

CONTENTS

<i>Preface</i>	ix
<i>List of Abbreviations</i>	xi
Chapter 1 Biomolecules	1
1.1 Amino Acids, Peptides and Proteins	1
1.1.1 Amino Acids	2
1.1.2 Peptides and Proteins	7
1.2 Nucleic Acids	14
1.2.1 The Structure of Nucleic Acids	15
1.2.2 Synthesis of Proteins	20
1.3 Biomolecules in Analytical Chemistry	22
1.3.1 Classical Analytical Chemistry	22
1.3.2 Limitations of Classical Analytical Chemistry	22
1.3.3 Bioanalytical Chemistry	23
Chapter 2 Chromatography	29
2.1 The Principle of Chromatography	29
2.2 Basic Chromatographic Theory	31
2.3 Application of Liquid Chromatography for Bioanalysis	34
2.3.1 Reversed Phase Liquid Chromatography (RP-LC)	34
2.3.2 Ion Exchange Chromatography (IEC)	37
2.3.3 Affinity Chromatography	40
2.3.4 Size Exclusion Chromatography (SEC)	42
Chapter 3 Electrophoresis	47
3.1 Principle and Theory of Electrophoresis	48
3.1.1 Electrophoretic Mobility	49
3.1.2 Joule Heating	50
3.1.3 Electroosmotic Flow (EOF)	50
3.1.4 Separation Efficiency and Resolution	54
3.2 Gel Electrophoresis (GE)	56
3.2.1 Instrumentation for Gel Electrophoresis	57
3.2.2 Modes of Gel Electrophoresis	63
3.2.3 Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis (SDS–PAGE)	63

3.2.4	Isoelectric Focussing (IEF)	64
3.2.5	Two-Dimensional Gel Electrophoresis (2D-GE)	67
3.3	Capillary Electrophoresis (CE)	69
3.3.1	Capillary Electrophoresis Instrumentation	70
3.3.2	Capillary Zone Electrophoresis (CZE)	75
3.3.3	Capillary Isoelectric Focussing (CIEF)	76
3.3.4	Micellar Electrokinetic Chromatography (MEKC)	77
3.3.5	Capillary Gel Electrophoresis (CGE)	82
Chapter 4 Mass Spectrometry		85
4.1	The Principle of Mass Spectrometry	85
4.1.1	Ionisation	86
4.1.2	Mass Analyser	86
4.1.3	Detector	87
4.2	Matrix Assisted Laser Desorption Ionisation – Time of Flight Mass Spectrometry (MALDI-TOF/MS)	87
4.2.1	Ionisation Principle	87
4.2.2	Mass Analysis in Time-of-Flight Analyser	90
4.2.3	Detection of Ions	92
4.2.4	Resolution	92
4.2.5	Sample Pretreatment	93
4.2.6	Applications of MALDI	94
4.3	Electrospray Ionisation Mass Spectrometry (ESI-MS)	97
4.3.1	Ionisation Principle	98
4.3.2	ESI – Source and Interface	99
4.3.3	Quadrupole Analyser	100
4.3.4	Applications of ESI-MS	101
Chapter 5 Molecular Recognition: Bioassays, Biosensors, DNA-Arrays and Pyrosequencing		109
5.1	Bioassays	110
5.1.1	Antibodies	111
5.1.2	Antigens	113
5.1.3	Antibody-Antigen Complex Formation	114
5.1.4	Assay Formats	115
5.1.5	Home Pregnancy Test	120
5.1.6	Enzyme Immunoassays (EI and ELISA)	121
5.2	Biosensors	125
5.2.1	Bioreceptors	126

5.2.2	Transducers	127
5.2.3	The Blood Glucose Sensor	128
5.3	DNA Binding Arrays	131
5.3.1	The Principle of DNA Arrays	131
5.3.2	Fabrication of DNA Arrays	132
5.3.3	Development and Analysis of a DNA Array	134
5.3.4	DNA Sequencing with Arrays	134
5.3.5	Other Applications of DNA Arrays	136
5.4	DNA Identification by Pyrosequencing	136
5.4.1	The Principle of Pyrosequencing	137
5.4.2	Sample Preparation and Instrumentation	140
5.4.3	Applications of Pyrosequencing	140

Chapter 6 Nucleic Acids: Amplification and Sequencing **143**

6.1	Extraction and Isolation of Nucleic Acids	143
6.1.1	CsCl Density Gradient Centrifugation	144
6.1.2	Total Cellular DNA Isolation	145
6.1.3	RNA Isolation – The Proteinase K method	145
6.2	Nucleic Acid Amplification – The Polymerase Chain Reaction (PCR)	146
6.2.1	The Principle of PCR	146
6.2.2	The Rate of Amplification During a PCR	149
6.2.3	Reagents for PCR	151
6.2.4	Real-Time PCR	153
6.2.5	Reverse Transcription – PCR (RT-PCR)	155
6.3	Nucleic Acid Sequencing	156
6.3.1	The Use of Restriction Enzymes in Sequencing	156
6.3.2	The Chemical Cleavage method (The Maxam-Gilbert method)	158
6.3.3	The Chain Terminator Method (The Sanger or Dideoxy method)	162
6.4	RNA Sequencing	166

Chapter 7 Protein Sequencing **169**

7.1	Protein Sequencing Strategy	170
7.2	End-group Analysis	170
7.2.1	<i>N</i> -terminal Analysis (Edman Degradation)	171
7.2.2	<i>C</i> -terminal Analysis	172
7.3	Disulfide Bond Cleavage	175

7.4	Separation and Molecular Weight Determination of the Protein Subunits	177
7.5	Amino Acid Composition	178
7.6	Cleavage of Specific Peptide Bonds	179
	7.6.1 Enzymatic Fragmentation	180
	7.6.2 Chemical Fragmentation Methods	183
7.7	Sequence Determination	183
7.8	Ordering of Peptide Fragments	186
7.9	Determination of Disulfide Bond Positions	186
7.10	Protein Sequencing by Mass Spectrometry	187
	Index	189