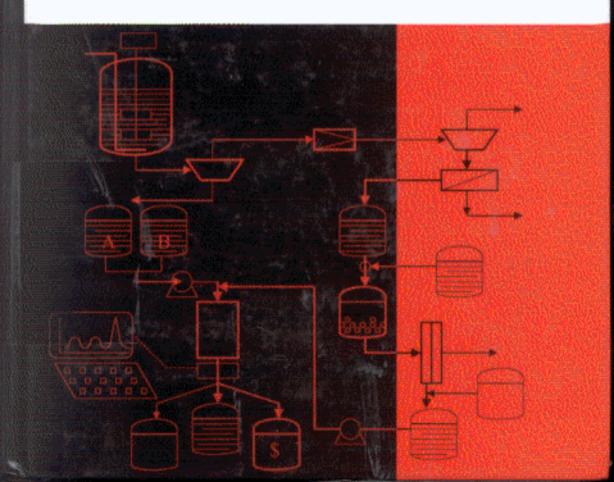
Bioseparations Engineering

Principles, Practice, and Economics

Michael R. Ludisch



CONTENTS

PREFACE

1. BIOSEPARATIONS

2.1

ACKNOWLEDGEMENTS

in Particle Size and Density / 17

| | Introduction / 1 |
|-----|---|
| 1.1 | The Manner in Which the Bioproduct is Associated with the Cell or Organism Defines Its Initial Recovery Characteristics / 2 |
| 1.2 | Physical Processing and Water Removal Steps Are Important Separation Methods for Large-Volume Products / 5' |
| 1.3 | Minimal Downstream Processing Is Characteristic of Some Large-Volume Extracellular Enzyme Products Used in the Food and Textile Industries / 6 |
| 1.4 | There Are Three Major Categories of Bioproducts: Cells, Intracellular Products, and Extracellular Products / 7 |
| 1.5 | Recombinant Proteins Derived from E. Coli Are Initially in the Form of Insoluble, Intracellular Inclusion Bodies / 9 |
| 1.6 | The Unit Operations of Bioseparations Are Grouped into Five Major Categories / 10 |
| 1.7 | Bioinformatics Will Lead to Products that Bioseparation Methods Must Purify / 13 |
| 1.8 | Bioseparations Engineering Plays a Major Role in the Successful Development of Bioprocesses for the Manufacture of Therapeutic Molecules and Specialty Chemicals / 14 |
| | References / 14 |
| 2. | SEDIMENTATION, CENTRIFUGATION, AND FILTRATION 16 |
| | Introduction / 16 |

Solid/Liquid Separations by Sedimentation or Centrifugation Are Based on Differences

XXİ

liixx

1

viii CONTENTS

- 2.2 Centrifugation Uses Mechanical Force to Amplify the Differences in Size and Density between Wet Biological Materials and the Aqueous Media in Which the Solids Are Found / 21
- 2.3 The Volumetric Rate of Clarified Supernatant Is Maximized by a Large-Density Difference and Low Viscosity / 22
- 2.4 Centrifuge Speed Is Limited by Stress in the Bowl's Wall and by Its Materials of Construction / 23
- 2.5 The Disc Stack Centrifuge Enables Continuous and Rapid Processing of Cell and Colloidal Suspensions / 25
- 2.6 A Decanter Centrifuge Is Less Efficient For Recovery of Microorganisms than a Disc Centrifuge / 32
- 2.7 Sterility, Containment, and Heat from Mechanical Work Affect Design of Process Centrifuges / 33
- 2.8 Centrifuge Design for Biotechnology Processes Incorporates Cleaning-in-Place and Sterilization Capabilities / 34
- 2.9 Centrifuge Containment Is Necessary for Processing of Some Types of Biotechnology Products / 35
- 2.10 Filtration / 36
- 2.11 A Fluid's Superficial Velocity, or Flux, through Filter Cake Is Proportional to a Permeability Coefficient Expressed in Units of Darcies / 36
- 2.12 Diatomaceous Earth and Perlites (Volcanic Rock) Serve to Enhance Permeability of Filter Cakes and Aid Filtration of Fermentation Broths / 40
- 2.13 Filtration for Streptomycin Recovery Requires Coagulation of the Mycelia and Addition of a Filter Aid / 41
- 2.14 Rotary Vacuum Filters Enable Continuous Filtration of Bioproducts Generated in Large Volumes of Fermentation Broth / 43
- 2.15 Penicillin G from *Penicillium Chrysogenum* Is Recovered by Rotary Filters prior to its Hydrolysis by Immobilized Penicillin Acylase / 46

52

References / 47

Problems / 48

3. MEMBRANE SEPARATIONS

Introduction / 52

- 3.1 Microfiltration Membranes Remove Particles Whose Sizes Range from 0.1 to 10 Microns / 52
- 3.2 Molecular Filtration by Ultrafiltration and Reverse Osmosis Utilizes Supported Membranes with Nanometer-Size Pores / 54
- 3.3 Flux, j_v , through a Membrane Follows Darcy's Law when the Osmotic Pressure Difference across the Membrane Is Small / 55
- 3.4 The Gibbs and van't Hoff Equations Provide a Basis for Calculating Estimates of Osmotic Pressures / 56
- 3.5 Dissociation of Salts in Aqueous Solutions Increases Osmotic Pressure / 64
- 3.6 Concentration Polarization Reduces Flux / 65

- 3.7 Flux Increases with Increasing Temperature and Fluid Velocity across the Membrane's Surface / 66
- 3.8 Pore Occlusion and Concentration Polarization Can Be More Important than Osmotic Pressure in Determining Flux for Membrane Filtration of Proteins / 69
- 3.9 Flux Equations Are Classified into Osmotic Pressure Dependent, Hydraulic Pressure Dependent, and Pressure Independent (Concentration Polarization) Regimes / 71
- 3.10 Dimensional Analysis of Momentum and Diffusive Transport Processes Enables Estimation of Flux from Membrane and Fluid Properties / 73
- 3.11 Solute Flux in Dialysis Is Based on a Concentration Gradient, not a Pressure Gradient / 80

Engineering Concepts of Membrane Applications

- 3.12 Membrane Separations of Small Particles Utilize Fibrous or Particulate Depth Filters and Isotropic (Symmetric) Screen Filters / 85
- 3.13 Membranes Are Packaged in Flat-Sheet or Hollow-Fiber Cartridge Configurations / 90
- 3.14 The Pressure at which Gas Flows through a Wetted Membrane Gives a Measure of Its Pore Size: Bubble-Point Test / 93
- 3.15 Sterilization of Human Plasma Proteins, Harvesting of Recombinant Microbial Cells, and Recovery of Cell Culture Products Are Applications of Microfiltration / 95
- 3.16 Aggregation of Proteins Promotes Membrane Fouling and Decreases Flux / 100
- 3.17 Dialysis and Evaporation or Reverse Osmosis Processes Remove Ethanol from Beer to Yield Beverages with Alcohol Content Reduced / 107

References / 113

Problems / 114

4. PRECIPITATION, CRYSTALLIZATION, AND EXTRACTION

116

Introduction / 116

- 4.1 The Addition of Neutral Salts, or an Acid or Base to Aqueous Solutions, Induces the Solute to Precipitate / 116
- 4.2 Alcohols Decrease Solvating Power of Water by Lowering the Dielectric Constant of the Solution / 118
- 4.3 Neutral Salts Added to Solutions of Amino Acids or Proteins Cause Precipitation by Hydrophobic Interactions / 120
- 4.4 The Logarithmic Decrease in Amino Acid and Protein Solubility Is Proportional to Increasing Salt Concentration: Cohn's Equation / 123
- 4.5 The Separation Factor for Two Proteins, Lysozyme and α-Chymotrypsin, Is Calculated from Their Distribution Coefficients: An Example / 128
- 4.6 Fractionation of Two Proteins by Precipitation Requires that Their Solubilities Are
 Significantly Different from Each Other / 130
- 4.7 pH, Temperature, and Initial Concentration Also Affect Protein Solubility / 131
- 4.8 Heat of Solution Effects Can Be Significant for Proteins / 134
- 4.9 The Salting-out Constant, K_s , Combines Salting-out and Salting-in Effects that Characterize Hydrophobic Interactions / 135

- 4.10 Hydrophobic Contact Areas Φ of Selected Proteins, Ranging from 20 to 42% of Total Surface Area, May Be Determined from K_s / 138
- 4.11 K_s May Be Calculated from the Protein's Dipole Moment (μ), Contact Area (Φ), and Surface Tension Increment (σ): Ovalbumin Example / 140
- 4.12 Graphing of K_s against Molal Surface Tension Increment (σ) Enables Calculation of Protein Solubility in Different Salt Solutions: An Example / 142
- 4.13 Thermodynamics Offer an Explanation for both Salting-in and Salting-out Effects / 143
- 4.14 Protein Micelles in Milk Precipitate by Enzyme-Induced Coagulation / 148

Crystallization

- 4.15 A Nucleus or Critical Cluster Known as an Embryo Is Required for Crystallization / 152
- 4.16 The Analysis of a Crystallization Process Is Based on Differences in Chemical Potential and the Saturation Ratio / 152
- 4.17 The Work for Forming an Embryo Is Associated with Building the Surface of a Crystal and Increasing Its Volume / 155
- 4.18 The Diameter of an Embryo at Equilibrium Represents the Critical Diameter at which Crystallization May Be Induced / 156
- 4.19 The Work Function Is a Thermodynamic Expression that Represents a Surface Tension and Temperature Dependent Activation Energy for Homogeneous Nucleation / 157
- 4.20 The Induction Period for Crystallization Is Proportional to the Cube of Surface Tension (γ^3) and Inversely Proportional to $(\ln S)^2 / 162$
- 4.21 Surface Tension May Be Estimated from the Ratio of Solute Concentration in the Crystal to Its Concentration in Solution / 164
- 4.22 Patterns of Particle Accumulation as a Function of Solute Concentration Sometimes Distinguish Heterogeneous from Homogeneous Nucleation: Cholesterol and Citric Acid Examples / 165
- 4.23 The Transition from Heterogeneous Nucleation at Low Supersaturation to Homogeneous Nucleation at High Supersaturation Facilitates Estimates of Interfacial Surface Tension / 168
- 4.24 Heterogeneous Nucleation Can Give Rise to Anomalies upon Scale-up / 169
- 4.25 Miers Plots Represent Crystallization Paths for Solutions Brought to Supersaturation by Cooling and through Solvent Removal by Evaporation / 171
- 4.26 Numerical Solutions of Material Balances Give Curves that Represent the Decrease in Solute as a Function of Time for Heterogeneous Crystallization / 172

Extraction And Leaching

- 4.27 "Leaching Is the Preferential Solution of One or More Constituents of a Solid Mixture by Contact with a Liquid Solvent" (Treybal, 1968) / 184
- 4.28 Supercritical Carbon Dioxide Is an Effective Extractor for Solid Bioproducts / 185 Summary and Perspectives / 187

References / 188

Suggested Reading / 189

Problems / 191

5. PRINCIPLES OF LIQUID CHROMATOGRAPHY

Introduction / 194

- 5.1 Liquid Chromatography Systems Are Classified by Pressures that Characterize Their Operation: HPLC, LPLC, and MPLC / 194
- 5.2 This Chapter Presents the Principles and Practices of Analyzing and Scaling-up Chromatography Column Performance from Experimental Measurements / 195
- 5.3 Liquid Chromatography Systems Consist of Columns, Injectors, Detectors, Pumps, Fraction Collectors, and Stationary and Mobile Phases / 196
- 5.4 The Target Molecule Is the Molecule that Is to be Recovered in a Purified Form / 200
- 5.5 The Nomenclature of Chromatography Is Summarized in Schematic Diagrams / 201
- 5.6 Gradient Chromatography Is a Form of Adsorption / 203
- 5.7 Gradients Are Formed by Combining Two or More Liquid Buffers to Give a Time-Varying Change in Displacer Concentration / 208
- 5.8 Liquid Chromatography Columns Are Packed Using Liquid Shurries / 215
- 5.9 Some Types of Stationary Phases Undergo Significant Swelling when Hydrated in Water or Buffer / 220
- 5.10 Convective Flow through Gigaporous Particles with Transecting Pores May Occur at High Pressures / 224
- 5.11 Pellicular Particles, Polymer Monoliths, Rolled Stationary Phases, and Bundles of Hollow Fibers Represent Other Forms of Stationary Phases / 226
- 5.12 Plate Count or Plate Height (HETP) Gives a First Indication of Packing Efficiency / 230
- 5.13 Poisson and Gaussian Distribution Equations May Be Used to Calculate Elution Profiles of Single Chromatography Peaks / 232
- 5.14 Many Peaks that Elute from Chromatography Columns Are Skewed due to Intracolumn and Extra Column Dispersion Effects: Exponentially Modified Gaussian Peaks / 237
- 5.15 One-Dimensional Model of Differential Chromatography Enables Simulation of Elution Profiles / 242
- 5.16 Sample (Feed) Volumes Affect the Calculation of Plate Count due to Contributions of the Feed Volume to Peak Width / 252
- 5.17 Contributions to Peak Broadening due to Particle Size and Flowrate Effects Are Given by the van Deemter Equation / 259
- 5.18 The Effect of Particle Size, Flowrate, Solute, and Temperature on Plate Height Is Modeled Using Dimensionless Numbers Re, Sc, Pe, Nu, and Da (Derivation of Athalye, Lightfoot et al.) / 261
- 5.19 Mass Transfer and Adsorption Kinetics Also Impact Plate Height / 271
- 5.20 Chromatographic Capacity Factors Are Determined from Peak Retention / 280
- 5.21 Chromatographic Separations Are Defined by Divergence of Peak Centers: Capacity Factors. Phase Ratios. and Resolution / 283

References / 288

Suggested Reading / 290

Problems / 293

LIQUID CHROMATOGRAPHY SCALE-UP

299

Introduction / 299

Linear Chromatography

- 6.1 Scale-up Rules Enable Initial Specification of Chromatography Columns / 300
- 6.2 Scale-up Rules for Size Exclusion Chromatography (SEC) Assume Pore Diffusion Controls / 304
- 6.3 Mass Transfer Can Be a Limiting Factor at Slow Flowrates, or for Solutes that Have Slow Diffusion Rates / 313
- 6.4 Scale-up Rules Are Similar for Pore Diffusion and Mass Transfer Limiting Cases / 318
- 6.5 Scale-up When Mass Transfer and Pore Diffusion Are of Comparable Magnitude Requires Combination of These Resistances / 318
- 6.6 A Material Balance Combined with Plate Count Facilitates Simulation of Elution Profiles for Linear Equilibrium: Size Exclusion and Ion Exclusion Examples (with contributions by Scott Rudge) / 321
- 6.7 Physical Properties of Stationary Phase, Mobile Phase, and Feed Sample Should Not Be Forgotten when Analyzing Column Performance / 328
- 6.8 Ion Exclusion Has Possible Applications for a Greener Chemical Industry / 337
- 6.9 Linear Chromatography May Depend on Particle Size, Temperature, or Solute Concentration Effects: Case Study for Linear Chromatography Scale-up / 339
- 6.10 The Craig Model May Be Used to Predict Elution Profiles for Strongly Retained Components (k' >> 0) (with Contributions by Ajoy Velayudhan) / 348
- 6.11 The Stirred-Tank-in-Series Model of Chromatography Is Based on a Material Balance (𝑢' ≥ 0) (with Contributions by Ajoy Velayudhan) / 354
- 6.12 The Craig and Stirred Tank in Series Models Give Similar Results: Glucose/Fructose Separation Example / 356

Nonlinear Chromatography

- 6.13 Local Equilibrium Theory Relates Elution Profiles for an Adsorbing or Desorbing Solute to Its Equilibrium Isotherm (with contributions by Juan Hong) / 360
- 6.14 Desorption Isotherms May Differ from Adsorption Isotherms: Hysteresis Effects and Local Equilibrium Theory / 365
- 6.15 Triangular Peaks Are Associated with Nonlinear Chromatography: Overload Conditions / 366
- 6.16 Equilibrium and Mass Transfer Theories Can Be Used to Calculate the Shape of the Front of a Single Peak for Nonlinear Chromatography / 369
- 6.17 Scale-up of Nonlinear Chromatography Is Based on Maintaining the Relative Peak
 Position and Overlap Two or More Peaks at a Fixed Ratio / 376
- 6.18 Ratios of the Width of Mass Transfer Zones at Process and Bench Scales Are the Basis of Scale-up for Nonlinear Chromatography / 378
- 6.19 Batch Equilibrium Experiments Are Needed for Determining Nonlinear Equilibria or Confirming Equilibrium Constants Obtained from Column Chromatography Measurements / 380

- 6.20 A Competition Factor in the Langmuir Equation Accounts for Cases where Adsorption of One Solute Affects the Other / 385
- 6.21 A Difference in Equilibrium Curves of Two Components Indicates that Separation Is Possible (Langmuir Isotherm) / 385
- 6.22 Differences in Rates of Adsorption May Enable a Separation to be Achieved when the Equilibrium Isotherms for Two Components Are Similar / 386

Hydrodynamics

- 6.23 Compression of Gel-Type Stationary Phases in Packed Beds May Cause Increased Pressure Drops: Case Study for Styrene/DVB Gel-Type Ion-Exchanger / 387
- 6.24 Column to Particle Diameter Should Exceed 80 in Order to Minimize Dispersion by Fingering / 392
- 6.25 Mixing and Dead Volumes Must Be Minimized in Liquid Chromatography Systems: Fittings, Injectors, Tubing, and Feed Distributors / 394
- 6.26 The Mobile Phase Is a Major Operational Cost for Process Liquid Chromatography: WFI Water and Other Solvents / 398
- 6.27 Special Operating Protocols Are Required for Storing the Mobile Phase until It Is Used / 399
- 6.28 Process Hygiene Affects Choice of Materials of Construction for Column Components (Use of NaOH for Cleaning-in-Place) / 399

Productivity and Costs

- 6.29 The Stationary Phase is the Single Most Important Factor for Purification Development / 403
- 6.30 Yield Represents Product Recovery Regardless of Its Extent of Purification / 404
- 6.31 The Productivity, P_{prod}, of a Column for Each Cycle Depends on the Acceptable Extent of Purification of the Product / 405
- 6.32 The Calculation of Costs Is Based on the Productivity of the Separation / 410
- 6.33 Recycle and Moving "Stationary" Phase Chromatography Increase Productivity / 414
- 6.34 A Moving Bed System Moves the "Stationary" Phase to Achieve Continuous Operation / 415
- 6.35 One Form of Continuous Chromatography Moves the Adsorbent by Rotating the Column / 417
- 6.36 Simulated Moving Beds Operate through a Sequential Switching Scheme to Move the Feed and Product Takeoff Points / 425

References / 430

Suggested Reading / 435

Problems / 438

7. PRINCIPLES OF GRADIENT ELUTION CHROMATOGRAPHY

449

Introduction / 449

7.1 The System for Carrying out Gradient Chromatography Is Similar to that for Isocratic Chromatography / 450

Ion Exchange Gradient Chromatography

- Linear Gradient Elution in Ion Exchange Chromatography Is Based on Exchange of a 7.2 Multivalent Protein for a Mono- Or Di-valent Salt / 452
- 7.3 Separation in Ion Exchange Gradient Chromatography Is Driven by the Time-Dependent Increase in Salt Concentration in the Mobile Phase / 453
- 7.4 Gradient Chromatography Is Often Carried out in the Middle of a Purification Sequence / 455
- 7.5 Purification of Recombinant Proteins from E. Coli Requires Steps that First Dissolve and then Refold the Proteins / 457
- 7.6 Anion Exchange Chromatography Is Prominent in the Purification of Blood Products: Pro- and Anticoagulant Factors / 457
- 7.7 Gene Therapy Vectors Can Be Purified by Anion Exchange Chromatography Using Phosphate Buffer and KCl Gradients / 458
- 7.8 Process-Scale Purification of Plasmid DNA Employs a Sequence of Anion Exchange and Size Exclusion Chromatography / 459
- 7.9 An Ion-Exchanger Is a Solid Material That Carries Exchangeable Cations or Anions / 461
- 7.10 Retention Times and Capacity Factors of Charged Species in Ion Exchange Chromatography Are Proportional to Their Charge (with contributions by A. Velayudhan) / 465
- 7.11 The Definition of the Separation Factor Is Based on Differences of Binding Charges of the Two Components A and B / 472
- 7.12 Definitions of Plate Height and Resolution for Linear Gradient Chromatography Are Analogous to Those for Isocratic Chromatography / 474
- 7.13 The Plate Height Increases with Increasing Interstitial Velocity in Linear Gradient Elution Chromatography / 479
- 7.14 Scale-up of Gradient Chromatography Is More Challenging than Isocratic Chromatography since Peak Retention as a Function of Gradient Characteristics Must Either Be Known or Calculated / 481
- 7.15 One Approach to Scale-up of Gradient Chromatography Is Based on Maintaining a Constant Gradient Duration / 482
- 7.16 Material Balances on Both Modulator and Protein Are Needed to Scale Up Linear Gradient Elution when Column Length and/or Gradient Slope Are Changed / 484
- 7.17 Adsorption of the Modulator on the Stationary Phase May Cause Deformation of the Gradient / 492
- 7.18 The Concepts of Gradient Chromatography Can Be Extended to Affinity Membranes / 502
- 7.19 Electrical Gradients May Also Be Used for Chromatographic Separations / 505 References / 508

Suggested Reading / 511

Problems / 511

8. PRINCIPLES OF BIOSEPARATIONS FOR BIOPHARMACEUTICALS AND RECOMBINANT PROTEIN PRODUCTS 514

Introduction / 514

- 8.1 New Biotechnology Products Are the Fastest Growing Area in Bioseparations / 515
- 8.2 Bioseparation Processes Have a Significant Impact on Manufacturing Costs / 515
- 8.3 Bioseparation Economics Are Secondary to Being First to Market . . . until after the New Product Is Introduced / 516
- 8.4 But Cost Is Important when Success of a New Product Generates Competition / 517
- 8.5 Manufacturing Processes for Biologics Are Part of the Product's Regulation / 517

Insulin Case Study

- 8.6 Biosynthetic Human Insulin Is the First Recombinant Polypeptide from E. coli Licensed for Human Use / 518
- 8.7 Recovery and Purification of Human Insulin Requires 27 Steps / 520
- 8.8 Recovery Process Equipment Volumes Are Modest by Chemical Industry Standards / 525
- 8.9 Yield Losses Are Amplified by the Number of Purification Steps / 527
- 8.10 Lys(B28), Pro(B29) Biosynthetic Human Insulin Is a Human Insulin Analog / 527
- 8.11 The Front-End Strategy for Manufacture of LysPro Insulin Is Different from Biosynthetic Human Insulin / 528

Tissue Plasminogen Activator

- 8.12 Tissue Plasminogen Activator Is the First Recombinant Protein Pharmaceutical from Mammalian Cell Culture for Treatment of Heart Attacks / 530
- 8.13 Recombinant Tissue Plasminogen Activator (t-PA) May Be Effective for Treatment of Strokes / 530
- 8.14 Tissue Plasminogen Activator Is a Proteolytic Enzyme / 531
- 8.15 Recombinant Technology Provides the Only Practical Means of t-PA Production / 532
- 8.16 Purification of Tissue Plasminogen Activator Must Remove Cells, Virus, and DNA / 532
- 8.17 Limits on Analytical Detection Require Independent Assays for Proving Virus Removal / 534
- 8.18 The Presence of u-PA Complicates the Purification of t-PA / 535
- 8.19 Process Changes in Manufacture of t-PA, a Biologic, Are Subject to Government Regulations / 537

Classes of Liquid Chromatography

8.20 Purification of Biologics and Drugs Are Based on Five Classes of Chromatography / 539

Ion Exchange Chromatography

8.21 Ion Exchange Is Based on Competition of Charged Species for Stationary Phase Binding Sites / 540

xvi CONTENTS

- 8.22 Titration Curves Give pK and Guide Selection of Stationary Phase / 542
- 8.23 pH, Ion Exchange, Hydrophobic Interactions, and Volatility Characteristics of Buffer Components Guide Specification of Buffer Composition / 544
- 8.24 Amino Acids that Flank Charged Residues Can Alter Protein Binding / 546
- 8.25 Protein Loading Capacity Is Based on Equilibrium Measurements / 546
- 8.26 Large Proteins and Small Pore Sizes Decrease Equilibrium Binding Capacity / 549
- 8.27 Dynamic (Operational) Capacities Are Significantly Lower than Equilibrium Capacities / 551
- 8.28 Ion Exchange Chromatography Separates Proteins by Desorption Using Increasing Salt Gradients / 551
- 8.29 Amphoteric Properties of Proteins Determine Conditions of Ion Exchange Chromatography / 553
- 8.30 Hydroxyapetite (Ca₁₀(PO₄)₆OH₂) Is a Mixed-Mode Ion-Exchanger with Both Weakly Anionic and Weakly Cationic Functional Groups / 555

Size Exclusion (Gel Permeation) Chromatography

- 8.31 Size Exclusion (Gel Permeation) Separates Proteins Based on Differences in Their Size and Shape / 557
- 8.32 Operational Definitions for Elution Volume in Gel Permeation Are Basic to All Types of Chromatography / 562
- 8.33 Representation of Elution Volume on a Chromatogram Depends on Injection Volume of Sample / 563
- 8.34 Distribution Coefficients Depend on Molecule Size and Gel Crosslinking / 564
- 8.35 The Distribution Coefficients, K_d , and K_{av} , Are Not Equivalent / 566
- 8.36 Selectivity Curves Plot Measured Values of K_{av} against Logarithms of Molecular Weight / 568
- 8.37 Buffer Exchange and Desalting Utilize Gel Permeation / 569
- 8.38 Buffer Exchange Can Also Be Achieved Using Membranes / 571
- 8.39 Gel Permeation Requires a Larger Column than Ion Exchange Chromatography / 573

Reversed Phase Chromatography

- 8.40 Reversed Phase Chromatography Can Separate Proteins by Solvophobic (Hydrophobic) and Silanophilic (Hydrophilic) Interactions / 574
- 8.41 Derivatized Silica Particles Are One Type of Reversed Phase Chromatographic Media / 574
- 8.42 Small Particle Silica Columns Benefit from Axial Compression / 576
- 8.43 Polymeric Reversed Phase Chromatographic Media Are Based on Polystyrene and Methacrylic Copolymers / 576
- 8.44 Experimentally Determined Chromatograms Are the Starting Points for Specifying Solvent Composition for Reversed Phase Chromatography / 579
- 8.45 Changes in Relative Peak Retention Occur with Changes in Mobile Phase Polarity / 581

- 8.46 Reversed Phase Chromatography Uses Hydrophobic Media and Increasing Gradients of Aqueous Methanol, Acetonitrile, or Isopropanol / 581
- 8.47 Insulin, a Polypeptide Hormone, Is Purified by Reversed Phase Process Chromatography / 582
- 8.48 Purification of Proteins by Reversed Phase Process Chromatography Is Not Common / 584
- 8.49 Reversed Phase Chromatography Is Widely Used for Analysis of Proteins and Peptide Mapping / 584
- 8.50 Reversed Phase Process Chromatography of Small Molecules Has a History of Use / 587

Hydrophobic Interaction Chromatography

- 8.51 Hydrophobic Interaction Chromatography Utilizes Decreasing Aqueous Gradients of Kosmotropes / 589
- 8.52 The Type and Concentration of Salt Affects Peak Retention in Hydrophobic Interaction Chromatography / 590
- 8.53 Hydrophobic Interaction Chromatography Is Suitable for Many Proteins / 592

Affinity Chromatography

- 8.54 Reversible Binding of Enzymes with Immobilized Substrates and Inhibitors Led to Affinity Chromatography / 593
- 8.55 Careful Selection of Affinity Stationary Phases Minimizes Nonspecific Binding / 594
- 8.56 Activation of the Stationary Phase Surface Precedes Covalent Attachment of Affinity Ligands / 594
- 8.57 Affinity Chromatography Will Not Be a One-Step Purification Process / 596

Bioseparations Process Development

- 8.58 Downstream Processing Requires Multiple Purification Steps Based on Different Molecular Properties / 598
- 8.59 Separation Goals Define Bioseparation Sequences / 602
- 8.60 Specification of Separation Goals Facilitates Selection of Separation Methods / 602
- 8.61 Separation Strategies Combine Categories, Objectives, and Methods into a Logical Sequence of Purification Steps: Example of SEP-OPS Method / 604
- 8.62 Biotechnology Process Development Differs from Chemical Industry Process
 Development / 607
- 8.63 Upstream Production Methods Affect Downstream Purification Strategies / 608
 References / 611
 Suggested Reading / 615
 Problems / 617

9. AFFINITY CHROMATOGRAPHY: BRIDGE BETWEEN MOLECULAR BIOLOGY, COMBINATORIAL METHODS, AND SEPARATIONS SCIENCE

621

Introduction / 621

Affinity Ligands from Combinatorial Libraries

- 9.1 Combinatorial Chemistry Creates Libraries of New Peptide Sequences / 623
- 9.2 Visual Identification Finds a Needle in the Haystack: One Affinity Ligand Among Thousands of Peptides: Factor IX Example / 625
- 9.3 Liquid Chromatography Using 300 mg of Beads Confirms Specificity of Factor IX Binding / 626
- 9.4 Cost-Effective Synthesis of Kilogram Quantities of an Affinity Peptide Remains a Challenge: Estimating Peptide Cost / 628
- 9.5 Combinatorial Synthesis of Carbohydrates and Screening with Biotin Labeled Lectin Gives New Affinity Ligands for Lectins / 629
- 9.6 Bovine Serum Albumin (BSA) Suppresses Nonspecific Binding of the Lectin on an Affinity Support / 633
- 9.7 The Combination of Combinatorial Chemistry and Computers Will Accelerate Drug Discovery and Catalyst Development / 633
- 9.8 Combinatorial Libraries of Small Molecules Can Be Generated by Solid Phase Synthesis or Solution-Based Chemistry / 634

Receptors, Affinity Ligands, and Acquired Immune Deficiency Syndrome (AIDS)

- 9.9 HIV-1 Initially Increases Antigens to HIV Core Protein p24 in the Serum of Infected Persons / 636
- 9.10 An Immobilized Human Antibody Against the Antigen for the HIV Core Protein, p24, Detects AIDS: ELISA Example / 637
- 9.11 HIV Is a Lentivirus, a Type of Retrovirus with Cytopathic Activity / 643
- 9.12 Azidothymidine (AZT) Stops HIV Replication by Inhibiting the Enzyme, Reverse Transcriptase / 644
- 9.13 Protease Inhibitors Prevent Cleavage of gag- and pol-Encoded Viral Proteins Required for Viral Replication / 646
- 9.14 Mutation of HIV to a Drug-Resistant Form Is Promoted by Rapid Turnover of a Large Pool of Infected T-Cells / 647
- 9.15 New Drug Discovery Is Necessitated by Rapidly Mutating HIV Virus / 648
- 9.16 Production Scale-up Is Part of Product Discovery: Case Study of Protease Inhibitors / 652
- 9.17 Coreceptors (Affinity Ligands) Facilitate HIV Binding and Infection: CXCR4, CCR5, and GP 120 / 654
- 9.18 Receptors Are Important to the Dynamics of HIV Transmission and Pathogenesis / 656
- 9.19 A Sensitive and Rapid Screen Helps Find the CXCR4 (Fusin) Protein Receptor in CD4 Cells / 656

Phage and Ribosome Display

- 9.20 Biocombinatorial Generation of Small Protein Ligands Utilizes Bacterial Viruses: Phages / 659
- 9.21 Binding Characteristics of Small Protein Ligands Are Determined by Adding the Phages to Target Molecules Immobilized on Beads / 661
- 9.22 Phage Display Technology Has Generated a Protease Inhibitor with High Affinity and Specificity for Plasmin / 662
- 9.23 Generation and Screening of Proteins by In Vitro Ribosome Display Separates and Amplifies mRNA for a Specific Protein / 663

Receptor Affinity Chromatography

- 9.24 Receptor Affinity Chromatography Selectively-Binds Active Forms of Protein / 665
- 9.25 Receptor Affinity Chromatography Combines Upstream and Downstream Processing Steps / 666
- 9.26 Interleukin-2 Is a Case Study in the Development of Receptor Affinity Chromatography / 666
- 9.27 Either Interleukin-2 or Its Receptor Is an Affinity Ligand for Chromatography of the Other / 667
- 9.28 Multiple Discrete Processing Steps Are Associated with Affinity Chromatography of the IL-2 Receptor / 668

Lectins as Ligands

- 9.29 Lectins Are an Important Laboratory Tool for Affinity Chromatography of Glycoproteins / 670
- 9.30 Concavalin-A Chromatography of α-Galactosidase Demonstrates Affinity Chromatography of a Plant Enzyme / 670

Protein A

- 9.31 Protein A Is One of Many Bacterial Immunoglobulin-Binding Proteins Used in Affinity Chromatography / 673
- 9.32 Protein A Is a Ligand for Process Affinity Chromatography of Immunoadhesions / 676
- 9.33 The Leaching of Ligands from Protein A Affinity Columns Is Difficult to Detect / 677

Immobilized Metal Ions

- 9.34 Counterions Bound to Sulfonic Acid Cation Exchange Resins Complex Sugars through Their Hydroxyl Groups / 678
- 9.35 Ca²⁺ Bound to Sulfonic Acid Resin Separates Stereoisomers of 2,3-Butanediol / 680
- 9.36 Copper (Cu²⁺) and Other Transition Metal Ions, Bound to Iminodiacetate, Complex Proteins through Their Histidine Residues / 680
- 9.37 Elution Buffer and pH Dissociate Metal-Protein Complexes to Achieve Separation / 682
- 9.38 A Chelating Peptide Must Exhibit Rapid Kinetics for Complex Formation to Be Useful as a Purification Handle / 683

XX CONTENTS

- 9.39 Recombinant Forms of Bovine Somatotropin Facilitated Studies of Immobilized Metal Affinity Chromatography / 684
- 9.40 Purification of Recombinant Bovine Somatotropin by Immobilized Metal Affinity Chromatography Requires Many Steps / 684
- 9.41 Heterogeneity of Protein Binding in Immobilized Metal Affinity Chromatography Follows the Temkin Isotherm / 687
- 9.42 Proteins Bind Less Randomly in Biological Receptor Systems than at Surfaces of Chromatographic Stationary Phases / 687

Cellulose Binding Domain Case Study

- 9.43 Cellulases Consist of a Cellulose-Binding Domain and a Catalytic Domain / 690
- 9.44 Cellulose-Binding Domains Modify Cotton Cellulose but Not Bacterial Cellulose nor Avicel / 692
- 9.45 Cellulosic Affinity Chromatography Stationary Phase Must Match Binding Preferences of the Binding Domain Protein and Give Good Flow Properties / 693
- 9.46 Combinations of Proteins with Cellulose Binding Domains Can Be Obtained through Genetic Engineering / 693
- 9.47 Noncovalent Bonding of Cellulose Binding Domain Proteins Could Couple Recombinant Affinity Ligands to Cellulose / 694
- 9.48 Protein A/Cellulose-Binding Domain Protein from E. coli Is an Easily Purified and Immobilized Affinity Ligand / 694
- 9.49 Protein Size Determines Optimum Immobilized Density of Protein A Ligands / 695
- 9.50 Additional Purification Steps Must Follow Affinity Chromatography of IgG over Protein A / 696
- 9.51 Streptavidin-Cellulose-Binding Domain Protein on Avicel Immobilizes Catalytically Active Enzymes / 696
- 9.52 Human Interleukin-2 Can Be Coupled to, Produced with, and Recovered from Cellulose Binding Domain Protein / 698

References / 700

Problems / 705

AUTHOR INDEX 707

SUBJECT INDEX 713