

Contents

Series Preface	v
Preface to the Third Edition	vii
Preface to the Second Edition	ix
Preface to the First Edition	xi
Chapter 1	
The Protein Purification Laboratory	1
1.1 Apparatus, Special Materials, and Reagents	1
1.2 Separation of Precipitates and Particulate Material	3
Filtration	3
Centrifugation	4
1.3 Principles of Column Chromatography	8
1.4 Manipulation of Protein Solutions	14
Concentration	15
Removal of Salts; Changing Buffers	17
Chapter 2	
Making an Extract	22
2.1 The Raw Material	22
Freshness and Storage	25
2.2 Cell Disintegration and Extraction	26
Mammalian Tissues	30
Erythrocytes	31
Soft Plant Tissues	31
Yeast	31
Bacteria	32
Fatty Tissues	34
2.3 Optimization and Clarification of the Extract	34
2.4 Extraction of Membrane Proteins	38

Chapter 3

Analysis—Measurement of Protein and Enzyme Activity	44
3.1 Methods for Measuring Protein Concentration	44
Biuret Reaction	45
Lowry Method	46
UV Absorption	46
Dye Binding	48
Bicinchoninic Acid	48
3.2 Measurement of Enzyme Activity—Basic Principles	50
Substrate Concentration, Activators, and Inhibitors	50
pH, Ionic Strength, and Temperature	55
3.3 Measurement of Enzyme Activity Using Stopped Methods	56
Incubation Conditions	57
Stopping Methods	58
Measurement of Product	59
3.4 Measurement of Enzyme Activity Using Continuous Methods	62
Coupled Methods	63
3.5 Practical Points in Enzyme Activity Determination	68

Chapter 4

Separation by Precipitation	71
4.1 General Observations	71
4.2 The Solubility of Proteins at Low Salt Concentrations	72
Points to Note in Practice	75
4.3 Salting Out at High Salt Concentration	76
4.4 Precipitation with Organic Solvents	85
General Theory	85
Choice of Solvent	87
Operating Procedures	89
4.5 Precipitation with Organic Polymers and Other Materials	92
4.6 Affinity Precipitation	93
4.7 Precipitation by Selective Denaturation	95
General Principles	95
Temperature Denaturation	96
pH Denaturation	98
Denaturation by Organic Solvents	100

Chapter 5

Separation by Adsorption I: General Principles	102
5.1 General Chromatographic Theory	103
Partition Coefficients	103
Zone Spreading, Resolution, and the Plate Height Concept	105
The Dissociation Constant for Protein-Adsorbent Interaction	111
Simplified Theory of Adsorption	112
5.2 Membrane Adsorbents; Radial Flow Columns	119
5.3 Batch Adsorption	121
General Principles	121
Practical Approaches	123
5.4 High-Performance Liquid Chromatography	126
General Principles	126

Relationships Between Bead Size, Flow Rate, Pressure, and Optimum Performance	128
5.5 Types of Adsorbent Used in Protein Chromatography	132
Nature of the Bead Matrix	132
Summary of Adsorbent Types	135
5.6 Operating Conditions for Column Chromatography	139
Sample Application	139
Overload and Displacement Chromatography	139
Flow Rates	142
 Chapter 6	
Separation by Adsorption II: Ion Exchangers and Nonspecific Adsorbents	146
6.1 Ion Exchangers—Principles, Properties, and Uses	146
General Principles	146
Adsorptive Capacities of Ion Exchangers	150
Types of Ion Exchangers	152
pH and Donnan Effects	153
Elution of Adsorbed Protein	154
6.2 Ion-Exchange Chromatography—Practical Aspects	157
Trials to Determine Ion-Exchange Behavior	159
Buffers for Use in Ion-Exchange Chromatography	160
Conditions of Adsorption	164
Size and Dimensions of the Column	165
Procedures for Elution	167
6.3 Inorganic Adsorbents	172
Hydroxyapatite and Calcium Phosphate Gels	173
6.4 Hydrophobic Adsorbents	175
Application of Sample to a Hydrophobic Column	176
Elution of Protein from Hydrophobic Columns	177
Reverse Phase Chromatography	178
Other Hydrophobic Techniques	179
6.5 Immobilized Metal Affinity Chromatography (IMAC)	180
General Principles	180
Operating Conditions for IMAC	182
6.6 Miscellaneous Adsorbents	183
Cationic Polymer-Nucleic Acid Complexes as Batch Adsorbents	183
Thiophilic Adsorbents	184
Mixed-Function Adsorbents	185
 Chapter 7	
Separation by Adsorption—Affinity Techniques	187
7.1 Principles of Affinity Chromatography	187
Synthesis of Affinity Adsorbents	188
Application of Chromatographic Theory to Affinity Adsorbents	196
General Techniques and Procedures in Affinity Adsorption Chromatography	200
7.2 Immunoadsorbents	204
Basic Principles	205
Methods Using Polyclonal Antibodies	205

	Methods Using Monoclonal Antibodies	208
	Relative Advantages of Polyclonal and Monoclonal Antibodies	209
7.3	Dye Ligand Chromatography	210
	Developmental History	210
	Preparation of Dye Ligand Adsorbents	214
	Dye–Protein Interactions	217
	Screening to Obtain a Suitable Adsorbent	219
	Elution of Proteins and Enzymes from Dye Columns	223
	Cleaning and Storage of Dye Adsorbents	224
7.4	Affinity Elution from Ion Exchangers and Other Adsorbents	226
	Affinity Elution from Ion Exchangers	227
	Affinity Elution from Other Adsorbents	231
	Practice and Theory of Affinity Elution	233
7.5	Commonly Used Affinity and Pseudo-Affinity Adsorbents	236
	Small Ligands	236
	Biopolymer Ligands	236
Chapter 8		
	Separation in Solution	238
8.1	Gel Filtration	238
	Practical Procedures	243
8.2	Electrophoretic Methods	250
	Electrophoresis Principles	251
	Methods for Preparative Electrophoresis—Horizontal Slabs	253
	Methods for Preparative Electrophoresis—Vertical Systems	255
	Buffer Systems for Electrophoresis	256
	Isoelectric Focusing	258
	Isotachophoresis	262
8.3	Liquid Phase Partitioning	264
8.4	Ultrafiltration	267
Chapter 9		
	Purification of Special Types of Proteins	270
9.1	Recombinant Proteins	270
	Terminology of Recombinant Proteins	271
9.2	Membrane Proteins	277
9.3	Purification of Antibodies	279
Chapter 10		
	Small-Scale and Large-Scale Procedures	283
10.1	Small-Scale Procedures—Proteins for Sequencing	283
10.2	Large-Scale Procedures	287
	Scaling Up in the Laboratory	287
	Commercial-Scale Protein Production	291
Chapter 11		
	Analysis for Purity	293
11.1	Electrophoretic Analysis	293
	Simple (Native) Gel Electrophoresis	294

Urea Gels	296
SDS Gels	296
Gradient Gels	298
Isoelectric Focusing	299
Two-Dimensional Systems	300
Capillary Electrophoresis	300
Staining and Detection of Proteins after Electrophoresis	302
Detection of Specific Proteins	303
11.2 Other Analytical Methods	307
Chapter 12	
Optimization of Procedures; Final Steps	310
12.1 Speed Versus Resolution: The Time Factor	311
12.2 Stabilizing Factors for Enzymes and Other Proteins	317
Prevention of Denaturation	317
Avoidance of Catalytic Site Inactivation	318
Avoidance of Proteolytic Degradation	321
Other Stabilizing Influences on Proteins	323
12.3 Control of pH: Buffers	324
Buffer Theory	324
Effect of Temperature, Ionic Strength, and Organic Solvents on pK_a Values	326
Making Up Buffer Solutions	330
12.4 Following a Published Procedure	333
12.5 Final Steps—Storage, Crystallization, and Publication	335
Crystallization for Purification	336
Methods for Crystallization for X-ray Diffraction Studies	337
Conditions for Storage of Purified Proteins	342
What is Important for Publication?	344
Appendix A	
Precipitation Tables	346
Appendix B	
Solutions for Measuring Protein Concentration	349
Appendix C	
Buffers for Use in Protein Chemistry	351
Appendix D	
Chromatographic Materials	353
References	356
Index	375