TABLE OF CONTENTS

Preface xiii	•
Acknowledg	ments xvii
About the A	uthor xix

Chapter 1	INTRODUCTION TO THE BIOCHEMISTRY
-	LABORATORY 1

A.	Safety	in th	e Labo	ratory	2
	Juicta		CLUNC		_

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 49 • 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 Immunoadsorption 152

Chromatographic Media 153

The Immobilized Ligand 154

Attachment of Ligand to Matrix 154

Immunoadsorption 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

Immunoelectrophoresis (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 II 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