Contents

Preface *xv*
About the Editors *xvii*
List of Abbreviations *xix*
Notation *xxiii*

1 Introduction *1*
Henner Schmidt-Traub and Reinhard Ditz
1.1 Chromatography, Development, and Future Trends *1*
1.2 Focus of the Book *4*
1.3 Suggestions on How to Read this Book *4*
References *6*

2 Fundamentals and General Terminology *9*
Andreas Seidel-Morgenstern
2.1 Principles and Features of Chromatography *9*
2.2 Analysis and Description of Chromatograms *13*
2.2.1 Voidage and Porosity *13*
2.2.2 Retention Times and Capacity Factors *16*
2.2.3 Efficiency of Chromatographic Separations *17*
2.2.4 Resolution *20*
2.2.5 Pressure Drop *23*
2.3 Mass Transfer and Fluid Dynamics *25*
2.3.1 Principles of Mass Transfer *25*
2.3.2 Fluid Distribution in the Column *27*
2.3.3 Packing Nonidealities *28*
2.3.4 Extra-Column Effects *29*
2.4 Equilibrium Thermodynamics *29*
2.4.1 Definition of Isotherms *29*
2.4.2 Models of Isotherms *31*
2.4.2.1 Single-Component Isotherms *31*
2.4.2.2 Multicomponent Isotherms Based on the Langmuir Model *33*
2.4.2.3 Competitive Isotherms Based on the Ideal Adsorbed Solution Theory *34*
2.4.2.4 Steric Mass Action Isotherms *37*

2.4.3 Relation Between Isotherms and Band Shapes *38*
2.5 Column Overloading and Operating Modes *44*
2.5.1 Overloading Strategies *44*
2.5.2 Beyond Isocratic Batch Elution *45*
References *46*

3 Stationary Phases *49*
Michael Schulte
3.1 Survey of Packings and Stationary Phases *49*
3.2 Inorganic Sorbents *50*
3.2.1 Activated Carbons *50*
3.2.2 Synthetic Zeolites *54*
3.2.3 Porous Oxides: Silica, Activated Alumina, Titania, Zirconia, and Magnesia *54*
3.2.4 Silica *55*
3.2.4.1 Surface Chemistry *57*
3.2.4.2 Mass Loadability *59*
3.2.5 Diatomaceous Earth *59*
3.2.6 Reversed Phase Silicas *60*
3.2.6.1 Silanization of the Silica Surface *60*
3.2.6.2 Silanization *60*
3.2.6.3 Starting Silanes *61*
3.2.6.4 Parent Porous Silica *61*
3.2.6.5 Reaction and Reaction Conditions *62*
3.2.6.6 Endcapping *62*
3.2.6.7 Chromatographic Characterization of Reversed Phase Silicas *63*
3.2.6.8 Chromatographic Performance *63*
3.2.6.9 Hydrophobic Properties Retention Factor (Amount of Organic Solvent for Elution), Selectivity *65*
3.2.6.10 Shape Selectivity *65*
3.2.6.11 Silanol Activity *67*
3.2.6.12 Purity *68*
3.2.6.13 Improved pH Stability Silica *68*
3.2.7 Aluminum Oxide *69*
3.2.8 Titanium Dioxide *70*
3.2.9 Other Oxides *71*
3.2.9.1 Magnesium Oxide *71*
3.2.9.2 Zirconium Dioxide *71*
3.2.10 Porous Glasses *72*
3.3 Cross-Linked Organic Polymers *73*
3.3.1 General Aspects *74*
3.3.2 Hydrophobic Polymer Stationary Phases *77*
3.3.3 Hydrophilic Polymer Stationary Phases *78*
3.3.4 Ion Exchange (IEX) *79*
3.3.4.1 Optimization of Ion-Exchange Resins *81*
3.3.5 Mixed Mode *88*
3.3.6 Hydroxyapatite *88*

3.3.7 Designed Adsorbents *91*
3.3.7.1 Protein A Affinity Sorbents *91*
3.3.7.2 Other IgG Receptor Proteins: Protein G and Protein L *96*
3.3.7.3 Sorbents for Derivatized/Tagged Compounds: Immobilized Metal Affinity Chromatography (IMAC) *96*
3.3.7.4 Other Tag-Based Affinity Sorbents *101*
3.3.8 Customized Adsorbents *102*
3.3.8.1 Low Molecular Weight Ligands *105*
3.3.8.2 Natural Polymers (Proteins, Polynucleotides) *108*
3.3.8.3 Artificial Polymers *111*
3.4 Advective Chromatographic Materials *111*
3.4.1 Adsorptive Membranes and Grafted-Polymer Membranes *114*
3.4.2 Adsorptive Nonwovens *115*
3.4.3 Fiber/Particle Composites *117*
3.4.4 Area-Enhanced Fibers *117*
3.4.5 Monolith *118*
3.4.6 Chromatographic Materials for Larger Molecules *121*
3.5 Chiral Stationary Phases *121*
3.5.1 Cellulose- and Amylose-Based CSP *122*
3.5.2 Antibiotic CSP *128*
3.5.3 Cyclofructan-Based CSP *128*
3.5.4 Synthetic Polymers *128*
3.5.5 Targeted Selector Design *130*
3.5.6 Further Developments *132*
3.6 Properties of Packings and Their Relevance to Chromatographic Performance *132*
3.6.1 Chemical and Physical Bulk Properties *132*
3.6.2 Morphology *133*
3.6.3 Particulate Adsorbents: Particle Size and Size Distribution *133*
3.6.4 Pore Texture *134*
3.6.5 Pore Structural Parameters *137*
3.6.6 Comparative Rating of Columns *137*
3.7 Sorbent Maintenance and Regeneration *138*
3.7.1 Cleaning in Place (CIP) *138*
3.7.2 CIP for IEX *140*
3.7.3 CIP of Protein A Sorbents *140*
3.7.4 Conditioning of Silica Surfaces *143*
3.7.5 Sanitization in Place (SIP) *145*
3.7.6 Column and Adsorbent Storage *145*
References *146*

4 Selection of Chromatographic Systems *159*
Michael Schulte
4.1 Definition of the Task *164*
4.2 Mobile Phases for Liquid Chromatography *167*
4.2.1 Stability *168*
4.2.2 Safety Concerns *172*

4.2.3 Operating Conditions *172*
4.2.4 Aqueous Buffer Systems *176*
4.3 Adsorbent and Phase Systems *178*
4.3.1 Choice of Phase System Dependent on Solubility *178*
4.3.2 Improving Loadability for Poor Solubilities *180*
4.3.3 Dependency of Solubility on Sample Purity *183*
4.3.4 Generic Gradients for Fast Separations *184*
4.4 Criteria for Choosing Normal Phase Systems *184*
4.4.1 Retention in NP Systems *186*
4.4.2 Solvent Strength in Liquid–Solid Chromatography *188*
4.4.3 Pilot Technique Thin-Layer Chromatography Using the PRISMA Model *190*
4.4.3.1 Step (1): Solvent Strength Adjustment *199*
4.4.3.2 Step (2): Optimization of Selectivity *199*
4.4.3.3 Step (3): Final Optimization of the Solvent Strength *200*
4.4.3.4 Step (4): Determination of the Optimum Mobile Phase Composition *200*
4.4.4 Strategy for an Industrial Preparative Chromatography Laboratory *202*
4.4.4.1 Standard Gradient Elution Method on Silica *203*
4.4.4.2 Simplified Procedure *204*
4.5 Criteria for Choosing Reversed Phase Systems *206*
4.5.1 Retention and Selectivity in RP Systems *208*
4.5.2 Gradient Elution for Small Amounts of Product on RP Columns *212*
4.5.3 Rigorous Optimization for Isocratic Runs *213*
4.5.4 Rigorous Optimization for Gradient Runs *217*
4.5.5 Practical Recommendations *222*
4.6 Criteria for Choosing CSP Systems *223*
4.6.1 Suitability of Preparative CSP *223*
4.6.2 Development of Enantioselectivity *224*
4.6.3 Optimization of Separation Conditions *226*
4.6.3.1 Determination of Racemate Solubility *226*
4.6.3.2 Selection of Elution Order *226*
4.6.3.3 Optimization of Mobile/Stationary Phase Composition, Including Temperature *226*
4.6.3.4 Determination of Optimum Separation Step *227*
4.6.4 Practical Recommendations *227*
4.7 Downstream Processing of mAbs Using Protein A and IEX *231*
4.8 Size-Exclusion Chromatography (SEC) *236*
4.9 Overall Chromatographic System Optimization *237*
4.9.1 Conflicts During Optimization of Chromatographic Systems *237*
4.9.2 Stationary Phase Gradients *241*
References *246*

5 Process Concepts *251*
Malte Kaspereit and Henner Schmidt-Traub
5.1 Discontinuous Processes *252*

5.1.1 Isocratic Operation *252*
5.1.2 Gradient Chromatography *253*
5.1.3 Closed-Loop Recycling Chromatography *256*
5.1.4 Steady-State Recycling Chromatography (SSRC) *258*
5.1.5 Flip-Flop Chromatography *259*
5.1.6 Chromatographic Batch Reactors *260*
5.2 Continuous Processes *261*
5.2.1 Column Switching Chromatography *262*
5.2.2 Annular Chromatography *262*
5.2.3 Multiport Switching Valve Chromatography (ISEP/CSEP) *263*
5.2.4 Isocratic Simulated Moving Bed (SMB) Chromatography *264*
5.2.5 SMB Chromatography with Variable Process Conditions *268*
5.2.5.1 Varicol *269*
5.2.5.2 PowerFeed *270*
5.2.5.3 Partial-Feed, Partial-Discard, and Fractionation-Feedback Concepts *271*
5.2.5.4 Improved/Intermittent SMB (iSMB) *271*
5.2.5.5 Modicon *273*
5.2.5.6 FF-SMB *273*
5.2.6 Gradient SMB Chromatography *274*
5.2.7 Supercritical Fluid Chromatography (SFC) *275*
5.2.7.1 Supercritical Batch Chromatography *276*
5.2.7.2 Supercritical SMB processes *277*
5.2.8 Multicomponent Separations *277*
5.2.9 Multicolumn Systems for Bioseparations *278*
5.2.9.1 Multicolumn Capture Chromatography (MCC) *279*
5.2.9.2 Multicolumn Countercurrent Solvent Gradient Purification (MCSGP) *286*
5.2.10 Countercurrent Chromatographic Reactors *288*
5.2.10.1 SMB Reactor *288*
5.2.10.2 SMB Reactors with Distributed Functionalities *290*
5.3 Choice of Process Concepts *292*
5.3.1 Scale *292*
5.3.2 Range of k' *292*
5.3.3 Number of Fractions *293*
5.3.4 Example 1: Lab Scale; Two Fractions *293*
5.3.5 Example 2: Lab Scale; Three or More Fractions *294*
5.3.6 Example 3: Production Scale; Wide Range of k′ *296*
5.3.7 Example 4: Production Scale; Two Main Fractions *297*
5.3.8 Example 5: Production Scale; Three Fractions *298*
5.3.9 Example 6: Production Scale; Multistage Process *300*
References *302*

6 Modeling of Chromatographic Processes *311*
Andreas Seidel-Morgenstern
6.1 Introduction *311*
6.2 Models for Single Chromatographic Columns *311*

6.2.1 Equilibrium Stage Models *312*
6.2.1.1 Discontinuous Model According to Craig *313*
6.2.1.2 Continuous Model According to Martin and Synge *315*
6.2.2 Derivation of Continuous Mass Balance Equations *316*
6.2.2.1 Mass Balance Equations *318*
6.2.2.2 Convective Transport *320*
6.2.2.3 Axial Dispersion *320*
6.2.2.4 Intraparticle Diffusion *321*
6.2.2.5 Mass Transfer Between Phases *321*
6.2.2.6 Finite Rates of Adsorption and Desorption *322*
6.2.2.7 Adsorption Equilibria *323*
6.2.3 Equilibrium Model of Chromatography *323*
6.2.4 Models with One Band Broadening Effect *329*
6.2.4.1 Equilibrium Dispersion Model *329*
6.2.4.2 Finite Adsorption Rate Model *331*
6.2.5 Continuous Lumped Rate Models *331*
6.2.5.1 Transport Dispersion Models *332*
6.2.5.2 Lumped Finite Adsorption Rate Model *333*
6.2.6 General Rate Models *333*
6.2.7 Initial and Boundary Conditions of the Column *335*
6.2.8 Dimensionless Model Equations *336*
6.2.9 Comparison of Different Model Approaches *338*
6.3 Including Effects Outside the Columns *343*
6.3.1 Experimental Setup and Simulation Flow Sheet *343*
6.3.2 Modeling Extra-Column Equipment *345*
6.3.2.1 Injection System *345*
6.3.2.2 Piping *345*
6.3.2.3 Detector *345*
6.4 Calculation Methods and Software *346*
6.4.1 Analytical Solutions *346*
6.4.2 Numerical Solution Methods *346*
6.4.2.1 Discretization *346*
6.4.2.2 General Solution Procedure and Software *349*
References *350*

7 Determination of Model Parameters *355*
Andreas Seidel-Morgenstern, Andreas Jupke, and Henner Schmidt-Traub
7.1 Parameter Classes for Chromatographic Separations *355*
7.1.1 Design Parameters *355*
7.1.2 Operating Parameters *356*
7.1.3 Model Parameters *356*
7.2 Concept to Determine Model Parameters *357*
7.3 Detectors and Parameter Estimation *359*
7.3.1 Calibration of Detectors *359*
7.3.2 Parameter Estimation *360*
7.3.3 Evaluation of Chromatograms *362*
7.4 Determination of Packing Parameters *363*

7.4.1 Void Fraction and Porosity of the Packing *363*
7.4.2 Axial Dispersion *363*
7.4.3 Pressure Drop *364*
7.5 Adsorption Isotherms *365*
7.5.1 Determination of Adsorption Isotherms *365*
7.5.2 Estimation of Henry Coefficients *365*
7.5.3 Static Isotherm Determination Methods *370*
7.5.3.1 Batch Method *370*
7.5.3.2 Adsorption–Desorption Method *370*
7.5.3.3 Circulation Method *371*
7.5.4 Dynamic Methods *371*
7.5.5 Frontal Analysis *371*
7.5.6 Analysis of Dispersed Fronts *378*
7.5.7 Peak Maximum Method *380*
7.5.8 Minor Disturbance/Perturbation Method *380*
7.5.9 Curve Fitting of the Chromatogram *383*
7.5.10 Data Analysis and Accuracy *384*
7.6 Mass Transfer Kinetics *386*
7.6.1 Correlations *386*
7.6.2 Application of Method of Moments *388*
7.7 Plant Parameters *389*
7.8 Experimental Validation of Column Models and Model Parameters *391*
7.8.1 Batch Chromatography *391*
7.8.2 Simulated Moving Bed Chromatography *394*
7.8.2.1 Model Formulation and Parameters *394*
7.8.2.2 Experimental Validation *400*
References *404*

8 Process Design and Optimization *409*
Andreas Jupke, Andreas Biselli, Malte Kaspereit, Martin Leipnitz, and Henner Schmidt-Traub
8.1 Basic Principles and Definitions *409*
8.1.1 Performance, Costs, and Objective Functions *409*
8.1.1.1 Performance Criteria *410*
8.1.1.2 Economic Criteria *411*
8.1.1.3 Objective Functions *412*
8.1.2 Degrees of Freedom *413*
8.1.2.1 Categories of Parameters *413*
8.1.2.2 Dimensionless Operating and Design Parameters *414*
8.1.3 Scaling by Dimensionless Parameters *418*
8.1.3.1 Influence of Different HETP Coefficients for Every Component *419*
8.1.3.2 Influence of Feed Concentration *420*
8.1.3.3 Examples for a Single-Column Batch Chromatography *421*
8.1.3.4 Examples for SMB Processes *424*
8.2 Batch Chromatography *426*
8.2.1 Fractionation Mode (Cut Strategy) *426*

8.2.2 Design and Optimization of Batch Chromatographic Columns *427*
8.2.2.1 Process Performance Depending on Number of Stages and Loading Factor *427*
8.2.2.2 Design and Optimization Strategy *432*
8.2.2.3 Other Strategies *436*
8.3 Recycling Chromatography *437*
8.3.1 Design of Steady-State Recycling Chromatography *437*
8.3.2 Scale-Up of Closed-Loop Recycling Chromatography *440*
8.4 Conventional Isocratic SMB Chromatography *445*
8.4.1 Considerations to Optimal Concentration Profiles in SMB Process *445*
8.4.2 Process Design Based on TMB Models (Shortcut Methods) *446*
8.4.2.1 Triangle Theory for an Ideal Model with Linear Isotherms *447*
8.4.2.2 Triangle Theory for an Ideal Model with Nonlinear Isotherms *449*
8.4.2.3 Shortcut to Apply the Triangle Theory on a System with Unknown Isotherms Assuming Langmuir Character *452*
8.4.3 Process Design and Optimization Based on Rigorous SMB Models *455*
8.4.3.1 Estimation of Operating Parameter *456*
8.4.3.2 Optimization of Operating Parameters for Linear Isotherms Based on Process Understanding *457*
8.4.3.3 Optimization of Operating Parameters for Nonlinear Isotherms Based on Process Understanding *458*
8.4.3.4 Optimization of Design Parameters *460*
8.5 Isocratic SMB Chromatography Under Variable Operating Conditions *465*
8.5.1 Performance Comparison of Varicol and Conventional SMB *466*
8.5.2 Performance Comparison of Varicol, PowerFeed, and Modicon with Conventional SMB *470*
8.5.3 Performance Trends Applying SMB Concepts Under Variable Operating Conditions *475*
8.6 Gradient SMB Chromatography *476*
8.6.1 Step Gradient *476*
8.6.2 Multicolumn Solvent Gradient Purification Process *482*
8.7 Multicolumn Systems for Bioseparations *487*
8.7.1 Design of Twin-Column CaptureSMB *488*
8.7.2 Modeling of Multicolumn Capture processes *490*
References *493*

9 Process Control *503*
Sebastian Engell and Achim Kienle
9.1 Standard Process Control *504*
9.2 Advanced Process Control *504*
9.2.1 Online Optimization of Batch Chromatography *505*
9.2.2 Advanced Control of SMB Chromatography *507*
9.2.2.1 Purity Control for SMB Processes *508*
9.2.2.2 Direct Optimizing Control of SMB Processes *510*

9.2.3 Advanced Parameter and State Estimation for SMB Processes *515*
9.2.4 Adaptive Cycle-to-Cycle Control *517*
9.2.5 Control of Coupled Simulated Moving Bed Processes for the Production of Pure Enantiomers *519*
References *521*

10 Chromatography Equipment: Engineering and Operation *525*
Henner Schmidt-Traub and Arthur Susanto
10.1 Challenges for Conceptual Process Design *525*
10.1.1 Main Cost Factors for a Chromatographic System *527*
10.1.2 Conceptual Process Design *528*
10.1.2.1 A Case Study: Large-Scale Biotechnology Project *529*
10.2 Engineering Challenges *533*
10.2.1 Challenges Regarding Sanitary Design *535*
10.2.2 Challenges During Acceptance Tests and Qualifications *539*
10.3 Commercial Chromatography Columns *540*
10.3.1 General Design *541*
10.3.1.1 Manually Moved Piston *542*
10.3.1.2 Electrically or Hydraulically Moved Piston *542*
10.3.2 High- and Low-Pressure Columns *543*
10.3.2.1 Chemical Compatibility *544*
10.3.2.2 Frit Design *546*
10.3.2.3 Special Aspects of Bioseparation *549*
10.4 Commercial Chromatographic Systems *551*
10.4.1 General Design Aspects: High-Pressure and Low-Pressure Systems *551*
10.4.2 Material *553*
10.4.3 Batch Low-Pressure Liquid Chromatographic (LPLC) Systems *553*
10.4.3.1 Inlets *553*
10.4.3.2 Valves to Control Flow Direction *555*
10.4.3.3 Pumps *556*
10.4.3.4 Pump- and Valve-Based and Gradient Formation *556*
10.4.4 Batch High-Pressure Liquid Chromatography *558*
10.4.4.1 General Layout *558*
10.4.4.2 Inlets and Outlets *559*
10.4.4.3 Pumps *559*
10.4.4.4 Valves and Pipes *562*
10.4.5 Continuous Systems: Simulated Moving Bed *563*
10.4.5.1 General Layout *563*
10.4.5.2 A Key Choice: The Recycling Strategy *565*
10.4.5.3 Pumps, Inlets, and Outlets *566*
10.4.5.4 Valves and Piping *566*
10.4.6 Auxiliary Systems *567*
10.4.6.1 Slurry Preparation Tank *567*
10.4.6.2 Slurry Pumps and Packing Stations *568*
10.4.6.3 Cranes and Transport Units *568*

10.4.6.4 Filter Integrity Test *568*
10.4.7 Detectors *569*
10.5 Packing Methods *571*
10.5.1 Column and Packing Methodology Selection *571*
10.5.2 Slurry Preparation *572*
10.5.3 Column Preparation *574*
10.5.4 Flow Packing *575*
10.5.5 Dynamic Axial Compression (DAC) Packing *577*
10.5.6 Stall Packing *577*
10.5.7 Combined Method (Stall + DAC) *578*
10.5.8 Vacuum Packing *580*
10.5.9 Vibration Packing *581*
10.5.10 Column Equilibration *582*
10.5.11 Column Testing and Storage *583*
10.5.11.1 Test Systems *583*
10.5.11.2 Hydrodynamic Properties and Column Efficiency *584*
10.5.11.3 Column and Adsorbent Storage *585*
10.6 Process Troubleshooting *585*
10.6.1 Technical Failures *586*
10.6.2 Loss of Performance *587*
10.6.2.1 Pressure Increase *587*
10.6.2.2 Loss of Column Efficiency *590*
10.6.2.3 Variation of Elution Profile *591*
10.6.2.4 Loss of Purity/Yield *592*
10.6.3 Column Stability *592*
10.7 Disposable Technology for Bioseparations *593*
10.7.1 Prepacked Columns *596*
10.7.2 Membrane Chromatography *597*
References *599*

Appendix A Data of Test Systems *601*
A.1 EMD53986 *601*
A.2 Tröger's Base *602*
A.3 Glucose and Fructose *604*
A.4 β-Phenethyl Acetate *606*
References *607*

Index *609*