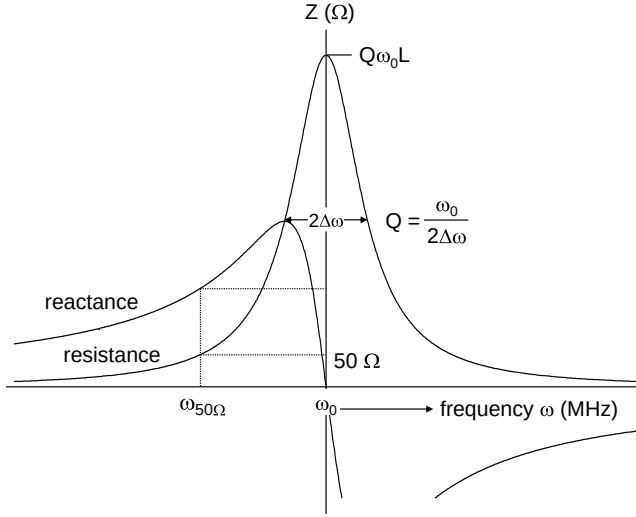


**Figure 10.23** The impedance of a parallel LCR circuit as a function of frequency. The impedance is clearly composed of a resistance and a reactance component. On-resonance, the reactance is zero and the impedance is maximum and equal to  $Q\omega_0 L$ . At a certain frequency  $\omega_{50\Omega}$  off-resonance, the circuit can be considered to be a  $50\ \Omega$  resistance plus a certain amount of reactance. The latter may be canceled by an appropriate capacitor  $C'$  (or inductor  $L''$ ) as shown in Figure 10.22D (and E).

Figure 10.23 reconfirms that impedance is a complex parameter, holding a resistance and a reactance component. On-resonance, i.e. at the Larmor frequency, the reactance is zero and the impedance is a pure resistance. However, this resistance is normally not  $50\ \Omega$ , such that the matching condition is not satisfied. The value of the resistance, together with the solution to match it to  $50\ \Omega$ , can easily be deduced by so-called series–parallel transformations. Consider a resistance  $R$  in series with an inductor  $L$ . The impedance  $Z$  is then given by:

$$Z = R + iX_L \quad (10.40)$$

The expression can be rewritten in a parallel form according to:

$$\frac{1}{Z} = \frac{1}{R + i\omega L} = \frac{R}{R^2 + \omega^2 L^2} + \frac{\omega L}{i(R^2 + \omega^2 L^2)} = \frac{1}{R'} + \frac{1}{i\omega L'} \quad (10.41)$$

Therefore, a resistance  $R$  in series with an inductance  $L$  can also be considered as a resistance  $R'$  in parallel with an inductance  $L'$ , i.e. an impedance transformation has been achieved. For a resistance  $R$  in series with a capacitance  $C$  a similar series–parallel transformation can be achieved:

$$\frac{1}{Z} = \frac{1}{R + \frac{1}{i\omega C}} = \frac{\omega^2 C^2 R}{1 + \omega^2 C^2 R^2} + \frac{i\omega C}{1 + \omega^2 C^2 R^2} = \frac{1}{R'} + i\omega C' \quad (10.42)$$



If one now places a capacitance  $C$  in parallel with an inductance  $L$  ('the coil') and a resistance  $R$  (Figure 10.22B), the total impedance  $Z$  is:

$$\frac{1}{Z} = \frac{1}{R'} + \frac{1}{i\omega L'} + i\omega C' \quad (10.43)$$

Since  $R \ll \omega L$ , one can deduce that  $R' \approx \omega^2 L^2 / R$  and  $L' \approx L$ . Therefore, when  $\omega = \omega_0 = 1/\sqrt{LC}$ , the imaginary part of Equation (10.43) vanishes, leaving a pure resistance  $R' \approx \omega^2 L^2 / R$  (which can be several  $k\Omega$ ). The frequency dependence of the impedance can be deduced from Equation (10.43) and is given by:

$$Z \approx \frac{R'}{1 + i\Delta\omega\tau} = \frac{R'}{1 + \Delta\omega^2\tau^2} - i \frac{R'\Delta\omega\tau}{1 + \Delta\omega^2\tau^2} \quad (10.44)$$

where  $\Delta\omega = \omega_0 - \omega$  and  $\tau = 2Q/\omega_0$ . Equation (10.44) describes the complex Lorentzian curve as shown in Figure 10.23, with a real (resistive) and imaginary (reactance) part. The quality factor of a coil can be experimentally measured for the curves of Figure 10.23 as  $Q = \omega_0/2\Delta\omega$ , where  $2\Delta\omega$  is the bandwidth of the circuit at half of the maximum impedance. Note the analogy of  $Q$  and  $T_2$  describing an exponentially decaying FID.

So far, nothing has been said about matching the probe to the desired  $50\ \Omega$ . From Figure 10.23 and Equation (10.44) it now follows that at a certain frequency  $\omega_{50\Omega}$  off resonance, the impedance equals  $50\ \Omega$  plus reactance. Therefore, if one is able to null the reactance at  $\omega_{50\Omega}$ , one has succeeded in providing the  $50\ \Omega$  impedance. This can be accomplished with a second capacitor  $C''$  (the matching capacitor) (Figure 10.22D). The tuning capacitor  $C$  can finally be readjusted to make  $\omega_{50\Omega}$  the Larmor frequency. The effect of the matching capacitance can again be described with series-parallel transformations. Consider the  $50\ \Omega$  resistance co-axial cable in series with a matching capacitance  $C''$  whose reactance  $1/\omega C'' \gg 50\ \Omega$ . With the aid of a series to parallel transformation it can be shown that this combination equals a resistance  $R'' \approx 1/(50\omega_0^2 C''^2)$  in parallel with a tuning capacitance and the tuned coil. Now if one makes  $R'' = R' = Q\omega_0 L$ , an impedance match has been established between the  $50\ \Omega$  co-axial cable and the coil circuit. The required matching capacitance can be deduced from:

$$R'' \approx \frac{1}{50\omega_0^2 C''^2} = Q\omega_0 L = \frac{Q}{\omega_0 C''}$$

such that

$$C'' \approx \sqrt{\frac{C}{50Q\omega_0}} \quad (10.45)$$

The introduction of the matching capacitor changes the reactance of the tuned circuit. However, this can easily be compensated by the tuning capacitor. Besides capacitive matching as shown in Figure 10.22D, the RF coil circuit can also be inductively matched, in which the distance between the two inductance coils,  $L$  and  $L''$ , and the mutual coupling determine the amount of impedance matching [41–44] (Figure 10.22E).

Since the resonance frequency (i.e. tuning) and complex impedance (i.e. matching) of a RF coil are influenced by the subject-specific coil load, tuning and matching must, in general, be performed for each load individually. Figure 10.24 shows a commonly employed method for coil tuning and matching. The set-up requires a variable frequency generator

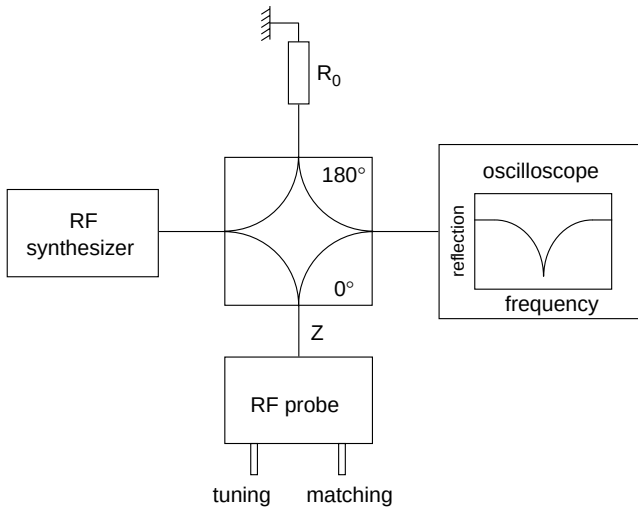


**Figure 10.24** RF coil (probe) tuning and matching using a hybrid junction. The RF synthesizer feeds the RF probe with a small signal. When the probe is properly matched, signals returned by the probe and a reference resistance  $R_0 = 50\ \Omega$  will be reflected with a  $180^\circ$  phase difference, leading to signal cancellation as can be detected on an oscilloscope. When the probe is correctly tuned and matched, the signal cancellation occurs at the desired resonance frequency.

with frequency sweep capabilities, a RF bridge that directs a small RF signal to the probe and an oscilloscope to monitor the output signal. When the impedance of the RF coil equals the impedance of the  $50\ \Omega$  load ( $R_0$ ), no signal is reflected to the oscilloscope and the curve is minimized. Therefore, by changing the matching capacitor, the oscilloscope shows a minimum when the impedances are matched. Tuning is then performed by adjusting the minimum to the Larmor frequency. The main advantage of this particular set-up for tuning and matching is that the coil impedance is known as function of frequency rather than at the Larmor frequency only, as would be the case for simpler methods. This makes measurement of other parameters, like the coil quality factor, possible.

### 10.5.2 RF Coil Performance

Upon loading of the RF probe with a subject or sample, the subject and the coil will exchange energy. There will be inductive (i.e. magnetic) and capacitive (i.e. electric) interactions between the subject and the RF probe. The inductive interaction consists of an inductive coupling between current loops in the conducting sample and the RF probe. The subject–probe interaction is responsible for an efficient exchange of energy during RF pulse transmission and signal reception. This desirable interaction can easily be verified with a test sample. For an unloaded (correctly tuned and matched) RF coil, the quality factor  $Q$  is:

$$Q_{\text{unloaded}} = \frac{\omega L}{R_c} \quad (10.46)$$

where  $R_c$  represents the resistance of the RF coil. Maximization of this value is desirable. Introducing a magnetically lossy test sample (i.e. the sample conductivity  $\sigma \neq 0$ ), like a physiological salt solution, reduces the  $Q$  value to:

$$Q_{\text{loaded}} = \frac{\omega L}{(R_c + R_m)} \quad (10.47)$$

(see Figure 10.25B) where  $R_m$  represents the effective resistance to magnetically induced currents within the sample/subject. A large drop in quality factor upon the introduction of a magnetically lossy sample indicates large magnetically induced currents in the sample (when dielectric losses can be excluded as will be explained shortly hereafter) and a high  $B_1$  magnetic field within the sample. A concentrated  $B_1$  is, through the principle of reciprocity (see Section 10.5.4), indicative of a sensitive coil making a large drop in  $Q$  value one of the design criteria for a well-constructed RF probe. As the coil sensitivity  $S$  is proportional to  $(Q_{\text{loaded}})^{1/2}$  [45], Equations (10.46) and (10.47) can be combined to give:

$$\frac{S}{S_0} = \sqrt{1 - \frac{Q_{\text{loaded}}}{Q_{\text{unloaded}}}} \quad (10.48)$$

where  $S$  is the experimental coil sensitivity and  $S_0$  represents the maximal sensitivity for an ideal RF probe (i.e.  $R_c \rightarrow 0$ ). Equation (10.48) provides a good basis to evaluate the sensitivity of a given RF coil. For instance, when  $Q$  drops by a factor of 5 upon introduction of the sample, the achieved S/N is to within 10 % of the maximum S/N, such that further coil optimization is not worthwhile. Loading a coil with a magnetically lossy sample reduces, besides the quality factor  $Q$ , also the frequency because the coupling between the coil

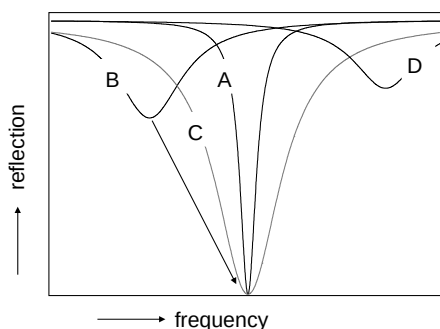


**Figure 10.25** Mismatching, detuning and quality factor (or selectivity) reduction of a given RF coil for different samples. (A) A correctly tuned and matched, unloaded (i.e. empty) RF coil. The quality factor  $Q$  (i.e. the selectivity) is high. (B) Introduction of a magnetically lossy sample (i.e. a biological sample) results in an impedance mismatch,  $Q$  reduction and a shift in the resonance frequency. (C) The loaded RF coil after retuning and impedance matching. Note that the quality factor is significantly reduced when compared to the unloaded coil. (D) Introduction of a metallic sample results in a mismatch, reduction of  $Q$  and a displacement of the resonance frequency towards higher frequency values. This effect is a consequence of eddy currents within the highly conductive metal, which results in a decrease of the inductance  $L$  of the RF coil (and hence a higher resonance frequency).

and the load is equivalent to a supplementary capacitance added to the coil capacitances. However, the tuning can easily be restored to the Larmor frequency by readjusting the tuning capacitance.

Besides inductive coupling, capacitive (i.e. electric) coupling (which explains the reduction in frequency upon coil loading) occurs between sample and probe because electric fields produced by the coil cause a current flow within the sample. Since electric fields do not contribute to signal excitation it is generally desirable to eliminate them as much as possible. The presence of significant electrical fields can easily be verified by introducing a dielectrically lossy test sample (e.g. a bottle of distilled water or a plastic) into the probe. The resulting  $Q$  value is identical to Equation (10.47) except that  $R_m$  should be replaced with  $R_e$ , the effective resistance to dielectric coil/load interactions, because dielectrically lossy samples only interact with the electric fields in the probe. A large  $Q$  drop is indicative of strong electrical fields, significant energy dissipation and a less efficient RF probe.

Therefore, a large  $Q$  drop upon introduction of a magnetically lossy sample only demonstrates the performance of a well-designed probe when there is no significant  $Q$  drop upon introduction of an exclusively dielectrically lossy sample. Electrical fields within the sensitive volume of the coil can be minimized for instance by proper (e.g. symmetrical) distribution of capacitors. Additional reductions in electrical fields may be obtained by distributing several capacitors along the coil wiring (instead of just using one).

### 10.5.3 Spatial Field Properties

The principles of a resonant LCR circuit as described in Section 10.5.1 form the basis of all RF probe designs. The wealth of known RF probe designs, all of which utilize a cylindrical symmetry, can roughly be divided into two categories, according to the  $B_1$  field orientation with respect to the main cylindrical axis. In this section the spatial field properties of different RF probe designs will be discussed. Spatial effects originating from the dielectric and conductive properties of the sample are ignored (i.e. the RF coils are empty), as these are the subject of Sections 10.5.4 and 10.5.5.

*Longitudinal Magnetic Fields.* A perfect homogeneous  $B_1$  field can be generated by running a homogeneous current tangentially on the surface of a (long) cylinder. In most practical applications this current distribution is generated by winding a wire equidistantly on a cylinder (Figure 10.26A). Within the cylinder, parallel with the cylinder axis a very homogeneous magnetic field is generated. This so-called solenoidal RF probe is, besides a completely spherical probe, the best probe design in terms of sensitivity and field homogeneity. Despite the outstanding electromagnetic properties of a solenoid, it is not frequently used for *in vivo* NMR studies because of the geometry of the coil relative to the main magnetic field  $B_0$ . Superconductive magnets themselves are normally built as solenoids (also because of the excellent field homogeneity), thereby offering a cylindrical bore with an axial main magnetic field. The  $B_1$  magnetic field created by the RF coil should be perpendicular to the main magnetic field, which results in a geometry conflict for solenoidal RF probes. Since the main cylindrical axis must be perpendicular to the  $B_0$  magnetic field, the sample can not easily be positioned in the RF probe. Furthermore, the large axial magnet bore space is not used efficiently.

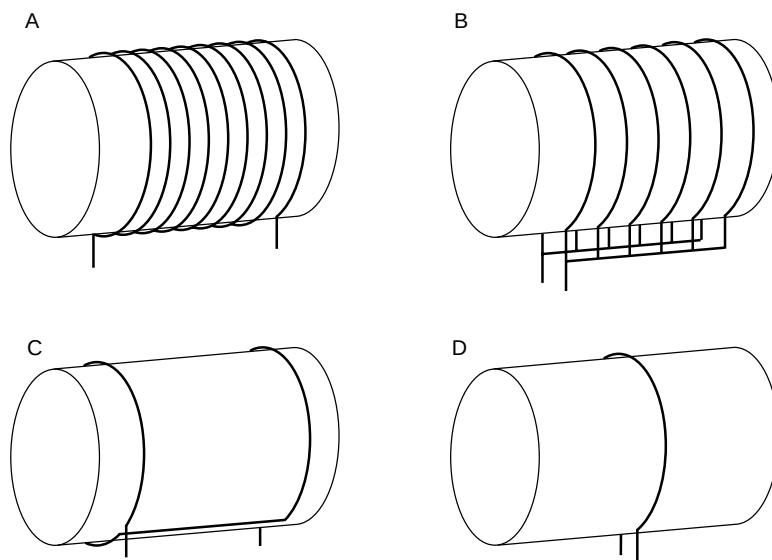


**Figure 10.26** Cylindrical RF coils that generate a longitudinal RF field. (A) A solenoidal coil approximates the ideal current distribution as close as possible. (B) A 'parallel' solenoidal coil shortens the conductor length/wavelength ratio for each conductor element, which makes the coil applicable at higher frequencies. (C) A Helmholtz coil is essentially a two-turn solenoidal coil. This design leaves a convenient radial access to the interior of the coil. The magnetic field homogeneity is optimal when the distance between the loops equals the loop radius. (D) Surface coil.

This problem can (partly) be alleviated by reducing the number of windings to two as shown in Figure 10.26C. The two parallel, in series connected windings with identical current direction, known as a Helmholtz coil [46], allow a much better access to the interior of the coil. When the distance between the two coil planes equals the coil radius, maximum field homogeneity is obtained. Even though the Helmholtz coil provides lower sensitivity and homogeneity than a solenoidal coil of the same dimensions, it is more frequently used for *in vivo* NMR applications since the sensitive volume is more accessible.

In situations where the total subject under investigation is much larger than the region of interest, the described coils are not optimal for several reasons. First of all, the sensitivity is compromised due to the low  $B_1$  magnetic field strength associated with large RF coils. Further, in case of localized spectroscopy an overwhelming amount of surrounding tissue needs to be suppressed in order to achieve adequate localization.

In these cases, the design of solenoidal coils can be further reduced to a single winding, known as a surface coil ([47–52], Figure 10.26D). Although the field homogeneity is extremely low, the filling factor and the sensitivity are very good, because the surface coil can be placed adjacent to the region of interest and can often be reshaped to exactly fit the object under investigation (e.g. wrist, knee). Furthermore, surface coils provide rough localization of signal which corresponds approximately to a hemisphere of one coil radius in which the sensitivity decreases with increasing distance from the coil. Figure 10.27 shows

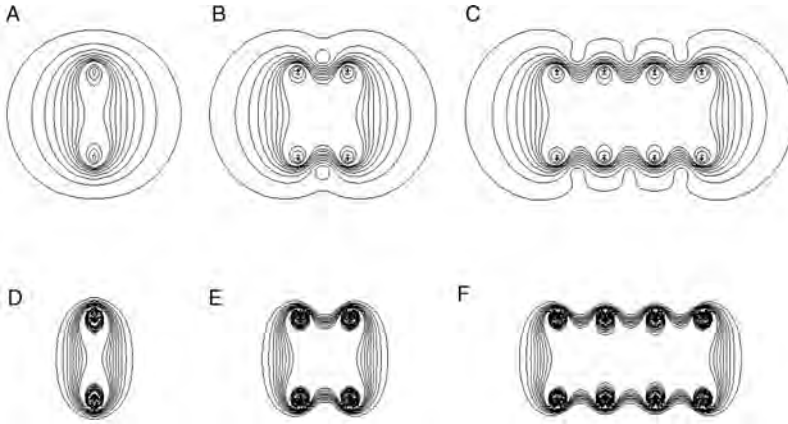


**Figure 10.27** Contour plot of the magnetic field distribution of (A, D) a single turn surface coil, (B, E) a two-turn solenoidal coil (Helmholtz coil) and (C, F) a four turn solenoidal coil. For the coils in (B) and (C), the winding separation equals the radius. The  $B_1$  magnetic field distribution of (A–C) corresponds to the sensitivity following homogeneous (i.e.  $B_1$  insensitive) excitation, for instance with adiabatic RF pulses. (D–F) show the sensitivity profiles of the coils following a  $90^\circ$  excitation with a conventional square RF pulse. The  $B_1$  dependence of the nutation angle significantly reduces the sensitive volumes of the coils.

RF field profiles of solenoidal coils. The  $B_1$  field distribution of RF coils (and with that the sensitivity) can be calculated by finite element analysis of the Maxwell equations, numerical or analytical evaluation of the Biot–Savart law. The  $B_1$  field homogeneity of a four-turn solenoidal coil is very high over a large part of its sensitive volume. The homogeneous part of the  $B_1$  field of a single turn surface coil is limited to the region between the wires. The  $B_1$  field distribution of surface coils, unlike other more complicated RF coils, can analytically be described in terms of axial ( $B_{1ax}$ ) and radial ( $B_{1rad}$ )  $B_1$  fields (Figure 10.28A) as derived through the Biot–Savart law:

$$B_{1rad} \propto \frac{y}{\sqrt{(x^2 + z^2)[(r + \sqrt{x^2 + z^2})^2 + y^2]}} \left[ -K(\kappa^2) + \frac{r^2 + x^2 + y^2 + z^2}{(r - \sqrt{x^2 + z^2})^2 + y^2} E(\kappa^2) \right] \quad (10.49)$$

$$B_{1ax} \propto \frac{1}{\sqrt{[(r + \sqrt{x^2 + z^2})^2 + y^2]}} \left[ K(\kappa^2) + \frac{r^2 - x^2 - y^2 - z^2}{(r - \sqrt{x^2 + z^2})^2 + y^2} E(\kappa^2) \right] \quad (10.50)$$

where  $K(\kappa^2)$  and  $E(\kappa^2)$  refer to the complete elliptic integrals of the first and second kind with  $\kappa^2 = 4r(x^2 + z^2)^{1/2}/[(r + (x^2 + z^2)^{1/2})^2 + y^2]$ . Figure 10.28B and C shows plots of the  $B_1$  field distribution of a 1 cm diameter single-turn surface coil in the  $xy$  and  $yz$  planes. The  $y$  direction is perpendicular to the plane of the coil. The  $B_1$  field strength is very high close to the coil. Note that the sensitivity in the  $yz$  plane is zero on two lines where the

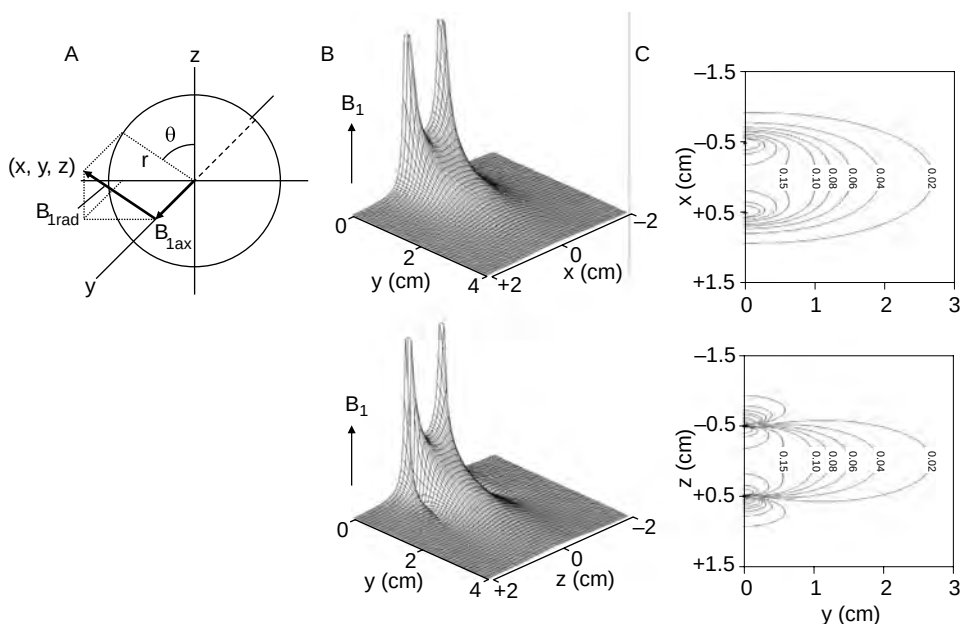


**Figure 10.28** (A) Definition of the two components describing the RF field generated by a circular surface coil as used in Equations (10.49) and (10.50). (B) Simulated  $B_1$  profiles of a 1 cm diameter single-turn surface coil for the xy ( $z = 0$ ) and yz ( $x = 0$ ) planes.  $B_{1ax}$ ,  $B_1 = (B_{1x}^2 + B_{1y}^2)^{1/2}$ , where  $B_{1x} = B_{1rad} \sin\theta$  and  $B_{1y} =$  (C) Contour plot representation of the simulations shown in (B). The highest  $B_1$  magnetic field amplitude was normalized to one. Note that the sensitive volume approximately equals one coil radius.

$B_1$  field direction is in the  $z$  direction, i.e. parallel with the longitudinal magnetization. For points on the coil axis (i.e.  $x = 0, z = 0$ ), Equation (10.50) can be reduced to:

$$B_{1ax} \propto \frac{r^2}{(r^2 + y^2)^{3/2}} \quad (10.51)$$

where it was taken that  $K(0) = E(0) = \pi/2$ . Equation (10.51) may be used to get a rough feeling for the sensitive volume and the effect of experimental parameters, like RF pulses.

One point of consideration for surface coils which has been described extensively [48–50] is the influence of experimental parameters, and in particular  $TR$ , on the shape and size of the sensitive volume. For  $TR \ll T_1$ , the amount of transverse magnetization becomes strongly dependent upon the nutation angle [see Equation (1.72)]. As a consequence lower nutation angles generate relatively more signal than higher nutation angles (due to  $T_1$  saturation). Since the nutation angle of regular, amplitude-modulated RF pulses decreases with increasing distance from the coil, the sensitive volume will be larger (and more homogeneous) when  $TR \ll T_1$ . This explains the high-quality FLASH images that can be obtained with surface coil transmission/reception. The signal dependence upon  $B_1$  and  $T_1$  can be eliminated altogether by using  $TR > 5T_1$  in combination with adiabatic RF pulses.

**Transverse Magnetic Fields.** Even though the previously described solenoidal RF probes are theoretically superior in terms of homogeneity and sensitivity, many probes supporting transverse magnetic fields have been designed for biomedical applications simply because their sensitive volume is better accessible in an axial  $B_0$  magnetic field. A transverse magnetic field can be generated by running a current on a cylindrical surface parallel to the cylindrical axis. Practical realization of such a current distribution is not trivial, leading to a compromise between the ideal transverse current distribution and realistic wire positioning. All RF probes supporting transverse magnetic fields have in common that the major part of the windings is running parallel with the cylinder axis. Wire running perpendicular to the cylinder axis is only used to connect the parallel running wires.

A reasonable approximation of the desired sinusoidal current distribution is obtained with the design shown in Figure 10.29A. By placing four parallel wires carrying equal current at angles  $60^\circ$  and  $-60^\circ$  and reverse current at  $120^\circ$  and  $-120^\circ$ , a reasonable homogeneous  $B_1$  magnetic field is generated within the cylinder. This design has been termed a saddle-shape coil due to the characteristic wire positioning [45]. Several modifications of this basic design have been described for optimal performance at different Larmor frequencies (Figure 10.29B shows a parallel designed saddle-shape coil).

As stated earlier, the ideal transverse current distribution could (theoretically) be generated by running current on a cylindrical surface parallel to the cylindrical axis. The Alderman–Grant or slotted tube RF probes [53–55] approximate this ideal by distributing the current in rectangular sheets as shown in Figure 10.29C.

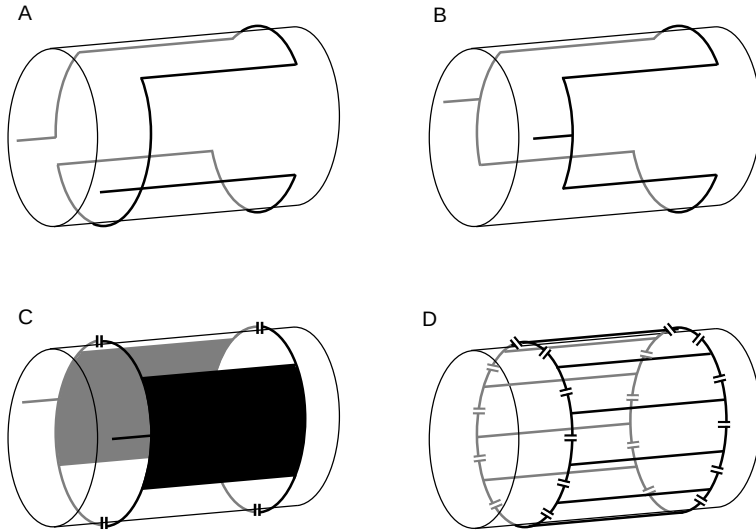


**Figure 10.29** Cylindrical RF coils which generate a transverse RF field. (A) The series and (B) the parallel saddle-shape coil. This coil approximates the ideal current distribution at four radial positions. Just as with the parallel solenoidal coil, the parallel saddle-shape coil is applicable at higher frequencies. (C) The Alderman–Grant or slotted tube RF coil. The ideal current distribution is approximated by the rectangular parallel sheets. (D) The birdcage RF coil.



A new dimension was added to the design of RF coils which produce transverse magnetic fields by an approach based on phase delay networks [45]. When a phase delay LC element, consisting of a capacitor and an inductor (i.e. the coil), is fed with an alternating input voltage, the output voltage will be of equal amplitude but will have a frequency-dependent phase difference relative to the input voltage of  $\Delta\phi(\omega)$ . If a number  $N$  of such elements is connected in series and the final element is reconnected to the first, a loop is created that supports currents at very precisely defined frequencies (Figure 10.29D). This is because only when the total phase shift over the entire collection of LC elements (filaments) equals  $2\pi$  [i.e.  $N\Delta\phi(\omega) = 2\pi$ ] does constructive interference (i.e. resonance) occur. For all other frequencies the phase relation between the filaments has a destructive effect. Increasing the number  $N$  of filaments yields an arbitrarily close approximation of the ideal sinusoidal current distribution. Unlike other RF coils, this sinusoidal current distribution rotates as a function of time around the cylinder axis. Therefore, this so-called birdcage resonator [45, 56, 57] is an inherent quadrature RF coil. If the proper orthogonal coupling to the RF transmitter amplifier and RF receiver preamplifier is provided (in order to suppress counter-rotating fields), the advantages of quadrature coils, i.e. a factor 2 in power reduction and a factor  $\sqrt{2}$  increase in S/N, can be fully exploited. Birdcage resonators provide excellent  $B_1$  homogeneity over the sensitive volume, provided that the number of filaments is large enough. Normally 16 to 32 filaments is a good compromise between  $B_1$  homogeneity and (design) complexity of the coil. Typically the  $B_1$  magnetic field variation is less than 10% over more than 70% of the sensitive volume. While highly effective at lower field, the birdcage design is increasingly replaced by the so-called transverse electromagnetic (TEM) coil for high-field human studies. The RF coil's field energy that is radiated to the far field environment is proportional to  $\omega^4$  [58, 59] and becomes a limiting factor for the RF coil performance for human applications at high magnetic fields. TEM coils greatly reduce radiation losses, while providing excellent  $B_1$  magnetic field homogeneity and strength [60, 61].

### 10.5.4 Principle of Reciprocity I

The emf induced in a given RF probe can be calculated by the principle of reciprocity [62, 63], which states that the emf  $\xi$  induced in a RF probe by a magnetic moment  $\mathbf{M}$  from a voxel  $dV$  at a specific position  $\mathbf{r}$  is determined by the magnetic field  $\mathbf{B}_1$  at that position when unit current flows through the coil. Mathematically this can be represented by:

$$d\xi(dV) = - \frac{d(\mathbf{B}_1 \mathbf{M}) dV}{dt} \quad (10.52)$$

Therefore, knowledge of the  $B_1$  field during transmission will give direct information about the induced emf in that same coil during reception. Application of a  $B_1$  field in the transverse plane (along the  $y$  axis) creates a torque which rotates the initially longitudinal magnetization towards the transverse plane over a nutation angle  $\theta = \gamma B_1 T$ , where  $T$  is the pulse length. The magnetization after the pulse and an evolution period  $t$  can be described as:

$$\mathbf{M} = \begin{pmatrix} M_x \\ M_y \\ M_z \end{pmatrix} = M_0 \begin{pmatrix} \sin \theta \cos \omega t \\ -\sin \theta \sin \omega t \\ \cos \theta \end{pmatrix} \quad (10.53)$$

where  $\omega t$  represents the phase angle acquired due to precession of transverse magnetization at the Larmor frequency  $\omega$ . Only components of  $\mathbf{B}_1$  perpendicular to  $\mathbf{M}$  contribute to the desired torque, such that  $\mathbf{B}_1$  can be described as:

$$\mathbf{B}_1 = \begin{pmatrix} B_{1x} \\ B_{1y} \\ 0 \end{pmatrix} = B_1 \begin{pmatrix} \sin \omega t \\ \cos \omega t \\ 0 \end{pmatrix} \quad (10.54)$$

Using Equations (10.52)–(10.54), the induced emf can be written as:

$$d\xi(dV) = -\frac{d}{dt} \left( \begin{pmatrix} B_{1x} \\ B_{1y} \\ 0 \end{pmatrix} \begin{pmatrix} \sin \theta \cos \omega t \\ -\sin \theta \sin \omega t \\ \cos \theta \end{pmatrix} M_0 dV \right)$$

or

$$d\xi(dV) = M_0 \omega \sin \theta (B_{1x} \sin \omega t + B_{1y} \cos \omega t) dV \quad (10.55)$$

As was already intuitively described, Equation (10.55) shows that  $M_z$  is not time-dependent and will therefore not contribute to the induced emf. By analogy with quadrature transmission, it is desirable to perform quadrature detection [64–66]. This can be achieved by receiving the induced emf along two orthogonal axes. The induced emf in coils positioned along the x and y directions are given by:

$$d\xi_x = M_0 \omega B_1 \sin \theta \sin \omega t dV$$

and

$$d\xi_y = M_0 \omega B_1 \sin \theta \cos \omega t dV \quad (10.56)$$

respectively. Combining Equations (10.56) gives the total complex signal given by:

$$\begin{aligned} d\xi &= d\xi_y + id\xi_x = M_0 \omega B_1 \sin \theta e^{+i\omega t} dV \\ &= M_0 \omega B_1 \sin(\gamma B_1 T) e^{+i\omega t} dV \end{aligned} \quad (10.57)$$

which displays the very characteristic  $\omega B_1$  product common to the principle of reciprocity [62, 63]. Equation (10.57) confirms the abstract formulation of Equation (10.52) that the observed emf during signal reception is directly proportional to the  $B_1$  field during transmission.

However, an important assumption underlying Equation (10.57) is that NMR can be treated as a near-field phenomenon (i.e. the wavelength is much longer than the sample dimensions). At the time of the original paper on the principle of reciprocity as applied to NMR [62], the magnetic field strengths were relatively low making this a perfectly valid approximation. However, as magnetic field strengths for *in vivo* applications steadily increase, the corresponding wavelengths become comparable or even smaller than the sample size. Under these conditions the wave behavior inside the sample must be considered, making it necessary to utilize the complete equations for the principle of reciprocity. As clearly pointed out by Hoult [63], the principle of reciprocity remains valid under all realistic conditions. Before the principle of reciprocity is derived for the intermediate and far-field conditions, a brief introduction into wave propagation is given.

**Wave Propagation.** A complete and concise treatment of electromagnetic wave propagation in materials can be found in all standard textbooks on electromagnetism (e.g. [6]). This section merely summarizes some of the most important findings in order to aid the reader in forming a basic understanding into the origins of wave behavior and its effects on high-field MR images of the human brain.

The magnetic induction  $\mathbf{B}$  in a homogeneous and isotropic medium must satisfy the wave equation:

$$\nabla^2 \mathbf{B} - \mu \epsilon \frac{\partial^2 \mathbf{B}}{\partial t^2} - \mu \sigma \frac{\partial \mathbf{B}}{\partial t} = 0 \quad (10.58)$$

which follows directly from Maxwell's equations for electromagnetism.  $\epsilon = \epsilon_0 \epsilon_r$  represents the dielectric permittivity and  $\epsilon_0$  and  $\epsilon_r$  are the dielectric permittivity of free space and the relative dielectric permittivity, respectively. Permittivity relates to the ability of a material to affect an electric field.  $\mu$  is the permeability of free space and  $\sigma$  is the conductivity (or resistivity  $\rho = 1/\sigma$ ). As detailed in Section 10.3, permeability relates the magnetic induction in a material to the applied auxiliary field. Flowing in the sample are both conduction currents associated with the sample conductivity and displacement currents associated with the dielectric permittivity constant. Note that the displacement current is not a real current (i.e. movement of charge), but rather refers to the fact that a changing electric field generates a changing magnetic field. These currents have a  $90^\circ$  phase difference and also produce their own fields. Thus the magnetic field inside the sample is modified and when a sinusoidal time-dependent magnetic induction field  $\mathbf{B}$  is applied to the sample (i.e.  $\mathbf{B} = B_{\max} e^{-i\omega t}$ ), the solution to Equation (10.58) inside a 1D sample is:

$$\mathbf{B} = B_{\max} e^{(-k_I x - i k_R x - i\omega t)} \quad (10.59)$$

where  $x$  is the direction of propagation and  $k_R$  and  $k_I$  are the real and imaginary parts of the complex wave number  $k$  given by:

$$k = \sqrt{\omega^2 \mu \epsilon - i \omega \mu \sigma} = k_R + i k_I \quad (10.60)$$

where

$$k_R = \omega \sqrt{\frac{\mu \epsilon}{2} \left( \sqrt{1 + \left( \frac{\sigma}{\omega \epsilon} \right)^2} + 1 \right)}^{1/2} \quad \text{and} \quad k_I = \omega \sqrt{\frac{\mu \epsilon}{2} \left( \sqrt{1 + \left( \frac{\sigma}{\omega \epsilon} \right)^2} - 1 \right)}^{1/2} \quad (10.61)$$

The imaginary part of  $k$  is often referred to as an attenuation factor. The reciprocal of  $k_I$  determines the distance over which the amplitude of the field is attenuated by a factor  $(1/e)$  and is commonly known as the skin depth of the medium. The real part of  $k$  is inversely proportional to the wavelength  $\lambda$  in the medium ( $\lambda = 2\pi/k_R$ ) and gives rise to a change in phase with position in the medium.

Figure 10.30 shows uniform plane waves in a lossy medium (i.e.  $\sigma \neq 0$ ) as a function of position and frequency at  $t = 0$ . It follows that at low frequency (1.5 T = 65 MHz) the wave propagates uniformly without significant phase changes or attenuation. However, when reaching higher magnetic fields (4 T and 7 T) and thus higher proton NMR frequencies (170 MHz and 300 MHz) the wave propagation experiences significant phase changes and attenuation. In a nonlossy medium (i.e.  $\sigma = 0$ ) the wave propagation is solely due to wave

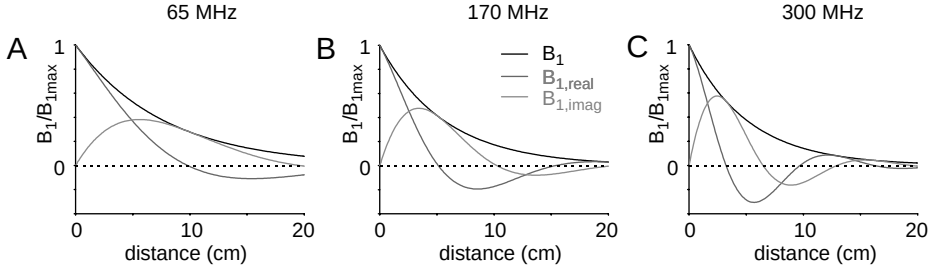


**Figure 10.30** Electromagnetic 1D wave propagation at 65, 170 and 300 MHz for a sample with conductivity  $\sigma = 0.8 \text{ Sm}^{-1}$  and relative permittivity  $\epsilon_r = 80$ . Note that the reduced wavelength at higher frequencies leads to a decreased skin depth (i.e. less sample penetration) as well as increased phase modulation across the sample.

behavior inside the sample, i.e.  $\lambda = 2\pi/(\omega\sqrt{\mu\epsilon})$ . In the case that the (pure dielectric) sample is a multiple of the wavelength  $\lambda$ , a resonance condition appears between the forward and reverse traveling waves. The RF magnetic field inhomogeneity observed in the human brain at higher magnetic fields [67, 68] has often been qualitatively described as being due to ‘dielectric resonances’. However, the simple 1D example (Figure 10.30) shows that the situation for a lossy medium, like brain tissue, is more complex and depends on wave behavior due to the dielectric permittivity characteristics as well as wave attenuation due to conductivity characteristics of the sample.

Now that the basic elements of wave propagation are established (i.e. reduced wavelength and phase changes due to high dielectric constants and wave attenuation due to sample conductivity), we can continue with the derivation of the generalized principle of reciprocity.

### 10.5.5 Principle of Reciprocity II

The theory of sample-induced  $B_1$  field perturbation on pulse transmission and signal reception have been covered in detail by many authors [69–74]. Here the observed perturbations will be described through the use of an analytical model, after which the use of the general principle of reciprocity for MR signal calculations will be outlined. As was shown in Section 10.5.4, the phase as well as the amplitude of wave propagation in lossy samples depends on the sample dielectric permittivity, conductivity and sample size. Based on those observations it is reasonable to expect that a linearly polarized transmit coil with a field  $B_1$  produced per unit current along the x axes generates both  $B_{1x}$  and  $B_{1y}$  components at polar coordinates  $(r, \phi)$  in a sample of a given dielectric permittivity and conductivity, i.e.:

$$\mathbf{B}_{1x}(r, \phi) = (b_{xx}\mathbf{x} + b_{yx}\mathbf{y})B_{1x} \quad (10.62)$$

Analytical expressions for  $b_{xx}$  and  $b_{yx}$  can be obtained for an infinitely long cylinder as described by Bottomley and Andrew [69] and Mansfield and Morris [70] and are given by:

$$b_{xx} = \frac{1}{J_0(kr_0)} \left( \frac{2J_1(kr)}{kr} - J_0(kr) \right) \cos 2\phi + \frac{J_0(kr)}{J_0(kr_0)} \quad (10.63)$$

$$b_{yx} = \frac{1}{J_0(kr_0)} \left( \frac{2J_1(kr)}{kr} - J_0(kr) \right) \sin 2\phi \quad (10.64)$$

where  $J_0$  and  $J_1$  are zero- and first-order Bessel functions of the first kind and  $r_0$  is the radius of the cylinder. In the case of nonconducting samples, the wave number  $k$  is real and hence  $b_{xx}$  and  $b_{yx}$  are real. Under this condition, the constant  $b_{xx}$  corresponds to the x component, and the constant  $b_{yx}$  to the y component produced by a field applied along the x direction. The  $b_{yx}$  component originates from the dielectric properties of the sample. For conducting samples,  $b_{xx}$  and  $b_{yx}$  are complex numbers, making a straightforward interpretation difficult.

The linearly varying components of Equations (10.62)–(10.64) can be rewritten in terms of rotating components according to:

$$\mathbf{B}_1 = \mathbf{B}_1^+ + \mathbf{B}_1^- = (b_{xx} + ib_{yx})\mathbf{B}_{1x}\mathbf{a}^+ + (b_{xx} - ib_{yx})^*\mathbf{B}_{1x}\mathbf{a}^- \quad (10.65)$$

where  $\mathbf{a}^+$  and  $\mathbf{a}^-$  are unit vectors rotating clockwise and anticlockwise at angular frequency  $\omega$ . By convention it is assumed that the positive, clockwise rotation component  $\mathbf{B}_1^+$  precesses in the same sense as the nuclear spins and it is therefore that this component can couple energy into the NMR response which leads to excitation. The counter-rotating component can under most conditions be ignored during the excitation process. By applying the principle of reciprocity, as outlined by Hoult [63], the received signal can be shown to be proportional to  $(\mathbf{B}_1^-)^*$  where  $*$  denotes complex conjugation, such that the total induced emf becomes:

$$\xi = M_0\omega\mathbf{B}_1^{-*} \sin(\gamma\mathbf{B}_1^+T)e^{+i\omega t} \quad (10.66)$$

At low magnetic field strengths, and hence low frequencies, the rotating fields are real and equal, i.e.  $\mathbf{B}_1^+ = \mathbf{B}_1^- = \mathbf{B}_1$ , such that Equation (10.66) reduces to Equation (10.57). When the sample is conductive,  $\mathbf{B}_1^+$  and  $(\mathbf{B}_1^-)^*$  are not identical and any spatial phase in  $\mathbf{B}_1^+$  will be doubled in the received signal  $\xi$ . Figure 10.31 shows simulations based on Equations (10.63)–(10.66) for a 10 cm diameter sphere at 3.0 T and 7.0 T. It follows that for signal strength calculations at higher magnetic fields it is crucial to separate  $\mathbf{B}_1^+$  during transmission from  $\mathbf{B}_1^-$  during reception. The measurement of  $\mathbf{B}_1^+$  is relatively straightforward and one possible method is detailed in Section 4.8.4, i.e. by measuring the signal at two different nutation angles, the  $\mathbf{B}_1^+$  field strength can be obtained from the ratio. Once  $\mathbf{B}_1^+$  is known,  $\mathbf{B}_1^-$  can be calculated using Equation (10.66). While this is straightforward on a homogeneous phantom, the *in vivo*  $\mathbf{B}_1^-$  calculation is complicated by  $T_1$ ,  $T_2$  and proton density contrast between tissues. Several methods have been proposed for the calculation of the *in vivo*  $\mathbf{B}_1^-$  [75, 76]. The distinction between  $\mathbf{B}_1^+$  and  $\mathbf{B}_1^-$  also becomes important during signal quantification when using external concentration references that may experience RF fields different from the compounds of interest. See Chapter 9 for more details.

## 10.6 Radiofrequency Coil Types

In the previous section the general design and operating criteria of a variety of RF coils have been described. In this section, a number of more specialized RF coils are described that have utility for *in vivo* NMR spectroscopy. These include RF coils in which RF transmission

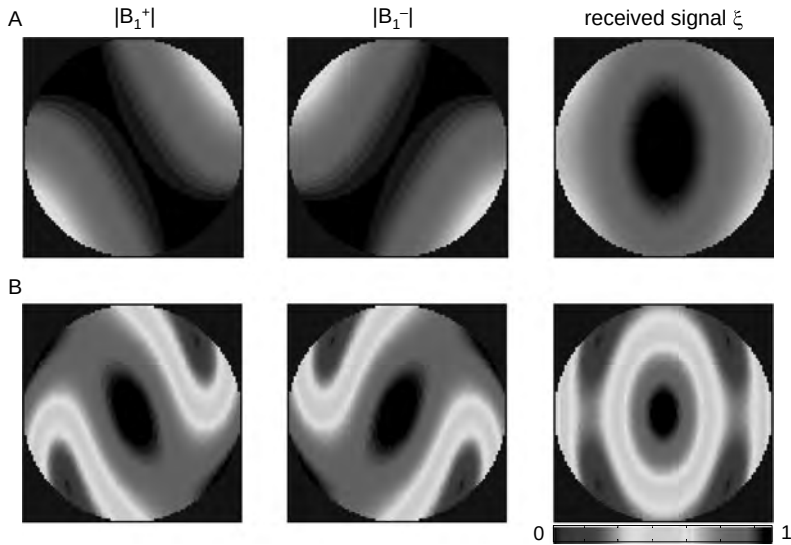


**Figure 10.31** Simulations for  $B_1^+$ ,  $B_1^-$  and the received signal  $\xi \propto B_1^+(B_1^-)^*$  of a linear polarized RF coil based on Equations (10.63)–(10.66) for a 10 cm diameter sphere at (A) 3.0 T and (B) 7.0 T with conductivity  $\sigma = 0.66 \text{ Sm}^{-1}$  and relative permittivity  $\epsilon_r = 51$ . While small differences between  $B_1^+$  and  $B_1^-$  are visible at 3.0 T, they become dominant contributors to the overall image intensity at 7.0 T. In order to adequately characterize the received signal  $\xi$ , separate knowledge of the transmit  $B_1^+$  and receive  $B_1^-$  magnetic fields is required. (See color plate 10)

and reception is achieved by separate coils, RF coils for heteronuclear applications, phased-array coils and cooled (superconducting) RF coils.

### 10.6.1 Combined Transmit and Receive RF Coils

The many different RF coil designs can partially be explained by the fact that each coil is often a compromise between its desirable and undesirable features. There is in general not a single coil optimal for all applications. A volume coil (e.g. a birdcage coil) provides a homogeneous  $B_1$  magnetic field, such that spin excitation is uniform across the sample. However, the large size and often poor filling factor of volume coils compromises their sensitivity. Surface coils on the other hand are very sensitive due to their high filling factor and their optimal size relative to the object under investigation. A drawback of surface coils is that the generated  $B_1$  field is extremely inhomogeneous which will lead to signal loss when conventional amplitude-modulated RF pulses are transmitted. In other words, a volume coil is desirable for RF transmission, but undesirable for signal reception. A surface coil, to the contrary, is desirable for signal reception, but performs poorly during RF transmission.

Therefore, a more optimal RF coil can be constructed when transmission and reception can be achieved by volume and surface coils, respectively. The construction of a volume coil transmission–surface coil reception combination is more complicated as any interaction

between the two RF coils will lead to artifacts, signal loss or inefficient power transfer. As a first step to minimize coil interactions, the coils should be positioned orthogonal relative to each other. Secondly, the mutual coupling between the coils is minimized by so-called active detuning. During RF transmission, the surface coil is actively detuned [for example by temporarily changing the capacitance of the surface coils circuitry through the use of a pin-diode, see Equation (10.39)], so that it becomes very inefficient to interact with RF fields in the Larmor frequency range. During signal acquisition, the surface coil is quickly returned to a state of resonance, so that signal can be efficiently received. Depending on the spatial resolution, the sensitivity obtained with this combined coil set-up as compared with a single transceiver surface coil in combination with adiabatic RF pulses could be slightly reduced. This is because the  $B_1$  flux lines between transmit and receive coils are spatially dependent [50], which will lead to some phase cancellation. For MRI this effect is minimal, as the phase variation across a MRI pixel will be negligible. For localized NMR spectroscopy, the effect can be minimized by limiting the voxel to the sensitivity volume of the surface coil where the variation in  $B_1$  flux lines is minimal. In analogy to signal reception with regular surface coils, the penetration depth of the dual coil set-up is limited to about one coil radius (see Figure 10.28).

## 10.6.2 Phased-array Coils

The primary advantage of surface coil reception is the high sensitivity that can be obtained immediately adjacent to the coil. This has made surface coils the primary choice for applications that are inherently sensitivity limited, like MRS. Besides the fact that the  $B_1$  magnetic field is highly inhomogeneous, the main disadvantage of surface coils is their limited spatial coverage. Hyde [77] proposed using multiple, non-interacting surface coils and receivers to independently and simultaneously obtain signals from multiple coils, achieving improved S/N from an expanded spatial region with no increase in time. Roemer [78] implemented a system using four receiver coils and four receiver channels, which was referred to as a NMR phased array and is schematically shown in Figure 10.32. In order to minimize noise correlation, each coil in a phased array assembly is connected to its own pre-amplifier and receiver. Under the assumption that the noise is uncorrelated, the combined signal  $S_c(x,y,z)$  can be calculated from the signals from the  $N$  individual coils,  $S_n(x,y,z)$ , multiplied by a weighting coefficient  $w_n(x,y,z)$  that is proportional to the S/N for coil  $n$  according to:

$$S_c(x, y, z) = \frac{\sum_{n=1}^N w_n(x, y, z) S_n(x, y, z)}{\sqrt{\sum_{n=1}^N (w_n(x, y, z))^2}} \quad (10.67)$$

Prior to the weighted addition given by Equation (10.67), the spectra must be phase-corrected in order to avoid phase cancellation. Phased-array receiver coils are often used in combination with a homogeneous volume transmission coil and can achieve significant improvements in the obtainable S/N ratio, especially in areas close to the surface coils. Current implementations of phased-array coils use 8–16 coils, while extensions to 32 coils and beyond have already been described [79]. Rather than using the phased-array assembly to increase the S/N of the experiment, the multiple coils can also be used to increase the temporal resolution of the experiment through parallel data acquisition strategies, like

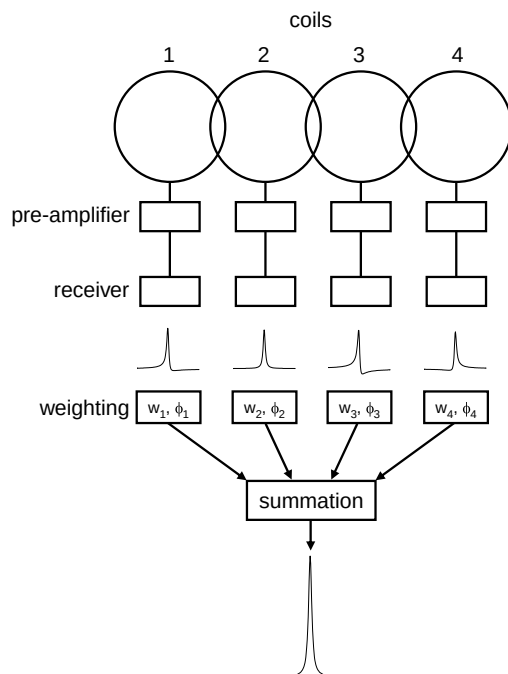


**Figure 10.32** General set-up for a four-coil phased-array experiment. Each coil has a dedicated pre-amplifier and receiver chain in order to minimize noise correlation between the coils. Since the signal amplitude and phase can be different between coils, a weighted, phase-corrected summation must be performed to ensure optimal sensitivity.

SENSE [80] and SMASH [81]. The principles of parallel data acquisition are discussed in Chapter 4.

### 10.6.3 $^1\text{H}$ - $^{13}\text{C}$ and $^{13}\text{C}$ - $^1\text{H}$ RF Coils

NMR spectroscopy is a unique tool to measure important metabolic fluxes like the TCA cycle flux noninvasively in human and animal brain *in vivo*. Chapter 3 provided a detailed description of the experimental considerations and analysis of flux measurements through the infusion of  $^{13}\text{C}$ -labeled substrates and the *in vivo* detection of the  $^{13}\text{C}$ -labeled metabolites. Chapter 8 provided a description of the NMR pulse sequences necessary to perform these state-of-the-art experiments. For almost all experiments, be it through indirect  $^1\text{H}$ - $^{13}\text{C}$ ] or direct  $^{13}\text{C}$  NMR detection, a double RF coil set-up is required. Figure 10.33 shows the most commonly used  $^{13}\text{C}$ - $^1\text{H}$ ] (Figure 10.33A) and  $^1\text{H}$ - $^{13}\text{C}$ ] RF coils (Figure 10.33B and C) for human and animal applications *in vivo*. Since direct or polarization transfer enhanced  $^{13}\text{C}$  NMR spectroscopy is hampered by low sensitivity, the  $^{13}\text{C}$  reception coil is typically a relatively small surface coil (circa 80–100 mm and 12–15 mm diameter for human occipital cortex and rat brain applications, respectively). The  $^1\text{H}$  RF coil, required for decoupling and other spin manipulations, was traditionally a larger surface coil



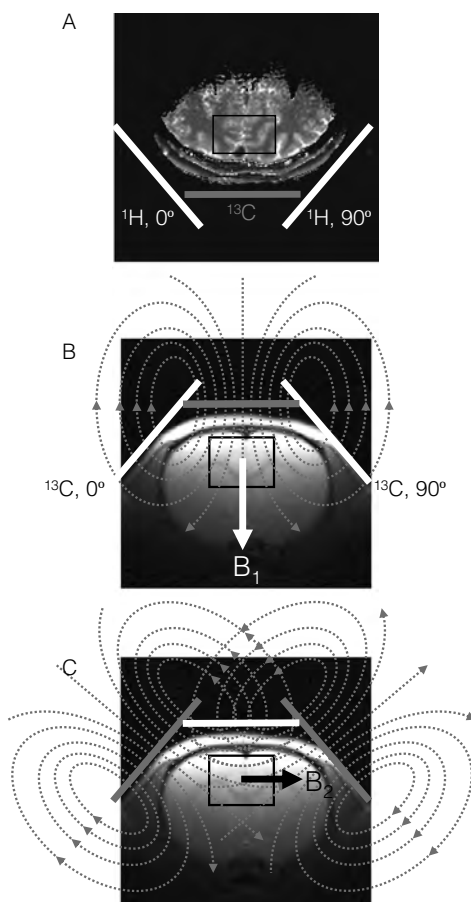


**Figure 10.33** (A) Typical coil set-up for  $^1\text{H}$ -decoupled,  $^{13}\text{C}$  NMR on human brain. The two  $^1\text{H}$  surface coils are  $90^\circ$  out of phase in order to achieve quadrature transmission. (B, C) RF coil set-up for  $^{13}\text{C}$ -decoupled,  $^1\text{H}$  NMR on rat brain. (B) Magnetic field flux lines of the  $^1\text{H}$  surface coil point approximately downwards in the volume of interest, whereas (C) the magnetic field flux lines of the quadrature  $^{13}\text{C}$  coils are roughly horizontal, thereby achieving an inherent, geometric decoupling of the  $^1\text{H}$  and  $^{13}\text{C}$  coils.

co-planar with the  $^{13}\text{C}$  RF coil. However, this geometric arrangement leads to significant coil interactions as well as unacceptable RF power deposition during proton decoupling. A more favorable, and currently the most commonly used, design was described by Adriany and Gruetter [82] in which the  $^1\text{H}$  RF coil is split into two surface coils driven in quadrature (Figure 10.33A). The quadrature requirement is met by a hybrid coupler in which the incoming RF power is split evenly over the two  $^1\text{H}$  coils, while simultaneously offsetting the RF phase by  $90^\circ$  for one  $^1\text{H}$  coil. During reception the hybrid splitter combines the two out-of-phase NMR signals, such that one in-phase signal arrives at the pre-amplifier. Besides the RF power advantage of a quadrature design, the two  $^1\text{H}$  RF coils also cover

the volume excited by the  $^{13}\text{C}$  RF coil more homogeneously. Furthermore, the  $B_1$  and  $B_2$  magnetic field flux lines of the  $^{13}\text{C}$  and  $^1\text{H}$  coils, respectively, are approximately perpendicular over a large fraction of the usable volume of the  $^{13}\text{C}$  RF coil, leading to an intrinsic decoupling of the mutual coil interactions (Figure 10.33B and C). The  $^1\text{H}$  and  $^{13}\text{C}$  RF coils in the  $^{13}\text{C}$ - $[^1\text{H}]$  design of Figure 10.33A can simply be reversed to give a  $^1\text{H}$ - $[^{13}\text{C}]$  RF coil (Figure 10.33B and C).

Despite the reduced interaction as a result of the geometric configuration of the two coils, the interference between the two coils is typically strong enough to cause a significant increase in noise level when broadband decoupling is attempted. This additional noise of the observe channel typically originates from noise on the decoupling channel.

For almost all heteronuclear experiments involving broadband decoupling it is therefore crucial to apply filters on the decoupling channel to filter out the frequencies that lead to noise in the observe channel. A typical set-up for  $^1\text{H}$ - $[^{13}\text{C}]$  NMR is shown in Figure 10.34A. At least one, but often two filters are required on the decoupling  $^{13}\text{C}$  channel to remove spurious signal at the  $^1\text{H}$  frequency. Even though band-pass filters can be used, low-pass filters typically have a better performance in terms of reduced signal loss at the desired frequency (this so-called insertion loss should be less than 0.5 dB for a good filter).

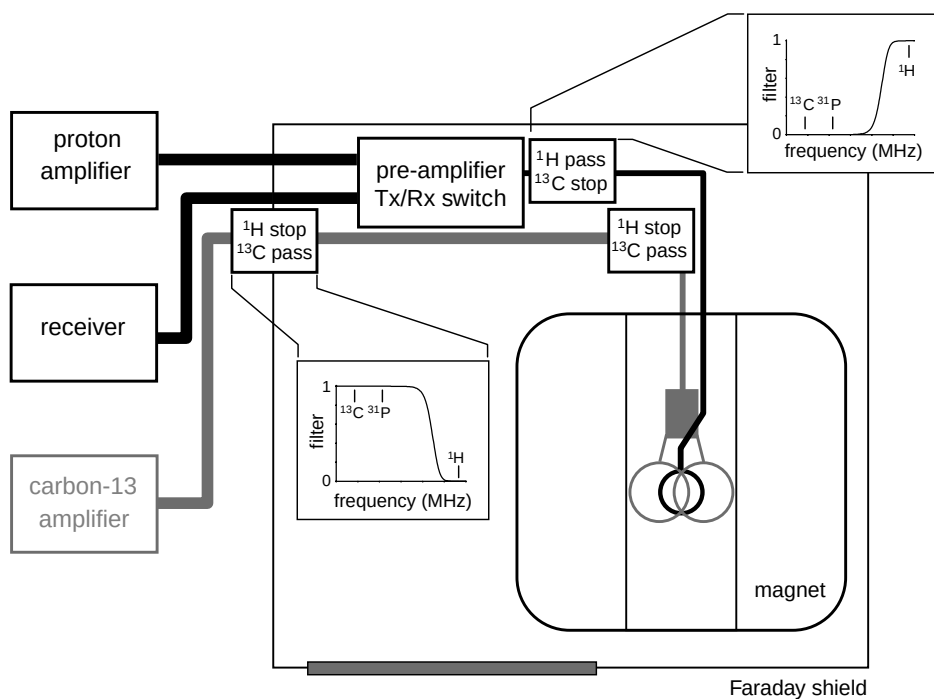


**Figure 10.34** Typical coil, filter and amplifier set-up for a  $^1\text{H}$ - $[^{13}\text{C}]$  NMR experiment. In order to minimize noise interference on the  $^1\text{H}$  receive channel during RF transmission on the  $^{13}\text{C}$  channel (i.e.  $^{13}\text{C}$  decoupling during  $^1\text{H}$  NMR reception), a combination of  $^{13}\text{C}$ -pass/ $^1\text{H}$ -stop and  $^{13}\text{C}$ -stop/ $^1\text{H}$ -pass filters are required as shown. See text for more details.

A good low-pass filter achieves at least 60 dB attenuation at the  $^1\text{H}$  NMR frequency. While somewhat counter-intuitive, a high-pass filter on the observe channel prior to the pre-amplifier often greatly decreases the noise injection during  $^{13}\text{C}$  decoupling. This is likely due to nonlinear elements in the pre-amplifier/TR-switch that can multiply spurious signals at the  $^{13}\text{C}$  NMR frequency to the observed  $^1\text{H}$  NMR frequency. While the exact filter combination and placement is often site-specific, the combination of filters should lead to negligible degradation of the S/N during decoupling.

### 10.6.4 Cooled (Superconducting) RF Coils

The available S/N is the dominant limiting factor in the majority of MR experiments, preventing the acquisition of higher-resolution images or the detection of metabolites from smaller volumes. Many strategies have evolved with the aim of increasing the detected signal in order to increase the S/N, including the use of higher magnetic fields, improved RF coil designs and hyperpolarized materials. However, being a ratio, the noise plays an equivalent role. As the size of the sample decreases, the thermal noise of the receiver coil represents a greater proportion of the total system noise. In the extreme case, the RF coil rather than the sample dictates the noise at the pre-amplifier. In an effort to limit receiver coil noise for small samples, as well as low magnetic field applications, the use of cooled [83–85] or superconducting [86–88] materials in the construction of low-noise RF coils have been explored. The overall aim is to reduce the noise contributions of the RF coil [i.e.  $R_c$  in Equations (10.46) and (10.47)] and pre-amplifier such that the sample noise once again becomes the dominant contributor of the S/N. Cooling the RF coil and pre-amplifier with liquid nitrogen can lead to improvements in S/N of 2 to 3. However, it should be realized that these improvements are only realized when the sample does not significantly load the coil at room temperature (i.e.  $Q_{\text{loaded}}/Q_{\text{unloaded}} \sim 1$ ). This typically limits the applications of cooled and superconducting RF coils to small surface coils or low frequency MR systems [84, 89].

## 10.7 Complete MR System

### 10.7.1 RF Transmission

Besides the commonly encountered components such as gradients, RF coils and magnet, a complete MR system holds many more essential components related to RF pulse transmission, signal reception and overall system integration. In Chapter 5 the characteristics of RF pulses were described, while Section 10.6 detailed the RF coils utilized to transfer the RF pulse power to a sample. Figure 10.35 shows a simplified flow chart for the steps involved in converting the desired shape into a high-powered RF pulse, i.e. the RF transmission chain. The synthesizer module generates a continuous frequency of constant amplitude. The exact frequency is under computer control and can thus be adjusted to coincide with the Larmor resonance frequency to create an on-resonance condition. Next, only the time period of the continuous frequency that corresponds to the length and position of a RF pulse is selected, after which the uniform pulse is modulated with the desired shape. Note that the pulse shape only determines the modulation of the base frequency, the actual pulse is still transmitted in the RF (MHz) range. The final step is an overall amplitude scaling

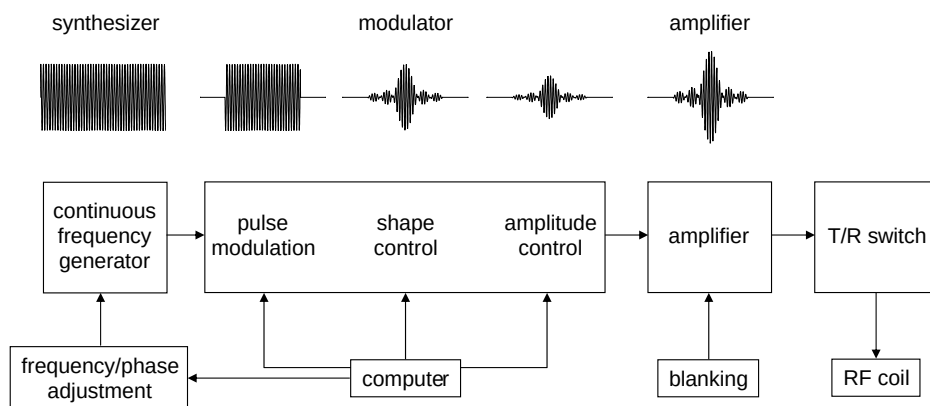


**Figure 10.35** RF transmission sub-system. A continuous frequency generator generates a signal of which the frequency and phase are under computer control. During a NMR sequence, a RF pulse is selected from the continuous signal (pulse modulation), shaped (e.g. sinc) and amplitude-adjusted, after which the signal is amplified to the kW range by a RF amplifier. Since many experiments use the same coil for RF pulse transmission and signal reception, a T/R switch is required to protect the sensitive receiver sub-system from the high-powered RF pulse that traverses along the same co-axial cable to the RF coil.

which allows the fine adjustment of the nutation angle. The scaled, shaped RF pulse is then multiplied to the kW range by a RF amplifier after which it can be transmitted to the sample via the RF coil. Note that, even in an idle mode, RF amplifiers generate noise in the Larmor frequency range which can quickly overwhelm the small NMR signal. It is therefore imperative that the RF amplifier is only on during the RF pulses and blanked (i.e. blocked) during the rest of the NMR sequence. In Section 5.8 the importance of RF amplifier linearity and phase stability has been discussed. In case of significant nonlinearities, RF pulses may be pre-distorted ('pre-warped') in order to compensate the distortions introduced by the RF amplifier (see also Figure 5.32).

### 10.7.2 Signal Reception

Following excitation the precessing magnetization can be detected as an induced voltage in a receiver coil, which is often the same coil as used for RF pulse transmission. The main task of the receiver chain is to amplify the small NMR signal with minimal degradation in the S/N, determine the absolute frequency by quadrature detection and digitize the analog signal for further computer processing. Figure 10.36 shows a simplified flow chart of the steps involved with NMR signal reception. The NMR signal, detected as an induced voltage in the receiver coil, is typically very weak in the  $\mu\text{V}$  range. The first, and arguably the most important step is to amplify the NMR signal to a more manageable range (mV) where it is less sensitive to sources of interference or additional noise. This amplification is achieved by a low-noise preamplifier. The low-noise characteristic can not be overstressed, as any noise introduced at this level directly degrades the S/N of the measurement. A measure of the spectrometer preamplifier to amplify the NMR response without the introduction of

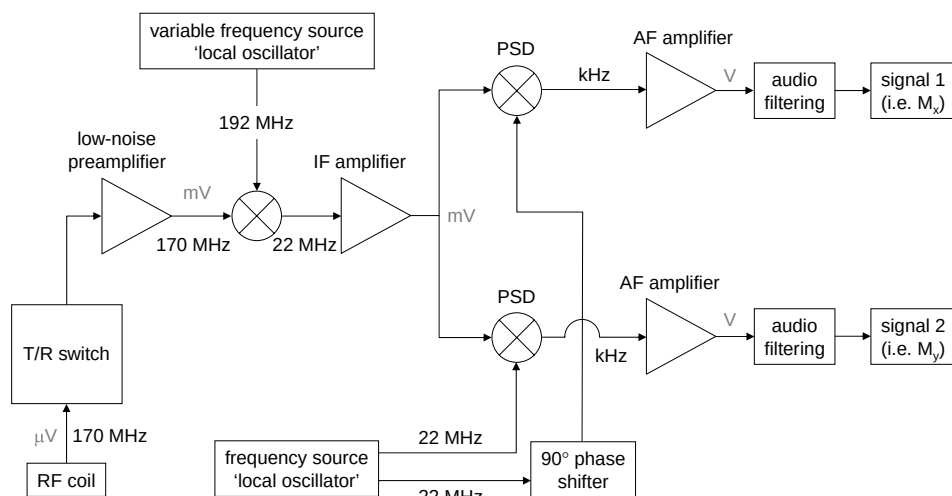


**Figure 10.36** Receiver sub-system. To minimize noise contributions from elements in the receiving chain the small NMR signal, which is typically in the  $\mu\text{V}$  range, is amplified to the mV range by a low-noise pre-amplifier. The RF signal is then down-sampled to a constant intermediate frequency (IF), where it is split for phase-sensitive quadrature detection. Following further down-sampling to the audio-frequency (AF) range (kHz), the signal can be digitized by an analog-to-digital converter (ADC). See text for more details.

additional noise is given by the noise figure. The noise figure  $F$  is defined as [90]:

$$F = 10 \log_{10} \left( \frac{N_A^2 + N_S^2}{N_S^2} \right) \quad (10.68)$$

where  $N_S$  is the RMS noise of the original NMR signal and  $N_A$  is the RMS noise introduced by the preamplifier. Good preamplifiers have a noise figure  $F < 1$  dB, corresponding to a S/N degradation of  $< 12\%$ . The measurement of the noise figure according to Equation (10.68) is difficult, as the RMS noise of the original NMR signal is not known. A practical method of determining the noise figure is through a so-called hot/cold resistor measurement during which the noise level produced by a  $50 \Omega$  resistor at cold and hot temperatures is measured. The noise figure can then be calculated according to:

$$F = -1.279 - 10 \log_{10} \left( 1 - \frac{N_{77K}^2}{N_{293K}^2} \right) \quad (10.69)$$

where  $N_{77K}$  and  $N_{293K}$  are the noise levels at 77 K (liquid nitrogen) and 293 K (room temperature).

Following pre-amplification the signal amplitude is in the mV range. Note that the frequency of the NMR signal following pre-amplification is still in the RF range (MHz). This poses several challenges for further processing of the signal. Firstly, current ADCs are not fast enough to directly digitize the signal in the RF range. Secondly, unwanted frequencies generated by the electronics at the Larmor frequency can lead to additional noise and artifacts. For these reasons the NMR frequency is often down-sampled to an intermediate frequency (e.g. 22 MHz) that does not coincide with the Larmor frequency of

any of the commonly studied nuclei. An additional advantage of working at an intermediate frequency is that all NMR frequencies, from protons to carbon-13, can be down-sampled to the same intermediate frequency such that any subsequent processing can be performed in the same manner. Down-sampling involves mixing of the NMR frequency  $\omega_0$  with a reference frequency  $\omega_{\text{ref}}$  according to:

$$\begin{aligned} \cos(\omega_0 t) &\xrightarrow{\text{mixers}} \cos(\omega_0 t) \cos(\omega_{\text{ref}} t) \\ &= \frac{1}{2} \cos[(\omega_0 + \omega_{\text{ref}})t] + \frac{1}{2} \cos[(\omega_0 - \omega_{\text{ref}})t] \end{aligned} \quad (10.70)$$

Note that the NMR signal at this stage only has a single component, which was arbitrarily chosen as  $\cos(\omega_0 t)$ . The mixing of two signals with frequency  $\omega_0$  and  $\omega_{\text{ref}}$  leads to signals oscillating at the sum and difference of  $\omega_0$  and  $\omega_{\text{ref}}$ . While real mixers give rise to additional frequencies, Equation (10.70) holds well to a first approximation [90]. The high frequency component  $(\omega_0 + \omega_{\text{ref}})$  can be removed by applying a low-pass filter to the data, such the final down-sampled or demodulated signal is given by:

$$\xrightarrow{\text{low-pass filter}} \frac{1}{2} \cos[(\omega_0 - \omega_{\text{ref}})t] = \frac{1}{2} \cos[\omega_{\text{IF}} t] \quad (10.71)$$

where the difference frequency is now an intermediate frequency (IF). Unfortunately, detecting only one component of the rotating magnetization (i.e. the cosine term) does not allow the discrimination between positive and negative frequencies. As is well known, both the cosine and sine components of a harmonic signal must be recorded in order to determine the sign of the frequency. Sampling a signal such that both components are recorded is known as quadrature detection.

### 10.7.3 Quadrature Detection

Quadrature detection during acquisition is accomplished by splitting the IF signal originating from a single coil into two separate channels. The two IF signals are compared with cosine- and sine-modulated reference signals according to:

$$\begin{aligned} \cos(\omega_{\text{IF}} t) &\xrightarrow{\text{mixers}} \cos(\omega_{\text{IF}} t) \cos(\omega_{\text{ref}} t) \\ &= \frac{1}{2} \cos[(\omega_{\text{IF}} + \omega_{\text{ref}})t] + \frac{1}{2} \cos[(\omega_{\text{IF}} - \omega_{\text{ref}})t] \end{aligned} \quad (10.72)$$

and

$$\begin{aligned} \cos(\omega_{\text{IF}} t) &\xrightarrow{\text{mixers}} \cos(\omega_{\text{IF}} t) \sin(\omega_{\text{ref}} t) \\ &= \frac{1}{2} \sin[(\omega_{\text{IF}} + \omega_{\text{ref}})t] - \frac{1}{2} \sin[(\omega_{\text{IF}} - \omega_{\text{ref}})t] \end{aligned} \quad (10.73)$$

Following further amplification and low-pass filtering, the two quadrature signals in the audiofrequency (AF) range are obtained which then uniquely define the absolute frequency, i.e.:

$$\frac{1}{2} \cos[(\omega_{\text{IF}} - \omega_{\text{ref}})t] + \frac{i}{2} \sin[(\omega_{\text{IF}} - \omega_{\text{ref}})t] = \frac{1}{2} \exp[i(\omega_{\text{IF}} - \omega_{\text{ref}})t] = \frac{1}{2} \exp[i\omega_{\text{AF}} t] \quad (10.74)$$

The AF signal undergoes a band pass filtering step to remove all signals, and in particular noise, outside the spectral bandwidth which would otherwise fold back into the spectrum

and degrade the attainable S/N. The amplitude of the analogue signals before analog-to-digital conversion is in the order of several volts and essentially insensitive to minor system noise sources. Note that with modern AD converters, the signal can be directly digitized at the IF, thereby eliminating the need for an AF stage.

### 10.7.4 Dynamic Range

The gain of a NMR receiver determines how well the dynamic range of a given ADC is utilized. An ADC is supplied with a limited range, e.g. a 16-bit ADC has a dynamic range of  $2^{16}$  components [i.e. the amplitude of the FID can be described by a maximum of  $2^{16}$  different numbers]. The optimal (maximum) dynamic range is obtained when the highest intensity data point of the FID corresponds to one of the highest bits (Figure 10.37 A and B). If the gain is set too low (Figure 10.37 C and D), only a small part of the available dynamic ADC range is used and the FID is described by only a few numbers (bits). Consequently, the accuracy of the digital representation of the FID is not optimal, leading to errors in peak heights and consequently a reduction in S/N. Furthermore, FID components of low

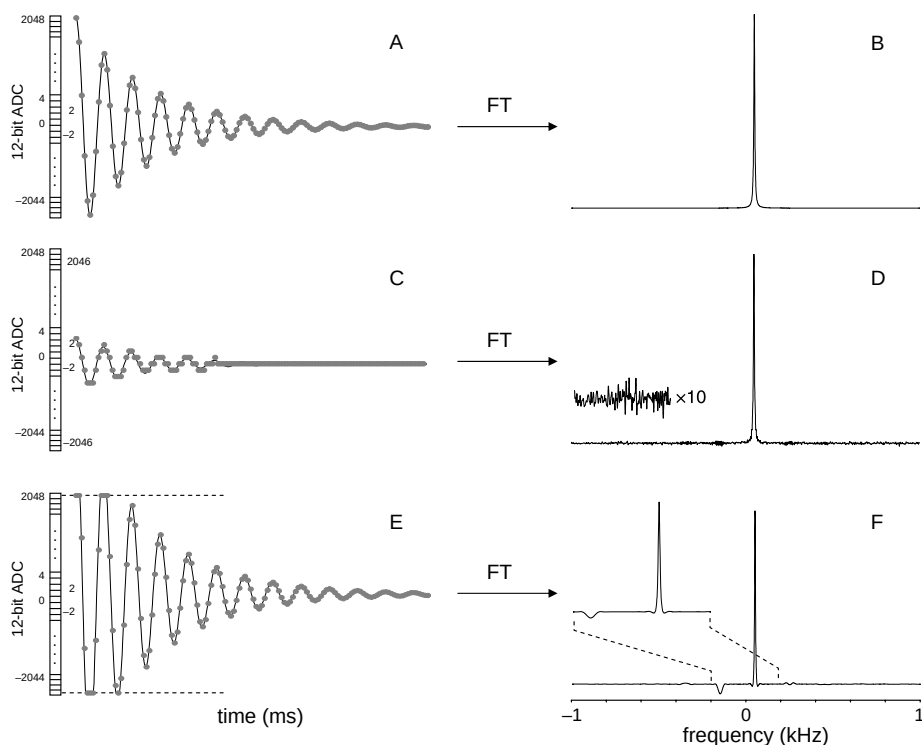


**Figure 10.37** Dynamic range and analog-to-digital conversion. A 12-bit ADC can digitize an analog signal over 4096 numbers. (A, B) Signal detection is optimal when the full ADC range is utilized. (C, D) In the case that only a few bits of the total range are used, the analog NMR signal (C) is not properly digitized resulting in a decrease in the observed S/N (D). (E, F) However, when the analog signal is larger than the largest ADC entry, the analog signal will be truncated (E), thereby leading to artifacts in the NMR spectrum (F).

intensity resonances may not be digitized at all, leading to a loss of information. On the other hand, when the gain is set too high (Figure 10.37 E and F) the ADC is said to be overloaded and the situation becomes even worse. With an ADC overload the strongest signal in the FID is larger than the highest number in the ADC and consequently the first few points of the FID are unreliable. The spectrum obtained after Fourier transformation of the FID shows strong fluctuations in all resonance lines and in the baseline, making for instance quantification of resonances impossible. The setting of the gain is therefore crucial for a proper digital representation of the analog FID after analog-to-digital conversion. A guideline for gain setting is that the highest signal should fill the ADC for 70–80 %. In this way, most resonances are digitized accurately, while maintaining some room for increase in signal intensities (e.g. due to less optimal water suppression) to prevent an ADC overload.

The Nyquist condition stated in Section 1.9.3 is a minimum requirement which also imposes the minimum hardware (i.e. memory) requirements. On many NMR spectrometers, the analog signal may be digitized at sampling rates much higher than the Nyquist frequency. This is referred to as oversampling and is typically performed in order to improve the dynamic range and sensitivity. NMR experiments on aqueous solutions typically encounter a large range of signals; small metabolite signals in the presence of an overwhelming water resonance. The receiver gain is typically adjusted to digitize the largest signal over the full range of ADC bits. However, with typical 12- or 16-bit ADCs the metabolite signals may be incompletely described by the lowest few bits, leading to loss of information and degradation of S/N ratio. While water suppression can minimize this problem for many applications, digital oversampling is a more general approach to increase the effective ADC size. It has been shown [91] that for an oversampling factor of  $n = (F_{\text{oversampling}}/F_{\text{Nyquist}})$  the gain in ADC dynamic range is  $\log_2(n)$ . For example, for an oversampling factor of 8 (i.e. digitize eight times faster than the minimum Nyquist frequency) the gain in ADC dynamic range is 3 bits.

### 10.7.5 Gradient and Shim Systems

The characteristics of magnetic field gradients and the corresponding coils were discussed in detail in Sections 10.4 and 10.5, whereas shimming-related considerations were the topic of Section 10.3. Figure 10.38 gives a schematic overview of the interfacing between gradient- and shim controllers and the currents ultimately produced in the gradient and shim coils.

The gradient controller determines the gradient timings, shapes and relative amplitudes. Due to the generation of eddy currents, the gradient input waveforms are modified in a pre-emphasis step to counteract the eddy currents generated within the magnet. The pre-emphasis unit is typically under computer control and can generate several exponential functions with variable amplitude and time-constants. In addition to pre-emphasis, the gradient waveforms also serve as input to a  $B_0$  compensation unit, which generates exponential modulations of the  $B_0$  field to counteract the  $B_0$  terms generated by eddy currents. See Section 10.4.1 for more information on eddy currents and their compensation. The pre-emphasized gradient waveforms are then amplified and sent to the corresponding gradient coil.

Unlike the gradient system, the shim system is much simpler as the shim controller directly operates on the shim coils. Note, however, that when shims are being used in a pulsed manner, as for example with dynamic shim updating ([15, 24], see also Figure



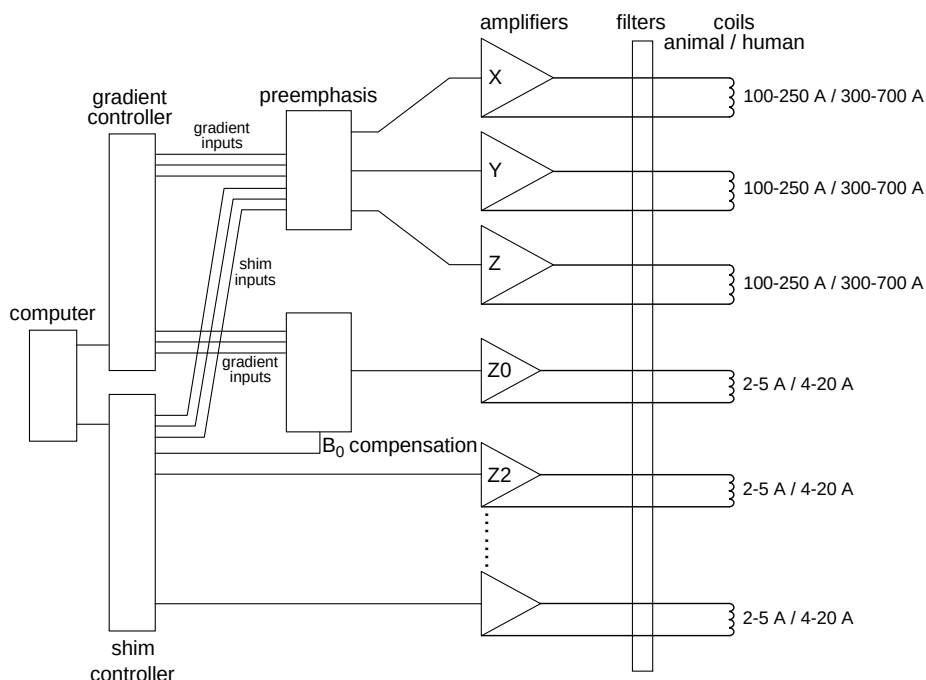


**Figure 10.38** Gradient and shim sub-systems. Most MRI systems are equipped with three linear X, Y, Z gradient coils, and 12 or more higher-order shim coils as well as a constant  $B_0$  coil. Each coil has its own independent amplifier, whereby the linear gradient coil amplifiers are significantly stronger than those for the other coils. Eddy currents generated by pulsed gradient fields require pre-emphasis and  $B_0$  field compensation. Note that shims normally do not require pre-emphasis and will as such be connected directly to the X, Y and Z gradient amplifiers. However, for dynamic shim applications, the linear as well as higher-order shims generate constant ( $B_0$ ) and linear eddy current field that can be corrected by pre-emphasis.

10.14), the shim system has to be extended with shim pre-emphasis,  $B_0$  compensation and possibly shim-to-gradient cross-term pre-emphasis.

## 10.8 Exercises

**10.1** Consider the following shim coil characteristics for a 100 % shim change:

$$\Delta Z^0 = 5000 \text{ Hz};$$

$$\Delta X, \Delta Y \text{ and } \Delta Z = 1000 \text{ Hz cm}^{-1} \text{ and associated } \Delta Z^0 = 20 \text{ Hz};$$

$$\Delta Z^2 = 500 \text{ Hz cm}^{-2} \text{ and associated } \Delta Z^0 = 200 \text{ Hz};$$

$$\Delta ZX = 400 \text{ Hz cm}^{-2} \text{ and associated } \Delta Z^0 = 40 \text{ Hz};$$

$$\Delta Z^3 = 80 \text{ Hz cm}^{-3} \text{ and associated } \Delta Z^0 = 150 \text{ Hz}.$$

**A** In a given spatial volume the required shim fields to counteract the  $B_0$  magnetic field inhomogeneity are  $\Delta Z = 250 \text{ Hz cm}^{-1}$ ,  $\Delta Z^2 = -450 \text{ Hz cm}^{-2}$  and  $\Delta Z^3 = 60 \text{ Hz cm}^{-3}$ . Calculate the required change in  $Z^0$  shim (in %) to maintain the water frequency on-resonance.

- B** Repeat the calculation in the presence of a  $Z^3$ -to- $Z$  coupling, i.e. a change in  $Z^3$  of  $80 \text{ Hz cm}^{-3}$  leads to a  $-40 \text{ Hz cm}^{-1}$  change in  $Z$ .
- C** For a pure  $Z^3$  magnetic field inhomogeneity measured in a 3D voxel located  $(x, y, z) = (+1, -2, +3) \text{ cm}$  calculate the required relative shim changes of all zero through third-order shims.
- 10.2** Calculate the nondegenerate first-through third order shims for infinitesimally thin slices in:
- A** the coronal plane (slice selection in the  $y$  direction);
  - B** the sagittal plane (slice selection in the  $x$  direction).
- 10.3** **A** For a 14 mm diameter surface coil calculate the distance along the coil axis at which the generated  $B_1$  field has dropped to 33 % of the  $B_1$  field at the coil center.
- B** For a given current  $I$ , the 14 mm diameter coil generates a  $B_1$  field at the coil center of 0.58 G. Calculate the required coil diameter to produce the same  $B_1$  field 10 mm away from the coil center, assuming an equal current.
- 10.4** **A** Equation (10.29) is valid for an infinitely long magnetic field gradient, such that eddy currents generated by the rising gradient edge can be neglected. Derive an expression for the  $B_0$  and gradient terms following a magnetic field gradient of finite length  $T$ . Assume instantaneous gradient switching.
- B** A NMR spectroscopist wants to investigate if gradient pre-emphasis and  $B_0$  magnetic field compensation can be eliminated by balancing each magnetic field gradient with an equal gradient of opposite amplitude. Derive an expression for this experiment (i.e. a gradient of amplitude  $+G$  for duration  $T$  followed by a gradient of amplitude  $-G$  for duration  $T$ ).
- C** For a gradient duration of 2 ms, calculate the range of eddy current time constants for which the proposed method gives at least a 10-fold suppression of the temporal  $B_0$  and gradient magnetic fields. Assume a single-exponential eddy current effect. Discuss the applicability of the proposed method.
- D** Following the optimization of pre-emphasis in all three directions, as well as the  $B_0$  compensation, the NMR spectroscopist is ready to perform high-quality MRS studies. However, while the magnetic field homogeneity achieved with room temperature shimming is excellent, the water spectrum obtained with a 3D LASER sequence is still very broad with spectral distortions pointing towards residual eddy currents. Give a likely explanation for this observation and propose a possible solution.
- 10.5** **A** In a vacuum the wavelength at 300 MHz is 100 cm. Calculate the wavelength in the human brain ( $\mu = 4\pi \times 10^{-7} \text{ H}\cdot\text{m}^{-1}$ ,  $\epsilon_r = 50$ ,  $\sigma = 0.66 \text{ S m}^{-1}$ ) at 300 MHz.
- B** Calculate the skin depth in human brain at 300 MHz.
- 10.6** Careless mounting of a second-order shim coil assembly inside a superconducting magnet has resulted in a  $15^\circ$  rotation in the  $x$ - $y$  plane and a 20 mm displacement in the  $z$  direction relative to the magnet isocenter and the first-order gradient coils.
- A** Derive expressions for the spherical harmonic fields generated by the five second-order shim coils in the reference frame of the magnet.
  - B** Magnetic field mapping reveals a magnetic field distribution given by  $+20Z^2 + 33XZ + 10Y$ . Calculate all first- and second order shim corrections necessary to achieve a homogenous magnetic field.

- 10.7 A** A volume coil tested on a phantom of distilled water arrives from the factory. In order to perform experiments on human brain, discuss the need for readjusting the tuning and matching capacitors.
- B** Attaching the coil, loaded with a human head, to a network analyzer reveals that the coil is well-matched and that the circuit resonance frequency appears at 123.4 MHz. For experiments at 3.0 T, discuss the need for readjustment of the tuning capacitor.
- C** A bench measurement of the coil reveals an unloaded Q value of 300. Upon loading the coil with a solid plastic human-sized phantom the loaded Q value drops to 290. Discuss the performance of this coil in light of these results.
- D** Further experiments reveal a loaded Q value of 120 when the a real human head is inserted. Discuss the performance of this coil in light of these additional results.
- E** Another, home-made coil shows an unloaded to loaded Q drop from 500 to 200. Discuss the preferred choice of RF coil.
- 10.8 A** given gradient coil has an inductance of 0.4 mH and is connected to a gradient power supply capable of delivering 350 V and 150 A. The efficiency is  $0.3 \text{ mT m}^{-1} \text{ A}^{-1}$ .
- A** Calculate the gradient ramp time. Note: perform the calculation in SI units.
- B** Calculate the gradient strength.
- C** When the gradient power amplifier is replaced with one capable of delivering 600 V and 200 A, calculate the new gradient ramp times and strengths.
- D** Discuss the advantage of the new amplifier for EPI.
- 10.9** Open MR magnets can be designed in a variety of patient-friendly configurations. By changing the direction of  $B_0$  relative to the head, open MR magnets could potentially reduce susceptibility-induced magnetic field inhomogeneity.
- A** Consider the nasal sinus cavity of a 3 cm diameter sphere located 5 cm below the frontal cortex (as measured from the center of the sphere). Given the susceptibilities of air and water as +0.37 and  $-9.05 \text{ ppm}$ , calculate the magnetic field offset at 7.05 T in the frontal cortex located in the magnetic isocenter for a regular patient orientation (i.e.  $B_0$  is parallel to the foot – head direction).
- B** Calculate the magnetic field offset when  $B_0$  is changed to the ear – ear orientation.
- C** Discuss the considerations involved with this approach to improve magnetic field homogeneity.
- D** It has been suggested that susceptibility-induced magnetic field inhomogeneity can be temporarily reduced by breathing nitrogen gas. Given that the magnetic susceptibility of nitrogen gas is  $-0.0063 \text{ ppm}$ , calculate the improvement in magnetic field homogeneity in the regular patient orientation when breathing nitrogen instead of oxygen.
- A considerable amount of research has been dedicated to the study of breathing-related magnetic field changes in the human brain. Assume that the human lungs can be approximated as a 19 cm diameter sphere filled with 18 %  $\text{O}_2$ /82 %  $\text{N}_2$  during expiration and a 23 cm diameter sphere filled with 22 %  $\text{O}_2$ /78 %  $\text{N}_2$  during inspiration.
- E** Giving the magnetic susceptibility of oxygen gas of +1.8 ppm, calculate the change in magnetic field between inhalation and expiration in a slice through the

human brain, 30 cm above the center of the 'lung sphere'. Ignore gas mixture changes in the nasal cavity.

**F** During a gradient-echo MRI sequence with an echo time TE of 25 ms, calculate the maximum phase difference between subsequent echoes.

**G** Discuss the importance of respiration-induced magnetic field perturbations in the human brain for *in vivo* NMR measurements.

**10.10** During phase-sensitive quadrature detection one channel produces a signal with a relative amplitude and phase of 100 and  $20^\circ$ , whereas the other channel produces a signal with a relative amplitude and phase of 104 and  $110^\circ$ .

**A** Calculate the relative size of the 'ghost' signal that appears at the negative frequency of the main resonance due to the amplitude/gain imbalance.

**B** Calculate the relative size and phase of the 'ghost' signal when the phase of the second channel was  $95^\circ$  instead of  $90^\circ$ . Ignore the receiver gain imbalance.

**C** Discuss the spectral effects when both channels have a constant receiver offset, i.e. a constant amplitude signal is generated even when no NMR signal is acquired.

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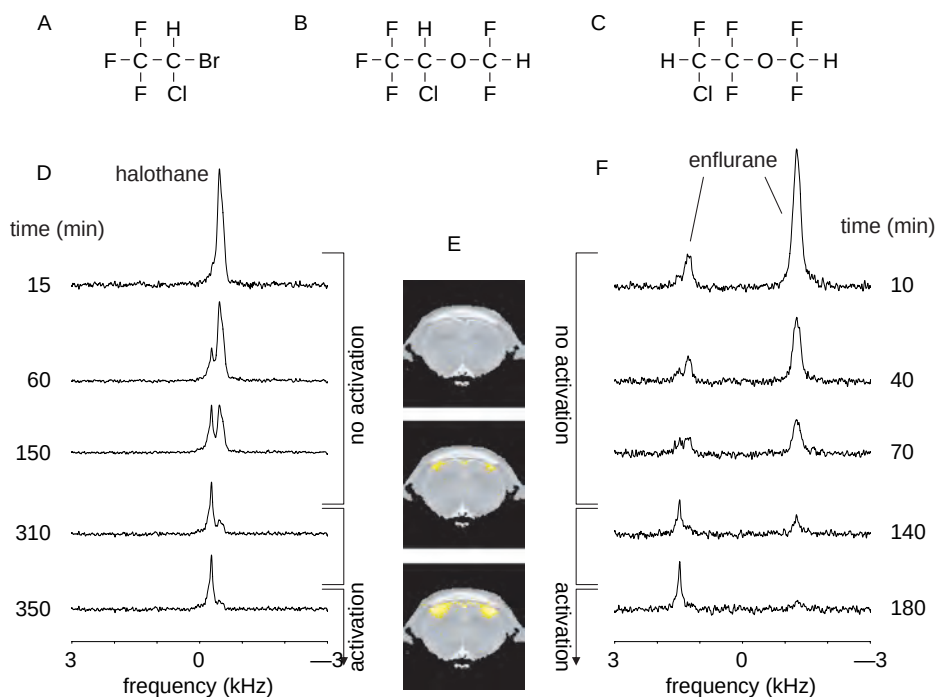


**Plate 1.** Chemical structures of commonly used fluorinated anaesthetics, namely (A) halothane, (B) isoflurane and (C) enflurane. (D) Halothane and (F) enflurane elimination from rat brain following the administration of 1 % of the corresponding anaesthetic for 2 h. Note that both anaesthetics break down over time, presumably into trifluoroacetate and difluoromethoxy-2-difluoroacetic acid for halothane and enflurane, respectively. Further note that the washout of enflurane is considerably faster than for halothane. (E) BOLD fMRI activation maps (yellow) overlaying an anatomical MR image through the somatosensory cortex during double forepaw stimulation. The appearance of BOLD activation is correlated with the washout of the fluorinated anaesthetics. (See figure 2.35)



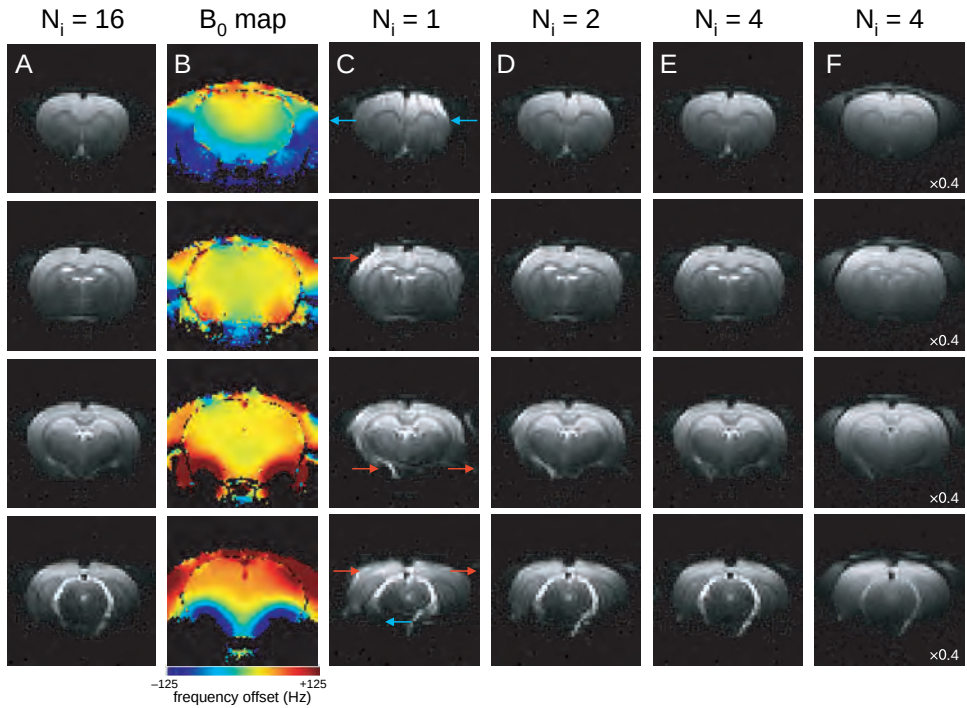


**Plate 2.** Spin-echo echo-planar images acquired from rat brain at 9.4 T with surface coil transmission and reception (coil diameter = 14 mm). All images (16 slices of 1 mm slice thickness) were acquired as a  $64 \times 64$  data matrix over a  $25.6 \times 25.6$  mm FOV, with the phase-encoding gradient blips in the x direction (horizontal) and an imaging bandwidth of 200 kHz. A single, globally optimized shim setting was used for all experiments. (A) Images acquired with 16 interleaves ( $TE = 60$  ms) closely resembling a conventional spin-echo MRI. (B) Magnetic field  $B_0$  map acquired over the same slices, see Section 4.8.3 for more details. (C–E) Echo-planar images acquired with (C) one, (D) two and (E) four interleaves ( $N_i$ ) and  $TE = 60$  ms. The single-shot EPI images in (C) show severe geometric distortion that is directly related to the magnetic field inhomogeneity shown in (B). Red and blue arrows indicate pixel shifts towards the right and left, respectively, and correspond to positive and negative magnetic field offsets. With (D) two and (E) four interleaves, the pixel shifts reduce two- and fourfold, respectively. However, even with four interleaves noticeable image distortion remains. (F) The shorter acquisition time per excitation with four interleaves allows a reduction of echo-time to 35 ms, thereby providing 2.5 times more signal. However, the geometric distortion between (E) and (F) is identical. (See figure 4.13)

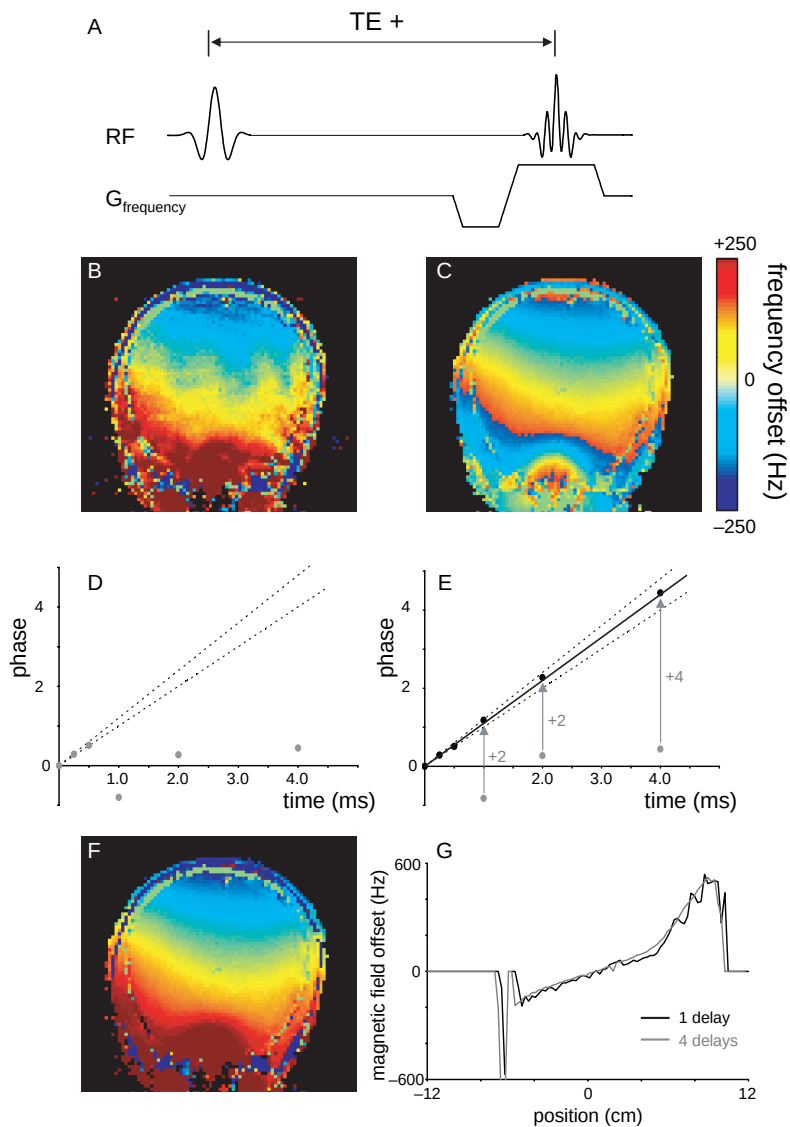


**Plate 3.** Principle of magnetic field  $B_0$  mapping. (A) Basic pulse sequence in which  $\tau$  is a variable delay in addition to the constant echo time  $TE$ . (B) For a short delay  $\tau = 0.33$  ms, no phase wraps occur at the expense of a relatively low SNR. (C) For a long delay  $\tau = 3.0$  ms, the SNR has greatly improved at the expense of phase wrapping artifacts. The advantages of short and long delays can be combined as shown in (D and E) through the procedure of 1D temporal unwrapping. Acquiring several images with increasing delays  $t$  allows the elimination of phase wrapping artifacts even when the majority of images is initially phase wrapped [MR pixels 3, 4 and 5 in (D) are wrapped]. Under the assumption that the first data point ( $\tau > 0$ ) is not wrapped, a linear trend can be established (D). All other data points can then simply be unwrapped by multiples of  $2\pi$  until they fall close to the line established by the previous points (E). Unwrapping followed by linear regression of a three delay dataset ( $\tau = 0.33, 1.0$  and  $3.0$  ms) gives a  $B_0$  map with excellent SNR and no phase wraps (F). (G) Comparison of a vertical trace through the field maps in (B) and (F) demonstrating the SNR advantage of additional longer delays. (See figure 4.17)

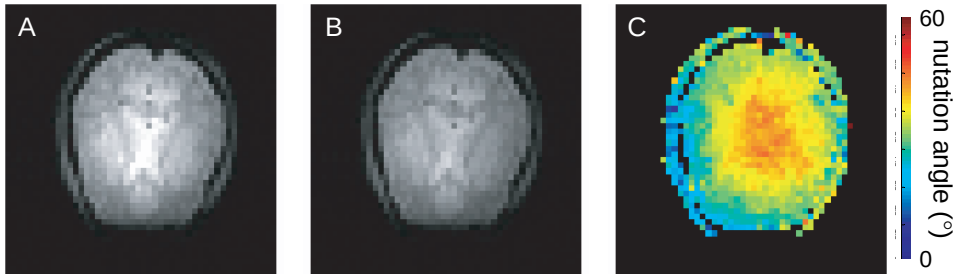


**Plate 4.** Principle of magnetic field  $B_1$  mapping. From the two acquired images (A and B) a nutation angle map (C) can be generated which is directly proportional to the transmit  $B_1$  magnetic field (see Exercise 4.9 for more details). (See figure 4.18)

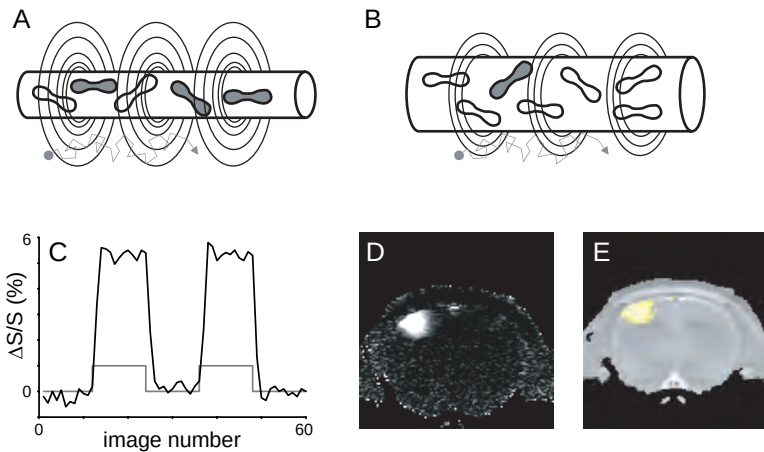


**Plate 5.** Principle of BOLD fMRI. (A) In a 'resting' state the paramagnetic deoxyhemoglobin in red blood cells generates a significant magnetic field disturbance surrounding the cells and blood vessels. Through a number of intra- and extravascular effects this magnetic field inhomogeneity leads to signal loss in  $T_2$ - and  $T_2^*$ -weighted MRI sequences. (B) As a result of the interplay between CBF, CBV and  $CMR_{O_2}$  changes following a stimulus there is a net decrease in the amount of deoxyhemoglobin, and thus less signal loss during a  $T_2^*$ -weighted sequence, giving rise to a positive BOLD effect. (C) BOLD response during single forepaw stimulation in the rat at 9.4 T. Each image was acquired in circa 5 s with  $TE = 15$  ms. A significant signal increase of 5–6% can be observed during the two stimulation blocks (gray line) relative to the baseline state. (D) Difference image, calculated as  $(S_{stimulated} - S_{baseline})/S_{baseline}$ , showing good localization of the forepaw region in the somatosensory cortex. (E) Cross-correlation map overlaid on an anatomical  $T_1$  map. Only pixels with a cross-correlation coefficient of  $>0.8$  are shown. (See figure 4.20)

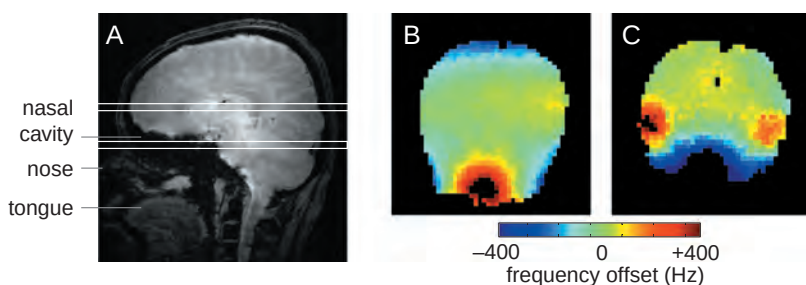


**Plate 6.** (A) Anatomical spin echo MRI of a human head in the sagittal plane. (B, C) Magnetic field  $B_0$  maps acquired at 4 T from the two slices shown in (A) following whole-brain second-order shimming. Significant magnetic field inhomogeneity can be observed (B) above the sinus cavity and (C) around the auditory tracts. (See figure 10.5)

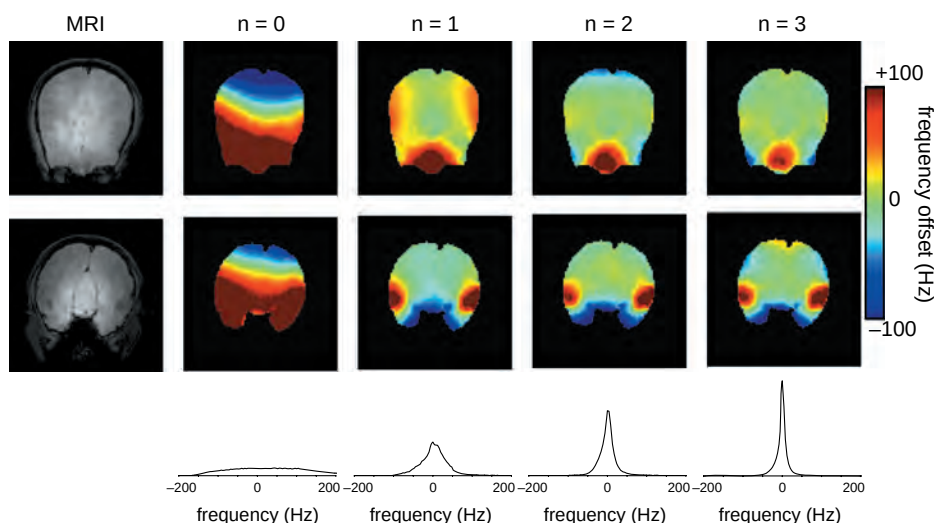


**Plate 7.** Effect of shimming with increasing order of compensation on human brain in vivo at 4.0 T. Without shimming ( $n = 0$ ), The magnetic field inhomogeneity caused by the magnet ( $n = 0$ ) can be largely eliminated by first( $n = 1$ )- and second( $n = 2$ )-order shimming. The residual magnetic field inhomogeneity following second-order shimming is largely caused by air–tissue magnetic susceptibility boundaries in the human head. The strength of third( $n = 3$ )- and higher-order shims available on current MR systems is insufficient to completely homogenize the magnetic field inside the human head. (See figure 10.12)

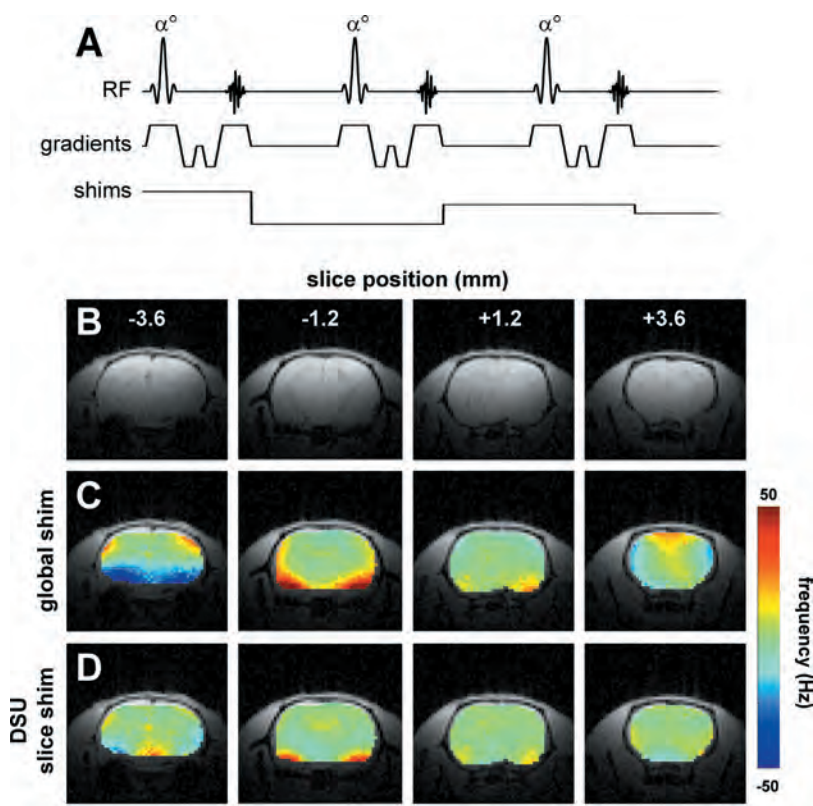


**Plate 8.** (A) Principle of DSU. Instead of a constant, global shim setting, DSU allows dynamic changes in the shim coils to achieve an optimal shim setting for each slice in a multislice MRI protocol. (B–D) Typical DSU results on rat brain at 4.0 T. (B) Anatomical spin-echo MR images and (C) corresponding magnetic field maps using a global shim settings. (D) Magnetic field maps with an optimal shim setting for each slice. Clear improvements can be observed in all slices. (See figure 10.14)

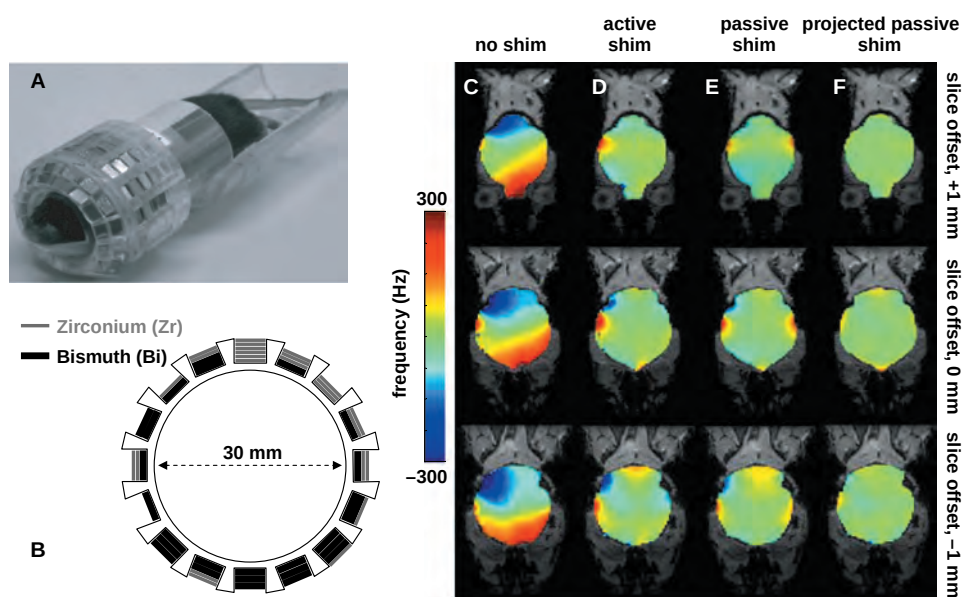


**Plate 9.** Passive shimming on mouse brain at 9.4 T. (A) Photograph of a mouse surrounded by a passive shim assembly. (B) Cross-sectional drawing of one ring through the eight-ring passive shim assembly in which 16 slots are available for the placement of paramagnetic zirconium and diamagnetic bismuth pieces. (C–F) Magnetic field maps acquired (C) without any (passive or active) shim, (D) with optimal first- and second-order active shims and (E) with an optimal passive shim setting. When the diameter of the passive shim assembly shown in (A, B) is reduced by 5 mm, the (simulated) results in (F) can be obtained. (See figure 10.15)

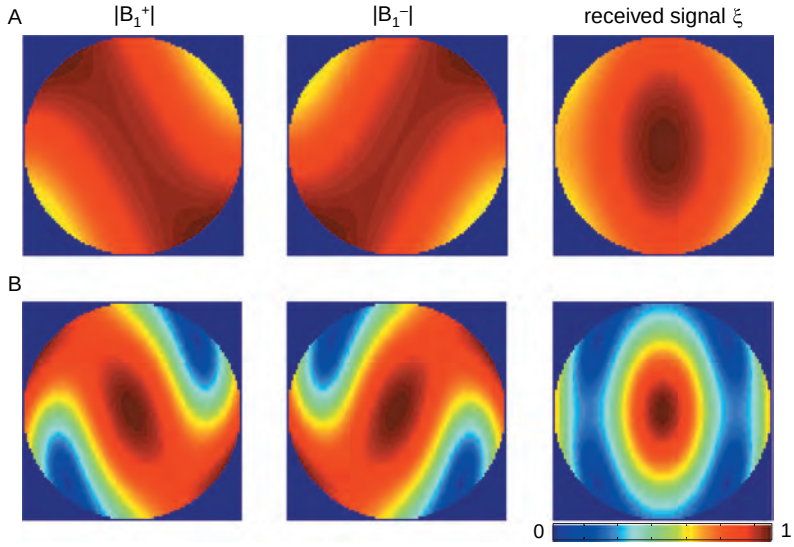


**Plate 10.** Simulations for  $B_1^+$ ,  $B_1^-$  and the received signal  $\xi \propto B_1^+(B_1^-)^*$  of a linear polarized RF coil based on Equations (10.63)–(10.66) for a 10 cm diameter sphere at (A) 3.0 T and (B) 7.0 T with conductivity  $\sigma = 0.66$  and relative permittivity  $\epsilon_r = 51$ . While small differences between  $B_1^+$  and  $B_1^-$  are visible at 3.0 T, they become dominant contributors to the overall image intensity at 7.0 T. In order to adequately characterize the received signal  $\xi$ , separate knowledge of the transmit  $B_1^+$  and receive  $B_1^-$  magnetic fields is required. (See figure 10.31)

# Appendix

## A1 Matrix Calculations

A matrix is a collection of numbers in rows and columns which characterize a set of linear equations. For example, the set of linear equations:

$$\begin{aligned}a_{11}x_1 + a_{12}x_2 + a_{13}x_3 &= b_1 \\a_{21}x_1 + a_{22}x_2 + a_{23}x_3 &= b_2 \\a_{31}x_1 + a_{32}x_2 + a_{33}x_3 &= b_3\end{aligned}\tag{A1.1}$$

is completely characterized by the matrices:

$$\begin{pmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ a_{31} & a_{32} & a_{33} \end{pmatrix}, \begin{pmatrix} x_1 \\ x_2 \\ x_3 \end{pmatrix} \quad \text{and} \quad \begin{pmatrix} b_1 \\ b_2 \\ b_3 \end{pmatrix}\tag{A1.2}$$

The elements in a matrix are normally labeled according to their column and row position. A matrix with  $k$  rows and  $n$  columns is normally referred to as a  $k$  by  $n$  (or  $k \times n$ ) matrix. When  $k = n$  the matrix is referred to as a square matrix of order  $n$ . One of the most common manipulations in matrix algebra is the product (multiplication) of two matrices. This is computed as follows:

$$\begin{pmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{pmatrix} \begin{pmatrix} b_{11} & b_{12} \\ b_{21} & b_{22} \end{pmatrix} = \begin{pmatrix} a_{11}b_{11} + a_{12}b_{21} & a_{11}b_{12} + a_{12}b_{22} \\ a_{21}b_{11} + a_{22}b_{21} & a_{21}b_{12} + a_{22}b_{22} \end{pmatrix}\tag{A1.3}$$

In general the product of a  $k_1 \times n_1$  and a  $k_2 \times n_2$  matrix is a  $k_1 \times n_2$  matrix. Note that  $n_1$  should always equal  $k_2$ . A diagonal matrix is a square matrix in which all off-diagonal elements (i.e. elements  $a_{ij}$  for which  $i \neq j$ ) are zero and at least one of the diagonal elements (i.e. elements  $a_{ij}$  for which  $i = j$ ) is nonzero. A diagonal matrix in which all diagonal elements are equal to one is called a unity matrix **I**. For example, a third-order



unity matrix is:

$$\mathbf{I} = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix} \quad (\text{A1.4})$$

The product of a unity matrix  $\mathbf{I}$  with another matrix  $\mathbf{A}$  does not have any net effect, i.e.  $\mathbf{I} \cdot \mathbf{A} = \mathbf{A}$ . Multiplication of  $\mathbf{A}$  with the inverse matrix  $\mathbf{A}^{-1}$  gives a unity matrix, i.e.  $\mathbf{A} \times \mathbf{A}^{-1} = \mathbf{I}$ .

To simplify calculations for particular NMR applications, it is often convenient to write the phenomenon under investigation in matrix form. As an example, consider the rotation of transverse magnetization about the z axis. The rotations are completely described by:

$$\begin{aligned} M_x &\rightarrow M_x \cos \theta + M_y \sin \theta \\ M_y &\rightarrow -M_x \sin \theta + M_y \cos \theta \\ M_z &\rightarrow M_z \end{aligned} \quad (\text{A1.5})$$

The set of linear equations of Equation (A1.5) can be expressed in matrix form as:

$$\begin{pmatrix} M_x \\ M_y \\ M_z \end{pmatrix} = \begin{pmatrix} \cos \theta & \sin \theta & 0 \\ -\sin \theta & \cos \theta & 0 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} M_x \\ M_y \\ M_z \end{pmatrix} \quad (\text{A1.6})$$

or

$$\mathbf{M} = \mathbf{R}_z(\theta)\mathbf{M} \quad (\text{A1.7})$$

where  $\mathbf{R}_z(\theta)$  is the rotation matrix for a rotation about the z axis through an angle  $\theta$  (see also Chapter 5). For some applications, like RF pulse simulation or quantitative description of diffusion, one would like to describe the rotations of  $\mathbf{M}$  in a frame  $\mathbf{A}'$  which is rotated with respect to the original frame  $\mathbf{A}$ . This can be achieved if the rotation matrix  $\mathbf{R}$  connecting both frames is known according to:

$$\mathbf{A}' = \mathbf{R}^{-1} \cdot \mathbf{A} \cdot \mathbf{R} \quad (\text{A1.8})$$

For other applications one is not interested in the rotations in either frame, but would like to obtain a parameter independent of the frame orientations. One parameter (among many others) that is rotationally invariant is the trace of the matrix and is defined as:

$$\text{Tr}(\mathbf{A}) = \text{Tr} \begin{pmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ a_{31} & a_{32} & a_{33} \end{pmatrix} = a_{11} + a_{22} + a_{33} \quad (\text{A1.9})$$

One can verify that the trace of a matrix is rotationally invariant, i.e.:

$$\text{Tr}(\mathbf{A}') = \text{Tr}(\mathbf{R}^{-1} \cdot \mathbf{A} \cdot \mathbf{R}) = \text{Tr}(\mathbf{A}) \quad (\text{A1.10})$$

Another feature encountered in matrix algebra that is frequently applied in NMR applications is transposition. The transpose matrix  $\mathbf{A}^T$  of a  $(k \times n)$  matrix  $\mathbf{A}$  is the  $(n \times k)$  matrix

in which the rows and columns of **A** have been interchanged, i.e.:

$$\mathbf{A}^T = \begin{pmatrix} a_{11} & a_{21} \\ a_{12} & a_{22} \\ a_{13} & a_{23} \end{pmatrix} \quad \text{when} \quad \mathbf{A} = \begin{pmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \end{pmatrix}$$

Besides the above cited characteristics of matrices, a wealth of other properties and rules exist for which the reader is referred to standard textbooks on mathematics.

## A2 Trigonometric Equations

In Fourier transform NMR many calculations and manipulations involve sums and products of sines and cosines and complex combinations thereof. Therefore, some of the most important trigonometric equations will be summarized here. The sums and differences of (co)sines are given by:

$$\sin(a + b) = \sin a \cos b + \cos a \sin b \quad (\text{A2.1})$$

$$\sin(a - b) = \sin a \cos b - \cos a \sin b \quad (\text{A2.2})$$

$$\cos(a + b) = \cos a \cos b - \sin a \sin b \quad (\text{A2.3})$$

$$\cos(a - b) = \cos a \cos b + \sin a \sin b \quad (\text{A2.4})$$

Other trigonometric equations which are frequently used are:

$$\sin^2 a + \cos^2 a = 1 \quad (\text{A2.5})$$

$$\cos^2 a - \sin^2 a = \cos 2a \quad (\text{A2.6})$$

$$2 \sin a \cos a = \sin 2a \quad (\text{A2.7})$$

$$\sin a = -\sin(-a) \quad (\text{A2.8})$$

$$\cos a = \cos(-a) \quad (\text{A2.9})$$

When complex notation is involved, the Euler relations are invaluable and are given by:

$$e^{+ia} = \cos a + i \sin a \quad (\text{A2.10})$$

$$e^{-ia} = \cos a - i \sin a \quad (\text{A2.11})$$

Many other useful relations can be derived from Equations (A2.1)–(A2.11). For a more complex overview of trigonometry the reader is referred to standard textbooks on mathematics.

## A3 Fourier Transformation

The Fourier transformation (FT) is at the heart of modern NMR. A thorough understanding of its characteristics and definitions is essential to describe the actions of many tools and techniques encountered in NMR, like data processing, RF pulses and MRI. Here some of the basic definitions concerning FT are given and how they relate to NMR. For more thorough discussions the reader is referred to the literature [1].

### A3.1 Introduction

Any periodic function  $f(t)$  can be decomposed into an infinite harmonic series according to:

$$f(t) = A_0 + \sum_{n=1}^{\infty} \left[ A_n \cos\left(\frac{2\pi nt}{T}\right) + B_n \sin\left(\frac{2\pi nt}{T}\right) \right] \quad (\text{A3.1})$$

Where  $T$  is the period and the Fourier components  $A_n$  and  $B_n$  are given by:

$$A_n = \frac{1}{T} \int_{-T/2}^{T/2} f(t) \cos\left(\frac{2\pi nt}{T}\right) dt \quad (\text{A3.2})$$

$$B_n = \frac{1}{T} \int_{-T/2}^{T/2} f(t) \sin\left(\frac{2\pi nt}{T}\right) dt \quad (\text{A3.3})$$

This decomposition is called a Fourier series. The frequency components  $\omega_n = 2\pi n/T$  and amplitude  $A_n$  and  $B_n$  can be retrieved from an unknown signal  $f(t)$  by performing a FT of signal  $f(t)$  according to:

$$F(\omega) = \int_{-\infty}^{+\infty} f(t) e^{-i\omega t} dt \quad (\text{A3.4})$$

The inverse FT is defined as:

$$f(t) = \frac{1}{2\pi} \int_{-\infty}^{+\infty} F(\omega) e^{+i\omega t} d\omega \quad (\text{A3.5})$$

The interpretation of Equations (A3.4) and (A3.5) is straightforward. The time domain function  $f(t)$  is a linear combination of orthonormal basis functions (i.e. sines and cosines)  $e^{-i\omega t} = \cos\omega t - i\sin\omega t$ . Each basis function denotes a circularly polarized oscillation (frequency). Corresponding to each frequency is an amplitude  $F(\omega)$ , which relates how much the component  $e^{-i\omega t}$  contributes to  $f(t)$ . FT of signal  $f(t)$  is therefore a decomposition of  $f(t)$  into its frequency components and their corresponding amplitudes.

A more practical, alternative way of looking at Equations (A3.4) and (A3.5) is the following. In mathematical terms, Equation (A3.4) expresses that the FID,  $f(t)$ , should be multiplied by a monochromatic reference frequency  $\omega$  and then integrated over the entire (infinite) time-domain. Only frequency components at or near the reference frequency give a finite integral, while the rest gets quickly out of phase with the reference signal giving a (near) zero integral. The procedure is repeated for different values of the reference frequency until all appropriate frequencies (i.e. the entire spectral bandwidth) have been explored.

### A3.2 Properties

Below several useful properties of the FT are summarized.

*Linearity.* For any integrable functions  $f(t)$  and  $g(t)$  and for any constant  $a$ , the FT is a linear operation:

$$\text{FT}(f(t) + g(t)) = \text{FT}(f(t)) + \text{FT}(g(t)) \quad (\text{A3.6})$$

$$\text{FT}(af(t)) = a \text{FT}(f(t)) \quad (\text{A3.7})$$

The same holds true for the integrable functions  $F(\omega)$  and  $G(\omega)$ .

*Time and frequency shifting.* The frequency-domain signal  $F(\omega)$  can be shifted to  $F(\omega - \omega_0)$  by applying a phase shift to the time-domain signal  $f(t)$  according to:

$$\text{FT}(f(t)e^{-i\omega_0 t}) = F(\omega - \omega_0) \quad (\text{A3.8})$$

A time domain signal  $f(t)$  can be shifted in an analogous manner:

$$\text{FT}(F(\omega)e^{+i\omega t_0}) = f(t - t_0) \quad (\text{A3.9})$$

The time and frequency properties have found widespread applications in NMR. In MRI, Equations (A3.8) and (A3.9) are regularly used to shift the object under investigation. In MRS, time-frequency shifting is often employed with RF pulses and localization. Adding a phase-ramp to a RF pulse shifts the frequency excitation profile without the need for frequency switching.

*Scaling.* When a time-domain function  $f(t)$  is scaled to  $f(at)$  then its Fourier transformation is given by:

$$\text{FT}(f(at)) = \frac{1}{|a|} F\left(\frac{\omega}{a}\right) \quad (\text{A3.10})$$

This phenomenon is also observed in many parts of *in vivo* NMR. For example, reducing the pulse length of a selective pulse by a factor of two ( $a = 1/2$ ) increases the excitation bandwidth  $F(\omega/a)$  by a factor two and doubles the required RF amplitude (proportional to  $|a|^{-1}$ ).

*Convolution.* The convolution  $h(t)$  of two functions  $f(t)$  and  $g(t)$  is a broadening of one function by the other. When convolution is used for filtering, one function  $g(t)$  is called the weighting function, which is convolved with the original data  $f(t)$  to give the filtered data  $h(t)$ . Mathematically this can be described as:

$$h(t) = f(t) * g(t) = \int_{-\infty}^{+\infty} f(\tau) \cdot g(t - \tau) d\tau \quad (\text{A3.11})$$

Convolutions are commutative, associative and distributive. In combination with the FT convolution leads to the following results:

$$\text{FT}(f(t) * g(t)) = \text{FT}(f(t)) \cdot \text{FT}(g(t)) \quad (\text{A3.12})$$

$$\text{FT}(f(t) \cdot g(t)) = \text{FT}(f(t)) * \text{FT}(g(t)) \quad (\text{A3.13})$$

Identical equations can be derived for an inverse FT. Equations (A3.12) and (A3.13) reveal that a convolution in one domain is a simple multiplication in the other domain. One of

the most commonly used convolutions is apodization of the FID signal as described in Chapter 1. To make a relative enhancement of the signal in the beginning of the FID over the noisy end, the original FID can be multiplied by an exponentially decaying function. This results in a convolution of the two functions in the frequency-domain, i.e. a broadening of resonances.

### A3.3 Discrete Fourier Transformation

All modern NMR experiments are performed with the aid of computers, which require that the FID signal is sampled (digitized) at discrete intervals. This consequently makes the FT also a discrete process in which the continuous integration of Equation (A3.4) is replaced by a discrete summation. The discrete Fourier transformation (DFT) of a discretely sampled time-domain signal  $f(t)$  is then given by:

$$F(\omega) = \sum_{j=0}^{N-1} f(t_j) \cdot e^{-i(\frac{\omega}{N})t_j} \quad (\text{A3.14})$$

The computation of Equation (A3.14) can be performed in an analogous manner to that described for the continuous FT. For simple 1D spectra this is an acceptable task, but for larger 1D datasets and all 2D and 3D MRI and MRS datasets, this would lead to unacceptable calculation times. By using symmetry and recursive characteristics of DFT, Cooley and Tukey proposed in 1965 [2] a new algorithm for the special case of  $N$  being a power of 2. This fast Fourier transform (FFT) algorithm requires  $N \log_2 N$  calculations on a dataset containing  $N$  data points, whereas the conventional DFT of Equation (A3.14) requires  $N^2$  calculations. For  $N = 256$  this already leads to a reduction in calculation time of 32, while for a  $256 \times 256$  2D dataset the efficiency is 1024 higher. Modern algorithms can also give substantial improvements for powers other than 2, although data acquired as a power of 2 remain the most efficient.

### A4 Product Operator Formalism

As has been demonstrated throughout the book, the use of classical vectors to describe NMR phenomena is a valuable method to visualize certain NMR experiments. However, a large number of NMR experiments, particularly those involving 2D techniques, polarization transfer and multiple quantum coherences, can not be adequately described by the classical vector model.

A variety of quantum mechanical methods exists which are capable of a quantitative description of all NMR phenomena. In particular, the density matrix formalism is universally applicable. In the density matrix formalism one is not directly concerned with magnetization, but rather with the energy states of the spin system under investigation. Therefore, a density matrix holds all possible energy states of a given spin system. Just as one is interested in the time evolution of magnetization during an NMR experiment, the time evolution of the density matrix needs to be calculated, which is governed by the Liouville–von Neumann equation. It is well outside the scope of the book to give a detailed treatment of density matrix calculations. For further reading, the reader is referred to the literature [3–10].

Because the density matrix formalism is concerned with energy states, it does not provide any intuitive insight to the NMR experiment during the calculation. Furthermore, for a spin system of more than a few spins, density matrix calculations become rapidly cumbersome. In certain cases, for instance in the case of non or weakly coupled spin systems, it is possible to simplify the density matrix formalism by expanding the density matrix into a linear combination of orthogonal matrices (or product operators) [11–13], each of which represents an orthogonal component of the magnetization (some of which are not directly observable as will be explained below). Orthogonality is defined using the trace relation, i.e. the trace of the product of two orthogonal matrices is zero. Various complete orthogonal basis matrix sets can be used, including single-transition operators, spherical tensor operators and product operators. The last two basis sets are most straightforward to use and will be discussed in more detail.

### A4.1 Cartesian Product Operators

The complete basis set for  $N$  spins  $\frac{1}{2}$  consists of  $2^{2N}$  product operators. For a two-spin system  $IS$ , the 16 product operators are (besides the unity operator)  $I_x, I_y, I_z, S_x, S_y, S_z, 2I_xS_x, 2I_xS_y, 2I_xS_z, 2I_yS_x, 2I_yS_y, 2I_yS_z, 2I_zS_x, 2I_zS_y$  and  $2I_zS_z$ . Each of these product operators corresponds to a particular physical state, according to:

|                    |                                                                         |
|--------------------|-------------------------------------------------------------------------|
| $I_z$              | Polarization of spin $I$ (longitudinal magnetization)                   |
| $I_x, I_y$         | In-phase $x$ and $y$ coherence of spin $I$ (transverse magnetization)   |
| $2I_xS_z, 2I_yS_z$ | $x$ and $y$ coherence of spin $I$ in antiphase with respect to spin $S$ |
| $2I_xS_x, 2I_xS_y$ |                                                                         |
| $2I_yS_x, 2I_yS_y$ | Two-spin coherence of spins $I$ and $S$                                 |
| $2I_zS_z$          | Longitudinal two-spin order of spins $I$ and $S$                        |

Using the product operator formalism [11–17], the rotation of magnetization due to a RF pulse applied along the  $x$  axis can be described as:

$$I_x \xrightarrow{\theta I_x} I_x \quad (\text{A4.1})$$

$$I_y \xrightarrow{\theta I_x} I_y \cos \theta + I_z \sin \theta \quad (\text{A4.2})$$

$$I_z \xrightarrow{\theta I_x} I_z \cos \theta - I_y \sin \theta \quad (\text{A4.3})$$

where  $\theta$  is the nutation angle. In an equivalent manner, the rotation about the  $y$  axis can be described by:

$$I_x \xrightarrow{\theta I_y} I_x \cos \theta - I_z \sin \theta \quad (\text{A4.4})$$

$$I_y \xrightarrow{\theta I_y} I_y \quad (\text{A4.5})$$

$$I_z \xrightarrow{\theta I_y} I_z \cos \theta + I_x \sin \theta \quad (\text{A4.6})$$

The effects of chemical shift evolution can be described by a rotation about the  $z$  axis over an angle  $\omega t$ :

$$I_x \xrightarrow{\omega t I_z} I_x \cos \omega t + I_y \sin \omega t \quad (\text{A4.7})$$

$$I_y \xrightarrow{\omega t I_z} I_y \cos \omega t - I_x \sin \omega t \quad (\text{A4.8})$$

$$I_z \xrightarrow{\omega t I_z} I_z \quad (\text{A4.9})$$

So far the transformations given by Equations (A4.1)–(A4.9) are similar to the rotation matrices given by Equations (5.5)–(5.7) for regular (nonscalar-coupled) magnetization. One of the most powerful characteristics of the product operator formalism is that it remains intuitive even for scalar-coupled spin systems. For a weakly coupled two-spin system IS, the evolution of coherence due to scalar coupling is given by:

$$I_x \xrightarrow{\pi J t 2 I_z S_z} I_x \cos \pi J t + 2 I_y S_z \sin \pi J t \quad (\text{A4.10})$$

$$I_y \xrightarrow{\pi J t 2 I_z S_z} I_y \cos \pi J t - 2 I_x S_z \sin \pi J t \quad (\text{A4.11})$$

$$I_z \xrightarrow{\pi J t 2 I_z S_z} I_z \quad (\text{A4.12})$$

Where  $J$  is the coupling constant between  $I$  and  $S$ . The evolution of anti-phase coherence due to  $J$  coupling can easily be derived from Equations (A4.10)–(A4.12), using the relation  $4I_z^2 = 4S_z^2 = 1$ , and is given by:

$$2 I_x S_z \xrightarrow{\pi J t 2 I_z S_z} 2 I_x S_z \cos \pi J t + I_y \sin \pi J t \quad (\text{A4.13})$$

$$2 I_y S_z \xrightarrow{\pi J t 2 I_z S_z} 2 I_y S_z \cos \pi J t - I_x \sin \pi J t \quad (\text{A4.14})$$

Up to this point, the product operator formalism has (superficially) not been more than an alternative to the classical vector model. However, consider a scalar-coupled two-spin system IS for which a pure antiphase coherence state  $2 I_x S_z$  has been created by a spin-echo sequence with  $t = TE = 1/(2J)$ . When at this point a nonselective  $90^\circ_x$  pulse is executed, a spin state described by  $-2 I_x S_y$  is created which can not be described by the classical vector model. The product operator formalism offer a convenient method to deal with these so-called multi quantum coherences, as will be described next.

## A4.2 Spherical Tensor Product Operators

Because terms such as  $2 I_x S_x$ ,  $2 I_x S_y$ ,  $2 I_y S_x$  and  $2 I_y S_y$  contain a combination of zero and double quantum coherences, that behave differently to external perturbations, it is convenient to replace the product operators by raising and lowering operators given by:

$$I^+ = I_x + i I_y \quad (\text{A4.15})$$

$$I^- = I_x - i I_y \quad (\text{A4.16})$$

$$I_0 = I_z \quad (\text{A4.17})$$

Making

$$I_x = \frac{1}{2}(I^+ + I^-) \quad (\text{A4.18})$$

$$I_y = -\frac{i}{2}(I^+ - I^-) \quad (\text{A4.19})$$

Alternatively, so-called spherical tensor operators can be used, which are directly proportional to the raising and lowering operators. Using raising and lowering operators one can easily keep track of the coherence order. Single quantum coherences (i.e. transverse magnetization) are those spin states with only one raising or lowering operator (e.g.  $I^+$ ,  $2I^-S_0$ ), zero quantum coherences are spin states in which the net quantum number is zero (i.e.  $I^+S^-$ ), while for double quantum coherences the spin quantum number adds to two (e.g.  $I^+S^+$ ). In this formalism, chemical shift evolution takes a simpler form:

$$I^+ \xrightarrow{\omega t I_z} I^+ e^{-i\omega t} \quad (\text{A4.20})$$

$$I^- \xrightarrow{\omega t I_z} I^- e^{+i\omega t} \quad (\text{A4.21})$$

$$I_0 \xrightarrow{\omega t I_z} I_0 \quad (\text{A4.22})$$

The effect of scalar evolution is described by:

$$I^+ \xrightarrow{\pi J t 2 I_z S_z} I^+ \cos \pi J t - 2i I^+ S_0 \sin \pi J t \quad (\text{A4.23})$$

$$I^- \xrightarrow{\pi J t 2 I_z S_z} I^- \cos \pi J t + 2i I^- S_0 \sin \pi J t \quad (\text{A4.24})$$

$$I_0 \xrightarrow{\pi J t 2 I_z S_z} I_0 \quad (\text{A4.25})$$

and

$$2I^+ S_0 \xrightarrow{\pi J t 2 I_z S_z} 2I^+ S_0 \cos \pi J t - i I^+ \sin \pi J t \quad (\text{A4.26})$$

$$2I^- S_0 \xrightarrow{\pi J t 2 I_z S_z} 2I^- S_0 \cos \pi J t + i I^- \sin \pi J t \quad (\text{A4.27})$$

The effect of a RF pulse with phase  $\phi$  (where 0 corresponds to the +x axis,  $\pi/2$  to the +y axis and so on) generating a nutation angle  $\theta$  is governed by:

$$I^+ \longrightarrow \frac{I^+}{2} (\cos \theta + 1) - \frac{I^-}{2} (\cos \theta - 1) e^{-2i\phi} + i I_0 \sin \theta e^{-i\phi} \quad (\text{A4.28})$$

$$I^- \longrightarrow \frac{I^-}{2} (\cos \theta + 1) - \frac{I^+}{2} (\cos \theta - 1) e^{-2i\phi} - i I_0 \sin \theta e^{+i\phi} \quad (\text{A4.29})$$

$$I_0 \longrightarrow \frac{i I^+}{2} \sin \theta e^{-i\phi} - \frac{i I^-}{2} \sin \theta e^{+i\phi} + I_0 \cos \theta \quad (\text{A4.30})$$



Up to this point only single quantum coherences ( $p = \pm 1$ ) have been discussed. However, the product operator formalism is particularly valuable when higher-order coherences are involved. The two-spin operators  $2I_xS_x$ ,  $2I_yS_y$ ,  $2I_xS_y$  and  $2I_yS_x$  can be written in terms of raising and lowering operators according to:

$$2I_xS_x = \frac{1}{2}(I^+S^+ + I^+S^- + I^-S^+ + I^-S^-) \quad (\text{A4.31})$$

$$2I_yS_y = -\frac{1}{2}(I^+S^+ - I^+S^- - I^-S^+ + I^-S^-) \quad (\text{A4.32})$$

$$2I_xS_y = -\frac{i}{2}(I^+S^+ - I^+S^- + I^-S^+ - I^-S^-) \quad (\text{A4.33})$$

$$2I_yS_x = -\frac{i}{2}(I^+S^+ + I^+S^- - I^-S^+ - I^-S^-) \quad (\text{A4.34})$$

Clearly all four two-spin terms contain both double ( $I^+S^+$  and  $I^-S^-$ ) and zero ( $I^+S^-$  and  $I^-S^+$ ) quantum coherences. Linear combinations of two-spin terms can reveal pure double and zero quantum coherences according to:

$$\frac{1}{2}(I^+S^+ + I^-S^-) = \frac{1}{2}(2I_xS_x - 2I_yS_y) = \text{DQC}_x \quad (\text{A4.35})$$

$$-\frac{i}{2}(I^+S^+ - I^-S^-) = \frac{1}{2}(2I_xS_y + 2I_yS_x) = \text{DQC}_y \quad (\text{A4.36})$$

$$\frac{1}{2}(I^+S^- + I^-S^+) = \frac{1}{2}(2I_xS_x + 2I_yS_y) = \text{ZQC}_x \quad (\text{A4.37})$$

$$-\frac{i}{2}(I^+S^- - I^-S^+) = \frac{1}{2}(2I_yS_x - 2I_xS_y) = \text{ZQC}_y \quad (\text{A4.38})$$

From Equations (A4.35)–(A4.38) it can be seen that selective inversion of one spin operator (e.g. I) results in the interconversion of double and zero quantum coherences. This is an important aspect in spectral editing using multiple quantum coherences. Double quantum coherences between spins I and S evolve in the transverse plane under the sum of the chemical shifts ( $\omega_I + \omega_S$ ):

$$\text{DQC}_x \xrightarrow{\omega_I t I_z + \omega_S t S_z} \text{DQC}_x \cos[(\omega_I + \omega_S)t] + \text{DQC}_y \sin[(\omega_I + \omega_S)t] \quad (\text{A4.39})$$

$$\text{DQC}_y \xrightarrow{\omega_I t I_z + \omega_S t S_z} \text{DQC}_y \cos[(\omega_I + \omega_S)t] - \text{DQC}_x \sin[(\omega_I + \omega_S)t] \quad (\text{A4.40})$$

Using Equations (A4.39) and (A4.40) it can be shown that double quantum coherences have twice the sensitivity to magnetic field gradients as compared with regular transverse

magnetization (i.e. single quantum coherences). Zero quantum coherences evolve under the difference of the chemical shift ( $\omega_I - \omega_S$ ):

$$ZQC_x \xrightarrow{\omega_I t_z + \omega_S t_{S_z}} ZQC_x \cos[(\omega_I - \omega_S)t] + ZQC_y \sin[(\omega_I - \omega_S)t] \quad (A4.41)$$

$$ZQC_y \xrightarrow{\omega_I t_z + \omega_S t_{S_z}} ZQC_y \cos[(\omega_I - \omega_S)t] - ZQC_x \sin[(\omega_I - \omega_S)t] \quad (A4.42)$$

Such that zero quantum coherences do not evolve under magnetic field gradients (i.e. ZQCs are *not* dephased by magnetic field gradients). A final aspect of multiple quantum coherence that needs to be taken into account when performing product operator calculations is the evolution of multiple quantum coherences under coupling with active and passive spins. For example, consider  $[3\text{-}^{13}\text{C}]\text{lactate}$  with heteronuclear scalar coupling between carbon-13 and the methyl protons, as well as homonuclear scalar coupling between the methyl and methane protons. Further consider a heteronuclear multiple quantum editing sequence that utilizes the heteronuclear multiple quantum coherences between  $[3\text{-}^{13}\text{C}]$  and  $[3\text{-}^1\text{H}]$ . These two nuclei are actively involved in the multiple quantum coherence transition and the evolution of multiple quantum coherences is not influenced by active spins. The homonuclear proton–proton couplings are not actively involved in the multiple quantum coherence transition and are therefore referred to as passive spins. Passive spins lead to an evolution of multiple quantum coherences according to:

$$ZQC_x^{IS} \xrightarrow{\pi J_{IL} t_{L_z} L_z + \pi J_{LS} t_{L_z} S_z} ZQC_x^{IS} \cos \pi K_{IS} t + 2ZQC_y^{IS} L_z \sin \pi K_{IS} t \quad (A4.43)$$

where  $K_{IS} = |J_{LS} - J_{IL}|$ , which is known as the zero-quantum splitting.

With the rules presented in this section for the evolution of coherences under chemical shifts, magnetic field gradients, scalar coupling and during RF pulses, the theoretical outcome of any NMR pulse sequence can be quantitatively calculated (ignoring relaxation and other physical processes).

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