

Contents

Preface XV

- 1 Introduction to NMR Spectroscopy 1**
- 1.1 Our First 1D Spectrum 1
- 1.2 Some Nomenclature: Chemical Shifts, Line Widths, and Scalar Couplings 2
- 1.3 Interpretation of Spectra: A Simple Example 5
- 1.4 Two-Dimensional NMR Spectroscopy: An Introduction 9

Part One Basics of Solution NMR 11

- 2 Basics of 1D NMR Spectroscopy 13**
- 2.1 The Principles of NMR Spectroscopy 13
- 2.2 The Chemical Shift 16
- 2.3 Scalar Couplings 17
- 2.4 Relaxation and the Nuclear Overhauser Effect 20
- 2.5 Practical Aspects 23
 - 2.5.1 Sample Preparation 23
 - 2.5.2 Referencing 25
 - 2.5.3 Sensitivity and Accumulation of Spectra 27
 - 2.5.4 Temperature Calibration 29
- 2.6 Problems 30
 - Further Reading 31

- 3 ^1H NMR 33**
- 3.1 General Aspects 33
- 3.2 Chemical Shifts 34
 - 3.2.1 Influence of Electronegativity of Substituents 35
 - 3.2.2 Anisotropy Effects 35
 - 3.2.3 Other Factors Affecting Chemical Shifts: Solvent, Temperature, pH, and Hydrogen Bonding 37

3.2.4	Shift Reagents	37
3.3	Spin Systems, Symmetry, and Chemical or Magnetic Equivalence	39
3.3.1	Homotopic, Enantiotopic, and Diastereotopic Protons	42
3.3.2	Determination of Enantiomeric Purity	43
3.4	Scalar Coupling	44
3.4.1	First-Order Spectra	45
3.4.2	Higher-Order Spectra and Chemical Shift Separation	47
3.4.3	Higher-Order Spectra and Magnetic Equivalence	49
3.5	^1H - ^1H Coupling Constants	50
3.5.1	Geminal Couplings	50
3.5.2	Vicinal Couplings	50
3.5.3	Long-Range Couplings	52
3.5.4	^1H Couplings to Other Nuclei	52
3.6	Problems	54
	Further Reading	55
4	NMR of ^{13}C and Heteronuclei	57
4.1	Properties of Heteronuclei	57
4.2	Indirect Detection of Spin-1/2 Nuclei	59
4.3	^{13}C NMR Spectroscopy	59
4.3.1	The ^{13}C Chemical Shift	60
4.3.2	X, ^{13}C Scalar Couplings	64
4.3.3	Longitudinal Relaxation of ^{13}C Nuclei	68
4.3.4	Recording ^{13}C NMR Spectra	68
4.4	NMR of Other Main Group Elements	70
4.4.1	Main Group Nuclei with $I = 1/2$	71
4.4.2	Main Group Nuclei with $I > 1/2$	75
4.5	NMR Experiments with Transition Metal Nuclei	78
4.5.1	Technical Aspects of Inverse Experiments with $I = 1/2$ Metal Nuclei	79
4.5.2	Experiments with Spin $I > 1/2$ Transition Metal Nuclei	81
4.6	Problems	82
	Further Reading	84

Part Two Theory of NMR Spectroscopy 85

5	Nuclear Magnetism – A Microscopic View	87
5.1	The Origin of Magnetism	87
5.2	Spin – An Intrinsic Property of Many Particles	88
5.3	Experimental Evidence for the Quantization of the Dipole Moment: The Stern–Gerlach Experiment	93
5.4	The Nuclear Spin and Its Magnetic Dipole Moment	94
5.5	Nuclear Dipole Moments in a Homogeneous Magnetic Field: The Zeeman Effect	96

5.5.1	Spin Precession	98
5.6	Problems	103
6	Magnetization – A Macroscopic View	105
6.1	The Macroscopic Magnetization	105
6.2	Magnetization at Thermal Equilibrium	106
6.3	Transverse Magnetization and Coherences	108
6.4	Time Evolution of Magnetization	109
6.4.1	The Bloch Equations	110
6.4.2	Longitudinal and Transverse Relaxation	112
6.5	The Rotating Frame of Reference	115
6.6	RF Pulses	117
6.6.1	Decomposition of the RF Field	118
6.6.2	Magnetic Fields in the Rotating Frame	119
6.6.3	The Bloch Equations in the Rotating Frame	120
6.6.4	Rotation of On-Resonant and Off-Resonant Magnetization under the Influence of Pulses	121
6.7	Problems	122
7	Chemical Shift and Scalar and Dipolar Couplings	125
7.1	Chemical Shielding	125
7.1.1	The Contributions to Shielding	127
7.1.2	The Chemical Shifts of Paramagnetic Compounds	131
7.1.3	The Shielding Tensor	132
7.2	The Spin–Spin Coupling	133
7.2.1	Scalar Coupling	134
7.2.2	Quadrupolar Coupling	140
7.2.3	Dipolar Coupling	141
7.3	Problems	144
	Further Reading	145
8	A Formal Description of NMR Experiments: The Product Operator Formalism	147
8.1	Description of Events by Product Operators	148
8.2	Classification of Spin Terms Used in the POF	149
8.3	Coherence Transfer Steps	151
8.4	An Example Calculation for a Simple 1D Experiment	152
	Further Reading	153
9	A Brief Introduction into the Quantum-Mechanical Concepts of NMR	155
9.1	Wave Functions, Operators, and Probabilities	155
9.1.1	Eigenstates and Superposition States	156
9.1.2	Observables of Quantum-Mechanical Systems and Their Measured Quantities	157

9.2	Mathematical Tools in the Quantum Description of NMR	158
9.2.1	Vector Spaces, Bra's, Ket's, and Matrices	158
9.2.2	Dirac's Bra–Ket Notation	159
9.2.3	Matrix Representation of State Vectors	160
9.2.4	Rotations between State Vectors can be Accomplished with Tensors	161
9.2.5	Projection Operators	162
9.2.6	Operators in the Bra–Ket Notation	163
9.2.7	Matrix Representations of Operators	165
9.3	The Spin Space of Single Noninteracting Spins	166
9.3.1	Expectation Values of the Spin-Components	168
9.4	Hamiltonian and Time Evolution	169
9.5	Free Precession	169
9.6	Representation of Spin Ensembles – The Density Matrix Formalism	171
9.6.1	Density Matrix at Thermal Equilibrium	173
9.6.2	Time Evolution of the Density Operator	173
9.7	Spin Systems	175
9.7.1	Scalar Coupling	176

Part Three Technical Aspects of NMR 179

10	The Components of an NMR Spectrometer	181
10.1	The Magnet	181
10.1.1	Field Homogeneity	182
10.1.2	Safety Notes	183
10.2	Shim System and Shimming	184
10.2.1	The Shims	184
10.2.2	Manual Shimming	185
10.2.3	Automatic Shimming	186
10.2.4	Using Shim Files	187
10.2.5	Sample Spinning	187
10.3	The Electronics	187
10.3.1	The RF Section	188
10.3.2	The Receiver Section	189
10.3.3	Other Electronics	189
10.4	The Probehead	189
10.4.1	Tuning and Matching	190
10.4.2	Inner and Outer Coils	191
10.4.3	Cryogenically Cooled Probes	191
10.5	The Lock System	192
10.5.1	The ^2H Lock	192
10.5.2	Activating the Lock	193
10.5.3	Lock Parameters	194

10.6	Problems	194
	Further Reading	194
11	Acquisition and Processing	195
11.1	The Time Domain Signal	197
11.2	Fourier Transform	199
11.2.1	Fourier Transform of Damped Oscillations	199
11.2.2	Intensity, Integral, and Line Width	200
11.2.3	Phases of Signals	201
11.2.4	Truncation	202
11.2.5	Handling Multiple Frequencies	202
11.2.6	Discrete Fourier Transform	203
11.2.7	Sampling Rate and Aliasing	204
11.2.8	How Fourier Transformation Works	205
11.3	Technical Details of Data Acquisition	209
11.3.1	Detection of the FID	209
11.3.2	Simultaneous and Sequential Sampling	210
11.3.3	Digitizer Resolution	213
11.3.4	Receiver Gain	214
11.3.5	Analog and Digital Filters	215
11.3.6	Spectral Resolution	216
11.4	Data Processing	217
11.4.1	Digital Resolution and Zero Filling	217
11.4.2	Linear Prediction	219
11.4.3	Pretreatment of the FID: Window Multiplication	220
11.4.4	Phase Correction	227
11.4.5	Magnitude Mode and Power Spectra	229
11.4.6	Baseline Correction	230
11.5	Problems	231
	Further Reading	232
12	Experimental Techniques	233
12.1	RF Pulses	233
12.1.1	General Considerations	234
12.1.2	Hard Pulses	235
12.1.3	Soft Pulses	236
12.1.4	Band-Selective RF Pulses	237
12.1.5	Adiabatic RF Pulses	238
12.1.6	Composite Pulses	240
12.1.7	Technical Considerations	241
12.1.8	Sources and Consequences of Pulse Imperfections	243
12.1.9	RF Pulse Calibration	244
12.1.10	Transmitter Pulse Calibration	245
12.1.11	Decoupler Pulse Calibration (^{13}C and ^{15}N)	246
12.2	Pulsed Field Gradients	247

12.2.1	Field Gradients	247
12.2.2	Using Gradient Pulses	248
12.2.3	Technical Aspects	250
12.3	Phase Cycling	251
12.3.1	The Meaning of Phase Cycling	251
12.4	Decoupling	255
12.4.1	How Decoupling Works	255
12.4.2	Composite Pulse Decoupling	256
12.5	Isotropic Mixing	257
12.6	Solvent Suppression	257
12.6.1	Presaturation	258
12.6.2	Water Suppression through Gradient-Tailored Excitation	259
12.6.3	Excitation Sculpting	260
12.6.4	WET	260
12.6.5	One-Dimensional NOESY with Presaturation	260
12.6.6	Other Methods	261
12.7	Basic 1D Experiments	262
12.8	Measuring Relaxation Times	262
12.8.1	Measuring T1 Relaxation – The Inversion-Recovery Experiment	262
12.8.2	Measuring T2 Relaxation – The Spin Echo	263
12.9	The INEPT Experiment	266
12.10	The DEPT Experiment	268
12.11	Problems	270
13	The Art of Pulse Experiments	271
13.1	Introduction	271
13.2	Our Toolbox: Pulses, Delays, and Pulsed Field Gradients	272
13.3	The Excitation Block	273
13.3.1	A Simple 90° Pulse Experiment	273
13.3.2	The Effects of 180° Pulses	273
13.3.3	Handling of Solvent Signals	274
13.3.4	A Polarization Transfer Sequence	275
13.4	The Mixing Period	277
13.5	Simple Homonuclear 2D Sequences	278
13.6	Heteronuclear 2D Correlation Experiments	279
13.7	Experiments for Measuring Relaxation Times	281
13.8	Triple-Resonance NMR Experiments	283
13.9	Experimental Details	284
13.9.1	Selecting the Proper Coherence Pathway: Phase Cycles	284
13.9.2	Pulsed Field Gradients	286
13.9.3	N-Dimensional NMR and Sensitivity Enhancement Schemes	288
13.10	Problems	289
	Further Reading	289

Part Four Important Phenomena and Methods in Modern NMR 291

- 14 Relaxation 293**
 - 14.1 Introduction 293
 - 14.2 Relaxation: The Macroscopic Picture 293
 - 14.3 The Microscopic Picture: Relaxation Mechanisms 294
 - 14.3.1 Dipole–Dipole Relaxation 295
 - 14.3.2 Chemical Shift Anisotropy 297
 - 14.3.3 Scalar Relaxation 298
 - 14.3.4 Quadrupolar Relaxation 298
 - 14.3.5 Spin–Spin Rotation Relaxation 299
 - 14.3.6 Paramagnetic Relaxation 299
 - 14.4 Relaxation and Motion 299
 - 14.4.1 A Mathematical Description of Motion: The Spectral Density Function 300
 - 14.4.2 NMR Transitions That Can Be Used for Relaxation 302
 - 14.4.3 The Mechanisms of T1 and T2 Relaxation 303
 - 14.4.4 Transition Probabilities 304
 - 14.4.5 Measuring Relaxation Rates 306
 - 14.5 Measuring ¹⁵N Relaxation to Determine Protein Dynamics 306
 - 14.5.1 The Lipari–Szabo Formalism 307
 - 14.6 Measurement of Relaxation Dispersion 310
 - 14.7 Problems 313

- 15 The Nuclear Overhauser Effect 315**
 - 15.1 Introduction 315
 - 15.1.1 Steady-State and Transient NOEs 318
 - 15.2 The Formal Description of the NOE: The Solomon Equations 318
 - 15.2.1 Different Regimes and the Sign of the NOE: Extreme Narrowing and Spin Diffusion 320
 - 15.2.2 The Steady-State NOE 321
 - 15.2.3 The Transient NOE 324
 - 15.2.4 The Kinetics of the NOE 324
 - 15.2.5 The 2D NOESY Experiment 325
 - 15.2.6 The Rotating-Frame NOE 327
 - 15.2.7 The Heteronuclear NOE and the HOESY Experiment 329
 - 15.3 Applications of the NOE in Stereochemical Analysis 330
 - 15.4 Practical Tips for Measuring NOEs 332
 - 15.5 Problems 333
 - Further Reading 334

- 16 Chemical and Conformational Exchange 335**
 - 16.1 Two-Site Exchange 335
 - 16.1.1 Fast Exchange 338
 - 16.1.2 Slow Exchange 340

16.1.3	Intermediate Exchange	340
16.1.4	Examples	342
16.2	Experimental Determination of the Rate Constants	344
16.3	Determination of the Activation Energy by Variable-Temperature NMR Experiments	346
16.4	Problems	348
	Further Reading	349
17	Two-Dimensional NMR Spectroscopy	351
17.1	Introduction	351
17.2	The Appearance of 2D Spectra	352
17.3	Two-Dimensional NMR Spectroscopy: How Does It Work?	354
17.4	Types of 2D NMR Experiments	357
17.4.1	The COSY Experiment	358
17.4.2	The TOCSY Experiment	359
17.4.3	The NOESY Experiment	362
17.4.4	HSQC and HMQC Experiments	364
17.4.5	The HMBC Experiment	365
17.4.6	The HSQC-TOCSY Experiment	366
17.4.7	The INADEQUATE Experiment	367
17.4.8	<i>J</i> -Resolved NMR Experiments	368
17.5	Three-Dimensional NMR Spectroscopy	370
17.6	Practical Aspects of Measuring 2D Spectra	370
17.6.1	Frequency Discrimination in the Indirect Dimension: Quadrature Detection	370
17.6.2	Folding in 2D Spectra	376
17.6.3	Resolution in the Two Frequency Domains	377
17.6.4	Sensitivity of 2D NMR Experiments	378
17.6.5	Setting Up 2D Experiments	379
17.6.6	Processing 2D Spectra	380
17.7	Problems	381
18	Solid-State NMR Experiments	383
18.1	Introduction	383
18.2	The Chemical Shift in the Solid State	384
18.3	Dipolar Couplings in the Solid State	386
18.4	Removing CSA and Dipolar Couplings: Magic-Angle Spinning	387
18.5	Reintroducing Dipolar Couplings under MAS Conditions	388
18.5.1	An Alternative to Rotor-Synchronized RF Pulses: Rotational Resonance	390
18.6	Polarization Transfer in the Solid State: Cross-Polarization	391
18.7	Technical Aspects of Solid-State NMR Experiments	393
18.8	Problems	394
	Further Reading	394

19	Detection of Intermolecular Interactions	395
19.1	Introduction	395
19.2	Chemical Shift Perturbation	397
19.3	Methods Based on Changes in Transverse Relaxation (Ligand-Observe Methods)	398
19.4	Methods Based on Changes in Cross-Relaxation (NOEs) (Ligand-Observe or Target-Observe Methods)	400
19.5	Methods Based on Changes in Diffusion Rates (Ligand-Observe Methods)	403
19.6	Comparison of Methods	404
19.7	Problems	405
	Further Reading	406
	Part Five Structure Determination of Natural Products by NMR	407
20	Carbohydrates	419
20.1	The Chemical Nature of Carbohydrates	419
20.1.1	Conformations of Monosaccharides	422
20.2	NMR Spectroscopy of Carbohydrates	423
20.2.1	Chemical Shift Ranges	423
20.2.2	Systematic Identification by NMR Spectroscopy	424
20.2.3	Practical Tips: The Choice of Solvent	429
20.3	Quick Identification	430
20.4	A Worked Example: Sucrose	430
	Further Reading	437
21	Steroids	439
21.1	Introduction	439
21.1.1	The Chemical Nature	440
21.1.2	Proton NMR Spectra of Steroids	441
21.1.3	Carbon Chemical Shifts	443
21.1.4	Assignment Strategies	444
21.1.5	Identification of the Stereochemistry	447
21.2	A Worked Example: Prednisone	449
	Further Reading	456
22	Peptides and Proteins	457
22.1	Introduction	457
22.2	The Structure of Peptides and Proteins	458
22.3	NMR of Peptides and Proteins	461
22.3.1	¹ H NMR	461
22.3.2	¹³ C NMR	464
22.3.3	¹⁵ N NMR	467
22.4	Assignment of Peptide and Protein Resonances	469

22.4.1	Peptides	470
22.4.2	Proteins	473
22.5	A Worked Example: The Pentapeptide TP5	476
	Further Reading	480
23	Nucleic Acids	481
23.1	Introduction	481
23.2	The Structure of DNA and RNA	482
23.3	NMR of DNA and RNA	486
23.3.1	^1H NMR	486
23.3.2	^{13}C NMR	489
23.3.3	^{15}N NMR	490
23.3.4	^{31}P NMR	490
23.4	Assignment of DNA and RNA Resonances	492
23.4.1	Unlabeled DNA/RNA	492
23.4.2	Labeled DNA/RNA	496
	Further Reading	498
	Appendix	499
A.1	The Magnetic H and B Fields	499
A.2	Magnetic Dipole Moment and Magnetization	500
A.3	Scalars, Vectors, and Tensors	501
A.3.1	Properties of Matrices	504
	Solutions	507
	Index	525