Contents

Preface *page* ix
Notation xi

1 Introduction to Biopharmaceutical Processes 1
1.1 Context 1
1.2 Single-Unit Operations 1
1.2.1 Cell Culture 2
1.2.2 Primary Recovery 3
1.2.3 Protein Capture 4
1.2.4 Polishing Steps 5
1.2.5 Viral Clearance 5
1.2.6 Formulation 6
1.2.7 Additional steps 6
1.3 Overview of the Impurities to Be Removed 7
1.3.1 Process-Related and Product-Related Impurities 7
1.3.2 Purity Specifications 8
1.4 Continuous Production Processes 9
1.4.1 Definition of Batch and Continuous Processes 10
1.4.2 Industrial Context 12
1.4.3 Some Engineering Considerations 13

2 Fundamentals of Protein Chromatography 27
2.1 Introduction 27
2.2 Interactions with Chromatographic Media 27
2.2.1 Steric Interactions 27
2.2.2 Hydrophobic Interactions 28
2.2.3 Electrostatic Interactions 29
2.2.4 Biospecific Interactions 30
2.2.5 Complexation 31
2.2.6 Multimodal Interactions 32
2.3 Modes of Operation 33
2.3.1 Single-Column Systems 33
2.3.2 Multicolumn Systems 35

2.4 Mechanistic Models 35
2.4.1 Overview 36
2.4.2 Thermodynamics of Fluid–Solid Equilibrium 39
2.4.3 Hydrodynamics 51
2.4.4 Kinetics of Mass Transfer 55
2.4.5 Column Efficiency 62
2.5 Model Parameter Estimation 67
2.5.1 Generalities 67
2.5.2 Thermodynamics of Fluid–Solid Equilibrium 68
2.5.3 Hydrodynamics 73
2.5.4 Kinetics of Mass Transfer 74
2.5.5 Case Study 74
2.6 Process Design 78
2.6.1 Process Performance Criteria 78
2.6.2 Process Optimization 79
2.6.3 Shortcut Design 80
2.7 Conclusion 83

3 Countercurrrent Separation Processes 84
3.1 Introduction 84
3.2 Countercurrent Separation for Idealized Systems 84
3.2.1 The Equilibrium Stage 84
3.2.2 Cascade of Equilibrium Stages 87
3.2.3 Two-Zone Countercurrent Separation Unit 88
3.2.4 Four-Zone Countercurrent Separation Unit 91
3.3 The Simulated Moving Bed Process 93
3.3.1 Principle 93
3.3.2 Dynamic Behavior 95
3.4 Separation Region for More Realistic Systems 95
3.4.1 Impact of Nonlinearities in the Fluid–Solid Equilibria 97
3.4.2 Impact of Dispersive Phenomena 99
3.5 Design of Countercurrent Chromatographic Processes 101
3.5.1 Unified Design Approach 101
3.5.2 Empirical Design of a Multicolumn Process from a Single-Column Chromatogram 104
3.5.3 Shortcut Design of Countercurrent Chromatographic Processes 105
3.5.4 Benefits from Countercurrent Chromatographic Processes 106
3.6 Conclusion 108

4 Countercurrent Chromatography for the Capture Step 110
4.1 Introduction 110
4.2 Process Operations and Associated Physical Phenomena 111
4.2.1 Typical Sequence of Process Operations 111

4.2.2 Thermodynamics of Fluid–Solid Equilibrium 112
4.2.3 Hydrodynamics and Kinetics of Mass Transfer 116
4.3 Design of Countercurrent Chromatographic Processes 125
4.3.1 Principle 125
4.3.2 Definition of the Process Variables 127
4.3.3 Processes with a Number of Columns per Zone Constant during One Cycle 128
4.3.4 Processes with a Number of Columns per Zone Changing during One Cycle 141
4.3.5 Process Optimization 146
4.3.6 Process Control 149
4.4 Conclusion 152

5 Countercurrent Chromatography for the Polishing Steps 153
5.1 Introduction 153
5.2 Process Operations and Associated Physical Phenomena 153
5.2.1 Chromatographic Modes 153
5.2.2 Thermodynamics of the Fluid-Solid Equilibrium 156
5.2.3 Hydrodynamics and Kinetics of Mass Transfer 161
5.3 Design of Countercurrent Chromatographic Processes 167
5.3.1 Binary Separations 167
5.3.2 Ternary Separations 179
5.3.3 Process Control 200
5.4 Conclusion 201

6 Protein Conjugation 203
6.1 Introduction 203
6.2 The Conjugation Reaction 204
6.2.1 Conjugation Chemistry 204
6.2.2 Kinetics of the Conjugation Reaction 206
6.2.3 Conjugation Reactors 216
6.3 Purification of Conjugated Proteins 225
6.3.1 Separation Challenges 225
6.3.2 Filtration 226
6.3.3 Chromatography 228
6.4 Process Integration 238
6.4.1 Motivations 238
6.4.2 Protein Recycling and Overall Conversion 239
6.4.3 Process Performances: Yield and Productivity 241
6.4.4 Batch or Continuous Operation? 244
6.5 Conclusion 245

7 Protein Aggregation in Biopharmaceutical Processes 247
7.1 Introduction 247
7.2 Experimental Characterization of Protein Solutions 248
7.2.1 Aggregate Content, Size, and Morphology 248
7.2.2 Protein–Protein Interactions 251
7.2.3 Protein Structure 254
7.2.4 Solution Viscosity 256
7.2.5 Summary 257
7.3 Protein Aggregation Mechanisms 257
7.3.1 Colloidal Stability and Conformational Stability 257
7.3.2 Aggregation Pathway 262
7.3.3 Aggregation Rate Constants 264
7.3.4 Population Balance Equations 266
7.4 Impact of Operating Conditions on Protein Aggregation 269
7.4.1 Solution pH 269
7.4.2 Salt Type and Concentration 271
7.4.3 Temperature 274
7.4.4 Protein Concentration 275
7.5 Critical Steps for Aggregation in Biopharmaceutical Processes 278
7.5.1 Upstream 278
7.5.2 Capture Step 279
7.5.3 Polishing Steps 279
7.5.4 Viral Inactivation 283
7.5.5 Filtration 284
7.5.6 Summary 287
7.6 Methods to Reduce the Aggregate Content 287
7.6.1 Limiting Aggregate Formation 287
7.6.2 Removing Aggregates 292
7.7 Conclusion 296

8 Conclusion 299

Bibliography 301
Index 326