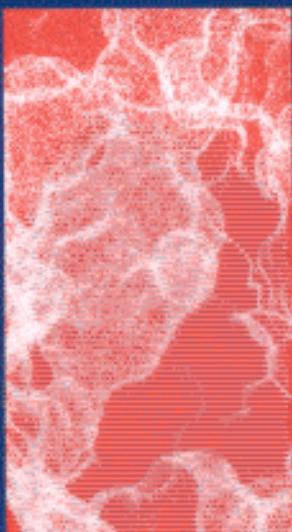
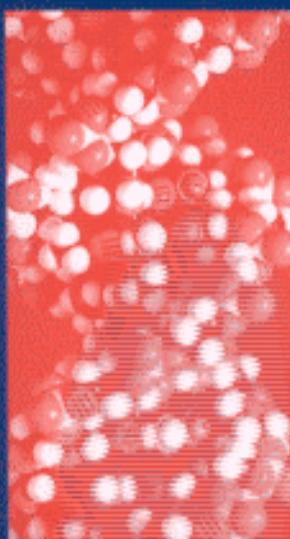


Physical Biochemistry:

Principles and Applications



Suranaree University of Technology



31051000616462

David Sheehan

 WILEY

Contents

Preface	xv
Acknowledgements	xvii
Chapter 1 Introduction	1
1.1 Special chemical requirements of biomolecules	1
1.2 Factors affecting analyte structure and stability	3
1.2.1 pH effects	3
1.2.2 Temperature effects	4
1.2.3 Effects of solvent polarity	6
1.3 Buffering systems used in biochemistry	7
1.3.1 How does a buffer work?	7
1.3.2 Some common buffers	8
1.3.3 Additional components often used in buffers	8
1.4 Quantitation, units and data handling	9
1.4.1 Units used in this text	9
1.4.2 Quantitation of protein and biological activity	9
1.5 Objectives of this book	10
Bibliography	11
Chapter 2 Chromatography	12
2.1 Principles of chromatography	12
2.1.1 The partition coefficient	12
2.1.2 Phase systems used in biochemistry	13
2.1.3 Liquid chromatography	14
2.1.4 Gas chromatography	15
2.2 Performance parameters used in chromatography	16
2.2.1 Retention	16
2.2.2 Resolution	16
2.2.3 Physical basis of peak broadening	17
2.2.4 Plate height equation	17
2.2.5 Capacity factor	23
2.2.6 Peak symmetry	23
2.2.7 Significance of performance criteria in chromatography	23

2.3	Chromatography equipment	23
2.3.1	Outline of standard system used	23
2.3.2	Components of a chromatography system	24
2.3.3	Stationary phases used	24
2.3.4	Elution	24
2.4	Modes of chromatography	26
2.4.1	Ion exchange	27
2.4.2	Gel filtration	30
2.4.3	Reversed phase	33
2.4.4	Hydrophobic interaction	34
2.4.5	Affinity	37
2.4.6	Immobilised metal affinity chromatography	39
2.4.7	Hydroxyapatite	43
2.5	Open-column chromatography	44
2.5.1	Equipment used	44
2.5.2	Industrial-scale chromatography of proteins	45
2.6	High-performance liquid chromatography	46
2.6.1	Equipment used	46
2.6.2	Stationary phases in HPLC	48
2.6.3	Liquid phases in HPLC	49
2.7	Fast protein liquid chromatography	50
2.7.1	Equipment used	50
2.7.2	Comparison with HPLC	51
2.8	Perfusion chromatography	51
2.8.1	Theory of perfusion chromatography	51
2.8.2	The practice of perfusion chromatography	52
2.9	Membrane-based chromatography systems	54
2.9.1	Theoretical basis	54
2.9.2	Applications of membrane-based separations	54
2.10	Chromatography of a sample protein	55
2.10.1	Designing a purification protocol	55
2.10.2	Ion exchange chromatography of a sample protein: Glutathione S-transferases	56
2.10.3	HPLC of peptides from glutathione S-transferases	57
	Bibliography	59

Chapter 3 Spectroscopic Techniques

3.1	The nature of light	61
3.1.1	A brief history of the theories of light	61
3.1.2	Wave-particle duality theory of light	64
3.2	The electromagnetic spectrum	65
3.2.1	The Electromagnetic Spectrum	65
3.2.2	Transitions in spectroscopy	66
3.3	Ultraviolet/visible absorption spectroscopy	66
3.3.1	Physical basis	66

3.3.2	Equipment used in absorption spectroscopy	71
3.3.3	Applications of absorption spectroscopy	72
3.4	Fluorescence spectroscopy	74
3.4.1	Physical basis of fluorescence and related phenomena	74
3.4.2	Measurement of fluorescence and chemiluminescence	78
3.4.3	External quenching of fluorescence	80
3.4.4	Uses of fluorescence in binding studies	83
3.4.5	Protein-folding studies	84
3.4.6	Resonance energy transfer	85
3.4.7	Applications of fluorescence in cell biology	86
3.5	Spectroscopic techniques using plane-polarised light	88
3.5.1	Polarised light	88
3.5.2	Chirality in biomolecules	89
3.5.3	Circular dichroism (CD)	90
3.5.4	Equipment used in CD	91
3.5.5	CD of biopolymers	92
3.5.6	Linear dichroism (LD)	94
3.5.7	LD of biomolecules	94
3.5.8	Plasmon resonance spectroscopy	95
3.6	Infrared spectroscopy	95
3.6.1	Physical basis of infrared spectroscopy	95
3.6.2	Equipment used in infrared spectroscopy	97
3.6.3	Uses of infrared spectroscopy in structure determination	98
3.6.4	Fourier transform infrared spectroscopy	98
3.6.5	Raman infrared spectroscopy	100
3.7	Nuclear magnetic resonance (NMR) spectroscopy	103
3.7.1	Physical basis of NMR spectroscopy	103
3.7.2	Effect of atomic identity on NMR	105
3.7.3	The chemical shift	106
3.7.4	Spin coupling in NMR	107
3.7.5	Measurement of NMR spectra	108
3.8	Electron spin resonance (ESR) spectroscopy	109
3.8.1	Physical basis of ESR spectroscopy	111
3.8.2	Measurement of ESR spectra	111
3.8.3	Uses of ESR spectroscopy in biochemistry	112
3.9	Lasers	113
3.9.1	Origin of laser beams	114
3.9.2	Some uses of laser beams	116
	Bibliography	117
Chapter 4	Mass spectrometry	121
4.1	Principles of mass spectrometry	121
4.1.1	Physical basis	121
4.1.2	Overview of MS experiment	123
4.1.3	Ionisation modes	126
4.1.4	Equipment used in MS analysis	131

4.2	Mass spectrometry of proteins and peptides	134
4.2.1	Sample preparation	134
4.2.2	MS modes used in the study of proteins/peptides	134
4.2.3	Fragmentation of proteins/peptides in MS systems	134
4.3	Interfacing MS with other methods	135
4.3.1	MS/MS	136
4.3.2	LC/MS	136
4.3.3	GC/MS	137
4.3.4	Electrophoresis/MS	138
4.4	Uses of mass spectrometry in biochemistry	138
4.4.1	MS and microheterogeneity in proteins	139
4.4.2	Confirmation and analysis of peptide synthesis	142
4.4.3	Peptide mapping	144
4.4.4	Post-translational modification analysis of proteins	145
4.4.5	Determination of protein disulphide patterns	146
4.4.6	Protein sequencing by MS	148
4.4.7	Analysis of DNA components	149
	Bibliography	150

Chapter 5 Electrophoresis 153

5.1	Principles of electrophoresis	153
5.1.1	Physical basis	153
5.1.2	Historical development of electrophoresis	155
5.1.3	Gel electrophoresis	155
5.2	Non-denaturing electrophoresis	160
5.2.1	Polyacrylamide non-denaturing electrophoresis	160
5.2.2	Protein mass determination by non-denaturing electrophoresis	160
5.2.3	Activity staining	161
5.2.4	Zymograms	164
5.3	Denaturing electrophoresis	164
5.3.1	SDS polyacrylamide gel electrophoresis	164
5.3.2	SDS polyacrylamide gel electrophoresis in reducing conditions	165
5.3.3	Chemical Crosslinking of Proteins — Quaternary Structure	165
5.3.4	Urea electrophoresis	167
5.4	Electrophoresis in DNA sequencing	169
5.4.1	Sanger dideoxynucleotide sequencing of DNA	169
5.4.2	Sequencing of DNA	170
5.4.3	Footprinting of DNA	173
5.4.4	Single-strand conformation polymorphism analysis of DNA	174
5.5	Isoelectric focusing (IEF)	177
5.5.1	Ampholyte structure	177
5.5.2	Isoelectric focusing	178
5.5.3	Titration curve analysis	179
5.5.4	Chromatofocusing	181
5.6	Two-dimensional SDS page	183

5.6.1	Basis of two-dimensional SDS PAGE	183
5.6.2	Equipment used in two-dimensional SDS PAGE	184
5.6.3	Analysis of cell proteins	185
5.7	Immunoelectrophoresis	186
5.7.1	Dot blotting and immunodiffusion tests with antibodies	186
5.7.2	Zone electrophoresis/immunodiffusion immunoelectrophoresis	188
5.7.3	Rocket immunoelectrophoresis	189
5.7.4	Counter-immunoelectrophoresis	190
5.7.5	Crossed immunoelectrophoresis (CIE)	190
5.8	Agarose gel electro-phoresis of nucleic acids	190
5.8.1	Formation of an agarose gel	190
5.8.2	Equipment for agarose gel electrophoresis	190
5.8.3	Agarose gel electrophoresis of DNA and RNA	191
5.8.4	Detection of DNA and RNA in gels	193
5.9	Pulsed field gel electrophoresis	193
5.9.1	Physical basis of pulsed field gel electrophoresis	193
5.9.2	Equipment used for pulsed field gel electrophoresis	195
5.9.3	Applications of pulsed field gel electrophoresis	197
5.10	Capillary electrophoresis	197
5.10.1	Physical basis of capillary electrophoresis	197
5.10.2	Equipment used in capillary electrophoresis	203
5.10.3	Variety of formats in capillary electrophoresis	203
5.11	Electroblotting procedures	204
5.11.1	Equipment used in electroblotting	206
5.11.2	Western blotting	206
5.11.3	Southern blotting of DNA	208
5.11.4	Northern blotting of RNA	209
5.11.5	Blotting as a preparative procedure for polypeptides	210
5.12	Electroporation of cells	211
5.12.1	Transformation of cells	211
5.12.2	Physical basis of electroporation	211
	Bibliography	211
Chapter 6	Three-dimensional structure determination of macromolecules	215
6.1	The protein-folding problem	216
6.1.1	Proteins are only marginally stable	216
6.1.2	Protein folding as a two-state process	220
6.1.3	Protein-folding pathways	221
6.1.4	Chaperonins	223
6.2	Structure determination by NMR	230
6.2.1	Relaxation in one-dimensional NMR	230
6.2.2	The Nuclear Overhauser Effect (NOE)	232
6.2.3	Correlation Spectroscopy (COSY)	234
6.2.4	Nuclear Overhauser Effect Spectroscopy (NOESY)	235
6.2.5	Sequential assignment and structure elucidation	237

5.6.1	Basis of two-dimensional SDS PAGE	183
5.6.2	Equipment used in two-dimensional SDS PAGE	184
5.6.3	Analysis of cell proteins	185
5.7	Immunoelectrophoresis	186
5.7.1	Dot blotting and immunodiffusion tests with antibodies	186
5.7.2	Zone electrophoresis/immunodiffusion immunoelectrophoresis	188
5.7.3	Rocket immunoelectrophoresis	189
5.7.4	Counter-immunoelectrophoresis	190
5.7.5	Crossed immunoelectrophoresis (CIE)	190
5.8	Agarose gel electro-phoresis of nucleic acids	190
5.8.1	Formation of an agarose gel	190
5.8.2	Equipment for agarose gel electrophoresis	190
5.8.3	Agarose gel electrophoresis of DNA and RNA	191
5.8.4	Detection of DNA and RNA in gels	193
5.9	Pulsed field gel electrophoresis	193
5.9.1	Physical basis of pulsed field gel electrophoresis	193
5.9.2	Equipment used for pulsed field gel electrophoresis	195
5.9.3	Applications of pulsed field gel electrophoresis	197
5.10	Capillary electrophoresis	197
5.10.1	Physical basis of capillary electrophoresis	197
5.10.2	Equipment used in capillary electrophoresis	203
5.10.3	Variety of formats in capillary electrophoresis	203
5.11	Electroblotting procedures	204
5.11.1	Equipment used in electroblotting	206
5.11.2	Western blotting	206
5.11.3	Southern blotting of DNA	208
5.11.4	Northern blotting of RNA	209
5.11.5	Blotting as a preparative procedure for polypeptides	210
5.12	Electroporation of cells	211
5.12.1	Transformation of cells	211
5.12.2	Physical basis of electroporation	211
	Bibliography	211

Chapter 6 Three-dimensional structure determination of macromolecules 215

6.1	The protein-folding problem	216
6.1.1	Proteins are only marginally stable	216
6.1.2	Protein folding as a two-state process	220
6.1.3	Protein-folding pathways	221
6.1.4	Chaperonins	223
6.2	Structure determination by NMR	230
6.2.1	Relaxation in one-dimensional NMR	230
6.2.2	The Nuclear Overhauser Effect (NOE)	232
6.2.3	Correlation Spectroscopy (COSY)	234
6.2.4	Nuclear Overhauser Effect Spectroscopy (NOESY)	235
6.2.5	Sequential assignment and structure elucidation	237

6.2.6	Multi-dimensional NMR	240
6.2.7	Other applications of multi-dimensional NMR	242
6.2.8	Limitations and advantages of multi-dimensional NMR	243
6.3	Crystallisation of biomacromolecules	245
6.3.1	What are crystals?	245
6.3.2	Symmetry in crystals	246
6.3.3	Physical basis of crystallisation	248
6.3.4	Crystallisation methods	252
6.3.5	Mounting crystals for diffraction	255
6.4	X-ray diffraction by crystals	257
6.4.1	X-rays	257
6.4.2	Diffraction of X-rays by crystals	258
6.4.3	Bragg's law	259
6.4.4	Reciprocal space	261
6.5	Calculation of electron density maps	263
6.5.1	Calculation of structure factors	263
6.5.2	Information available from the overall diffraction pattern	264
6.5.3	The phase problem	265
6.5.4	Isomorphous replacement	266
6.5.5	Molecular replacement	268
6.5.6	Anomalous scattering	269
6.5.7	Calculation of electron density map	275
6.5.8	Refinement of structure	276
6.5.9	Synchrotron sources	278
6.6	Other diffraction methods	279
6.6.1	Neutron diffraction	279
6.6.2	Electron diffraction	280
6.7	Comparison of X-ray crystallography with multi-dimensional NMR	281
6.7.1	Crystallography and NMR are complementary techniques	281
6.7.2	Different attributes of crystallography- and NMR-derived structures	282
6.8	Structural databases	282
6.8.1	The protein database	283
6.8.2	Finding a protein structure in the database	283
	Bibliography	285

Chapter 7 Hydrodynamic methods

7.1	Viscosity	287
7.1.1	Definition of viscosity	287
7.1.2	Measurement of viscosity	288
7.1.3	Specific and intrinsic viscosity	289
7.1.4	Dependence of viscosity on characteristics of solute	290
7.2	Sedimentation	292
7.2.1	Physical basis of centrifugation	292
7.2.2	The Svedberg equation	293
7.2.3	Equipment used in centrifugation	294

7.2.4	Subcellular fractionation	295
7.2.5	Density gradient centrifugation	296
7.2.6	Analytical ultracentrifugation	299
7.2.7	Sedimentation velocity analysis	300
7.2.8	Sedimentation equilibrium analysis	302
7.3	Methods for varying buffer conditions	305
7.3.1	Ultrafiltration	305
7.3.2	Dialysis	307
7.3.3	Precipitation	309
7.4	Flow cytometry	310
7.4.1	Flow cytometer design	311
7.4.2	Cell sorting	312
7.4.3	Detection strategies in flow cytometry	313
7.4.4	Parameters measurable by flow cytometry	315
	Bibliography	315
Chapter 8	Biocalorimetry	317
8.1	The main thermodynamic parameters	318
8.1.1	Activation energy of reactions	318
8.1.2	Enthalpy	318
8.1.3	Entropy	319
8.1.4	Free energy	320
8.2	Isothermal titration calorimetry	321
8.2.1	Design of an isothermal titration calorimetry experiment	321
8.2.2	ITC in binding experiments	322
8.2.3	Changes in heat capacity determined by isothermal titration calorimetry	323
8.3	Differential scanning calorimetry	323
8.3.1	Outline design of a differential scanning calorimetry experiment	325
8.3.2	Applications of differential scanning calorimetry	325
8.4	Determination of thermodynamic parameters by non-calorimetric means	326
8.4.1	Equilibrium constants	326
	Bibliography	327
Appendix 1	SI units	329
Appendix 2	The Fourier transform	330
Index		335