

TABLE OF CONTENTS

Preface xiii

Acknowledgments xvii

About the Author xix

Chapter 1 INTRODUCTION TO THE BIOCHEMISTRY LABORATORY 1

A. Safety in the Laboratory 2

Safety First 2

Material Safety Data Sheets 2

Safe Practices in the Biochemistry Laboratory 3

B. Keeping Records and Communicating Experimental Results 6

The Laboratory Notebook 6

Details of the Experimental Write-Up 7

Communicating Results from Biochemistry Research 9

C. Using Biochemical Reagents and Solutions 14

Water Purity 14

Cleaning Laboratory Glassware 15

Solutions: Concentrations and Calculations 15

Preparing and Storing Solutions 17

D. Quantitative Transfer of Liquids 18

Pipets and Pipetting 18

Automatic Pipetting Devices 21

E. Statistical Analysis of Experimental Data 23

Defining Statistical Analysis 23

The Mean, Sample Deviation, and Standard Deviation 24

Spreadsheet Statistics 28

Statistical Analysis in Practice 28

Study Problems 30 • Further Reading 32

Chapter 2 USING THE COMPUTER AND INTERNET FOR RESEARCH IN BIOCHEMISTRY 35

A. What Is Research and How Is It Done in Biochemistry? 35

What Is Research? 35

The Scientific Method 36

B. Using Computers in Biochemistry 38

Accessing the Internet	39
The World Wide Web	40
C. Web Sites Useful in Biochemistry	40
Directories, Library Resources, Databases, and Tools	40
Viewing Structures of Biomolecules	43
Searching the Biochemical Literature	44
Literature Searches on the Web	45
Sequence Homology in Proteins	47
Virtual Biochemistry Laboratories	47
Study Problems	48 • Further Reading
Computer Glossary	50

Chapter 3 GENERAL LABORATORY PROCEDURES 53

A. pH, Buffers, Electrodes, and Biosensors	53
Measurement of pH	54
Using the pH Electrode	54
Biochemical Buffers	56
Selection of a Biochemical Buffer	57
Buffer Dilutions	63
The Oxygen Electrode	64
Biosensors	66
B. Measurement of Protein Solutions	67
The Biuret and Lowry Assays	67
The Bradford Assay	69
The BCA Assay	70
The Spectrophotometric Assay	70
C. Measurement of Nucleic Acid Solutions	71
The Spectrophotometric Assay	71
Other Assays for Nucleic Acids	72
D. Techniques for Sample Preparation	73
Dialysis	73
Ultrafiltration	74
Lyophilization and Centrifugal Vacuum Concentration	77
E. Radioisotopes in Biochemistry	80
Origin and Properties of Radioactivity	80
Detection and Measurement of Radioactivity	85
Radioisotopes and Safety	90
Study Problems	91 • Further Reading
	92

Chapter 4 CENTRIFUGATION TECHNIQUES IN BIOCHEMISTRY 95

- A. Basic Principles of Centrifugation 96**
- B. Instrumentation for Centrifugation 99**
 - Low-Speed Centrifuges 99
 - High-Speed Centrifuges 101
 - Ultracentrifuges 105
- C. Applications for Centrifugation 106**
 - Preparative Techniques 106
 - Analytical Measurements 108
 - Care of Centrifuges and Rotors 112
 - Study Problems 113 • Further Reading 114

Chapter 5 PURIFICATION AND ANALYSIS OF BIOMOLECULES BY CHROMATOGRAPHY 115

- A. Introduction to Chromatography 116**
 - Partition versus Adsorption Chromatography 117
- B. Planar Chromatography (Paper and Thin-Layer Chromatography) 118**
 - Preparation of the Sorbent 118
 - Solvent Development 119
 - Detection and Measurement of Components 120
 - Applications of Planar Chromatography 121
 - Advanced Planar Chromatography 121
- C. Column Chromatography 122**
 - Operation of a Chromatographic Column 123
 - Packing the Column 124
 - Loading the Column 125
 - Eluting the Column 125
 - Collecting the Eluent 126
 - Detection of Eluting Components 126
- D. Ion-Exchange Chromatography 126**
 - Ion-Exchange Resins 127
 - Selection of the Ion Exchanger 128
 - Choice of Buffer 130
 - Preparation of the Ion Exchanger 130
 - Using the Ion-Exchange Resin 130
 - Storage of Resins 131

E. Gel-Exclusion Chromatography 132

Theory of Gel Filtration 132

Physical Characterization of Gel Chromatography 133

Chemical Properties of Gels 133

Selecting a Gel 135

Gel Preparation and Storage 136

Operation of a Gel Column 136

Applications of Gel-Exclusion Chromatography 138

F. High-Performance Liquid Chromatography (HPLC) 140

Instrumentation 142

Stationary Phases in HPLC 144

Chiral Chromatography 148

The Mobile Phase 150

Sample Preparation and Selection of HPLC Operating Conditions 150

FPLC—A Modification of HPLC 150

Perfusion Chromatography 151

G. Affinity Chromatography and Immunoabsorption 152

Chromatographic Media 153

The Immobilized Ligand 154

Attachment of Ligand to Matrix 154

Immunoabsorption 156

Experimental Procedure for Affinity Chromatography 157

H. Membrane-Based Chromatography 159

Study Problems 161 • Further Reading 163

Chapter 6 CHARACTERIZATION OF PROTEINS AND NUCLEIC ACIDS BY ELECTROPHORESIS 165

A. The Theory of Electrophoresis 166

Introduction 166

Theory and Practice 166

B. Methods of Electrophoresis 167

Polyacrylamide Gel Electrophoresis (PAGE) 167

Discontinuous Gel Electrophoresis 172

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) 174

Nucleic Acid Sequencing Gels 176

Agarose Gel Electrophoresis	177
Pulsed Field Gel Electrophoresis (PFGE)	180
Isoelectric Focusing of Proteins	182
Two-Dimensional Electrophoresis (2-DE) of Proteins	184
Capillary Electrophoresis (CE)	185
Immuno-electrophoresis (IE)	186

C. Practical Aspects of Electrophoresis 188

Instrumentation	188
Reagents	189
Staining and Detecting Electrophoresis Bands	189
Protein and Nucleic Acid Blotting	192
The Western Blot	194
Analysis of Electrophoresis Results	197
Study Problems	198 • Further Reading 199

Chapter 7 SPECTROSCOPIC ANALYSIS OF BIOMOLECULES 201

A. Ultraviolet-Visible Absorption Spectrometry 202

Wavelength and Energy	202
Light Absorption	204
Electronic Transitions in Biomolecules	205
The Absorption Spectrum	207
The Beer-Lambert Law	207
Instrumentation	209
Applications of UV-VIS Spectroscopy	212

B. Fluorescence Spectrometry 220

Principles	220
Quantum Yield	221
Instrumentation	222
Applications of Fluorescence Spectroscopy	223
Difficulties in Fluorescence Measurements	224

C. Nuclear Magnetic Resonance Spectroscopy 225

NMR Theory	226
NMR in Biochemistry	226
NMR and Protein Structures	227

D. Mass Spectrometry 230

Ionization and Analysis of Proteins	230
MS Applications in Biochemistry	232

E. X-Ray Crystallography 233

Methodology of X-ray Crystallography 233

Study Problems 234 • Further Reading 235

Chapter 8 BIOMOLECULAR INTERACTIONS: LIGAND BINDING AND ENZYME REACTIONS 239

A. Ligand-Macromolecule Interactions (Molecular Recognition) 239

Properties of Noncovalent Binding Interactions 240

Quantitative Characterization of Ligand Binding 242

Scatchard's Equation 244

Cooperative Binding of Ligands 245

Experimental Measurement of Ligand-Binding Interactions 245

The Bradford Protein Assay as an Example of Ligand Binding 247

Computer Software for Analysis of LM Binding 249

B. Biological Catalysis (Enzymes) 250

Classes of Enzymes 250

Kinetic Properties of Enzymes 252

Significance of Kinetic Constants 254

Inhibition of Enzyme Activity 255

Units of Enzyme Activity 256

Specific Activity 258

Design of an Enzyme Assay 258

Kinetic versus Fixed-time Assay 259

Applications of an Enzyme Assay 260

Computer Software for Analysis of Enzyme Kinetic Data 262

Study Problems 262 • Further Reading 264

Chapter 9 MOLECULAR BIOLOGY I: STRUCTURES AND ANALYSIS OF NUCLEIC ACIDS 267

A. Introduction to the Nucleic Acids 268

Chemical Components of DNA and RNA 268

DNA Structure and Function 270

RNA Structure and Function 272

B. Laboratory Methods for Investigation of DNA and RNA 275

Isolation of Chromosomal DNA 275

Isolation of Plasmid DNA 277

Characterization of DNA	279
Ethidium Bromide Binding and Fluorescence	280
Agarose Gel Electrophoresis	282
Sequencing DNA Molecules	282
Isolation and Characterization of RNA	284
Study Problems	285 • Further Reading 286

Chapter 10 MOLECULAR BIOLOGY II: RECOMBINANT DNA, MOLECULAR CLONING, AND ENZYMOLOGY 289

A. Recombinant DNA Biotechnology	290
Molecular Cloning	290
Steps for Preparing Recombinant DNA	292
Cloning Vectors	294
B. Important Enzymes in Molecular Biology and Biotechnology	297
The Restriction Endonucleases	297
Applications of Restriction Enzymes	298
Practical Aspects of Restriction Enzyme Use	299
The Polymerase Chain Reaction	301
C. Nucleic Acid Blotting	304
Study Problems	304 • Further Reading 305

Chapter 11 PROTEIN PRODUCTION, PURIFICATION, AND CHARACTERIZATION 307

A. Procedures for the Purification of Proteins	308
Composition of Proteins	308
Amount of Protein versus the Purity of Protein versus Expense	308
Basic Steps in Protein Purification	309
Preparation of the Crude Extract	311
Stabilization of Proteins in a Crude Extract	312
Separation of Proteins Based on Solubility Differences	315
Selective Techniques in Protein Purification	316
B. Production of Proteins by Expression of Foreign Genes	317
Gene Expression in Prokaryotic Organisms	317
Gene Expression in Eukaryotic Cells	320
C. Protein Characterization	322
D. Determination of Primary Structure	323

	Amino Acid Composition	323
	Sequencing DNA Instead of the Protein	328
	Study Problems	328 • Further Reading 328
Appendix I	List of Software Programs and Web Sites Useful for Each Chapter	331
Appendix II	Properties of Common Acids and Bases	334
Appendix III	Properties of Common Buffer Compounds	335
Appendix IV	pK_a Values and pH_i Values of Amino Acids	337
Appendix V	Molecular Weight of Some Common Proteins	338
Appendix VI	Common Abbreviations Used in This Text	339
Appendix VII	Units of Measurement	342
Appendix VIII	Table of the Elements	344
Appendix IX	Answers to Odd-Numbered Study Problems	348
Index		353