Continuous Processing in Pharmaceutical Manufacturing

Edited by Ganapathy Subramanian
Related Titles

Subramanian, G. (ed.)

Biopharmaceutical Production Technology
2012
Print ISBN: 978-3-527-33029-4 (Also available in a variety of electronic formats)

Flickinger, M.C. (ed.)

Encyclopedia of Industrial Biotechnology
Bioprocess, Bioseparation, and Cell Technology, 7V
2010
Print ISBN: 978-0-471-79930-6 (Also available in a variety of electronic formats)

Flickinger, M.C. (ed.)

Downstream Industrial Biotechnology
Recovery and Purification
2013
Print ISBN: 978-1-118-13124-4 (Also available in a variety of electronic formats)

Carta, G., Jungbauer, A.

Protein Chromatography
Process Development and Scale-Up
2010
Print ISBN: 978-3-527-31819-3 (Also available in a variety of electronic formats)

Eibl, R., Eibl, D. (eds.)

Single-Use Technology in Biopharmaceutical Manufacture
2011
Print ISBN: 978-0-470-43351-5 (Also available in a variety of electronic formats)
Contents

List of Contributors  XVII
Preface  XXIII

1  Proteins Separation and Purification by Expanded Bed Adsorption and Simulated Moving Bed Technology  1
   Ping Li, Pedro Ferreira Gomes, José M. Loureiro, and Alirio E. Rodrigues
   1.1 Introduction  1
   1.2 Protein Capture by Expanded Bed Technology  3
   1.2.1 Adsorbent Materials  3
   1.2.2 Expanded Bed Adsorption/Desorption of Protein  10
   1.2.3 Modeling of the Expanded Bed  13
   1.3 Proteins Separation and Purification by Salt Gradient Ion Exchange SMB  15
   1.3.1 Adsorption Isotherms and Kinetics of BSA and Myoglobin on Ion Exchange Resins  16
   1.3.2 Salt Gradient Formation and Process Design for IE-SMB Chromatography  20
   1.3.3 Separation Region of Salt Gradient IE-SMB Chromatography  21
   1.3.4 Proteins Separation and Purification in Salt Gradient IE-SMB with Open Loop Configuration  24
   1.4 Conclusion  26
   References  27

2  BioSMB Technology as an Enabler for a Fully Continuous Disposable Biomanufacturing Platform  35
   Marc Bisschops
   2.1 Introduction  35
   2.2 Integrated Continuous Bioprocessing  36
   2.3 Multicolumn Chromatography  39
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4 BioSMB Technology</td>
<td>40</td>
</tr>
<tr>
<td>2.5 Fully Disposable Continuous Processing</td>
<td>44</td>
</tr>
<tr>
<td>2.6 Case Studies</td>
<td>46</td>
</tr>
<tr>
<td>2.7 Regulatory Aspects</td>
<td>47</td>
</tr>
<tr>
<td>2.8 Conclusions</td>
<td>50</td>
</tr>
<tr>
<td>References</td>
<td>51</td>
</tr>
<tr>
<td>3 Impact of Continuous Processing Techniques on Biologics</td>
<td></td>
</tr>
<tr>
<td><strong>Supply Chains</strong></td>
<td>53</td>
</tr>
<tr>
<td>Aloke Das</td>
<td></td>
</tr>
<tr>
<td>3.1 Introduction</td>
<td>53</td>
</tr>
<tr>
<td>3.1.1 The Biologics Industry</td>
<td>53</td>
</tr>
<tr>
<td>3.1.2 The Biologics Value Chain</td>
<td>54</td>
</tr>
<tr>
<td>3.1.3 Downstream Purification Costs</td>
<td>54</td>
</tr>
<tr>
<td>3.2 Chromatography Techniques Used in Downstream Purification</td>
<td>55</td>
</tr>
<tr>
<td>3.2.1 Need for Continuous Manufacturing in Downstream Purification</td>
<td>56</td>
</tr>
<tr>
<td>3.2.2 The Multicolumn Countercurrent Solvent Gradient Purification</td>
<td>58</td>
</tr>
<tr>
<td>Chromatography System</td>
<td></td>
</tr>
<tr>
<td>3.3 Next-Generation Biologic Products – Bispecific Monoclonal Antibodies</td>
<td>59</td>
</tr>
<tr>
<td>3.3.1 Major Biopharma Companies and Their Interest in Bispecific Mabs</td>
<td>59</td>
</tr>
<tr>
<td>3.3.2 Challenges in Purification of Bispecific Monoclonal Antibodies</td>
<td>60</td>
</tr>
<tr>
<td>3.4 Improving the Downstream Processing of Bispecific Mabs</td>
<td></td>
</tr>
<tr>
<td>by Introduction of MCSGP in the Value Chain</td>
<td>61</td>
</tr>
<tr>
<td>3.4.1 Advantages of Utilizing MCSGP Process in Bispecific Mabs Purifica-</td>
<td>61</td>
</tr>
<tr>
<td>tion as Compared to Batch Chromatography</td>
<td></td>
</tr>
<tr>
<td>3.4.2 Impact of MCSGP System on Biologic Supply Chains</td>
<td>62</td>
</tr>
<tr>
<td>3.4.3 Impact on Patent Approval Structure of Biologic Drugs</td>
<td>62</td>
</tr>
<tr>
<td>3.4.3.1 For a Manufacturer Who Already has a Biologic Drug in the</td>
<td>62</td>
</tr>
<tr>
<td>Market</td>
<td></td>
</tr>
<tr>
<td>3.4.3.2 For a Manufacturer Who is Developing a Biologic Drug</td>
<td>62</td>
</tr>
<tr>
<td>3.4.4 Impact on Big Biopharma Companies</td>
<td>63</td>
</tr>
<tr>
<td>3.4.5 Impact on the Chromatography Market</td>
<td>64</td>
</tr>
<tr>
<td>3.4.6 Limitations of the MCSGP System</td>
<td>64</td>
</tr>
<tr>
<td>3.5 Conclusion</td>
<td>64</td>
</tr>
<tr>
<td>3.6 Further Research</td>
<td>65</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>66</td>
</tr>
<tr>
<td>3.A Appendix/Additional Information</td>
<td>66</td>
</tr>
<tr>
<td>3.A.1 Regulatory Structure for Bispecific Monoclonal Antibodies</td>
<td>67</td>
</tr>
<tr>
<td>3.A.1.1 Regulatory Compliance Comparison between US, EU, and Emerging</td>
<td>67</td>
</tr>
<tr>
<td>Economies</td>
<td></td>
</tr>
<tr>
<td>References</td>
<td>68</td>
</tr>
</tbody>
</table>
4 Integrating Continuous and Single-Use Methods to Establish a New Downstream Processing Platform for Monoclonal Antibodies 71
Christopher Gillespie, Mikhail Kozlov, Michael Phillips, Ajish Potty, Romas Skudas, Matthew Stone, Alex Xenopoulos, Alison Dupont, Jad Jaber, and William Cataldo

4.1 Introduction 71
4.2 Harvest and Clarification 74
4.2.1 The Challenge and Technology Selection 74
4.2.1.1 Centrifugation 76
4.2.1.2 Filtration 76
4.2.1.3 Impurity Precipitation 77
4.2.2 Summary 77
4.3 Capture 78
4.3.1 Background 78
4.3.1.1 Protein A Chromatography 78
4.3.2 Chromatographic Methods 79
4.3.2.1 Slurried Bed Methods 79
4.3.2.2 Continuous Chromatography 79
4.3.3 Capture Case Studies 82
4.3.3.1 Continuous Protein A Chromatography Capture Case Study 82
4.3.3.2 Effect of Clarification Method on Protein A Performance 83
4.4 Polishing 84
4.4.1 Background 84
4.4.2 Technology Selection Strategy 86
4.4.3 Complete Flow-Through Polishing Case Study 87
4.5 Cost of Goods Analysis 89
4.5.1 Methodology 89
4.5.2 Clarification 89
4.5.3 Capture 90
4.5.4 Polishing 91
4.5.5 Overall 91
4.6 Summary 92
References 93

5 Modeling of Protein Monomer/Aggregate Purification by Hydrophobic Interaction Chromatography: Application to Column Design and Process Optimization 97
Mark-Henry Kamga, Hae Woo Lee, Namjoon Kim, and Seongkyu Yoon

5.1 Introduction 97
5.2 Mathematical Model 99
5.2.1 The Rate-Limiting Step in the HIC Process 99
5.2.2 Dimensional Considerations 100
5.2.2.1 Adsorption Capacity vs. Concentration of Vacant Sites \( q_m vs. C_v \) 100
5.2.2.2 Concentration of Protein Adsorbed on Resin \( q_i vs. C_i \) 100
5.2.3 Mathematical Model 101
5.3 Experimentation 103
5.3.1 Protein Solutions 103
5.3.2 Determination of Adsorption and Desorption Kinetic Constants 104
5.3.3 Column Chromatography 104
5.4 Results and Discussion 105
5.4.1 Kinetic Constants 105
5.4.2 Protein Denaturation 107
5.4.3 Model vs. Experimental Results 108
5.4.4 Applications 109
5.5 Conclusion 112
Acknowledgments 112
References 113

6 Continuous Animal Cell Perfusion Processes: The First Step Toward
Integrated Continuous Biomanufacturing 115
Leda R. Castilho
6.1 Introduction 115
6.2 The Basics of Perfusion Processes 116
6.3 Cell Banking and Inoculum Development in the Context of Perfusion Processes 117
6.4 Culture Conditions 120
6.5 Cell Retention Devices 125
6.5.1 Gravitational Settlers 126
6.5.2 Centrifuges 130
6.5.3 Hydrocyclones 131
6.5.4 Acoustic (Ultrasonic) Separators 134
6.5.5 Tangential Flow-Filtration 134
6.5.6 ATF Systems 136
6.5.7 Floating Membrane Devices 138
6.5.8 Spin-Filters 138
6.5.9 Rotating Cylindrical Filters (Vortex-Flow Filters or External Spin-Filters) 140
6.5.10 Rotating Disc Filters (Controlled-Shear Filters) 141
6.6 Integrated Perfusion–Purification Processes for Continuous Biomanufacturing 142
6.7 Concluding Remarks 144
References 145

7 Perfusion Process Design in a 2D Rocking Single-Use Bioreactor 155
Nico M.G. Oosterhuis
7.1 Introduction 155
7.2 Production Costs 155
7.3 Equipment Requirements for a Single-Use Perfusion Process 157
8 Advances in the Application of Perfusion Technologies to *Drosophila* S2 Insects Cell Culture  
*Lars Poulsen and Willem A. de Jongh*

8.1 Introduction 165
8.2 Case Study 1: Acoustic Separation 167
8.2.1 The Perfusion Setup (BioSep) 167
8.2.2 Results and Discussion 168
8.2.2.1 Development 168
8.2.2.2 Cell Count in the Bioreactor 168
8.2.2.3 Effects of BioSep Settings on Cell Loss and Viability 169
8.2.2.4 Controlling the Cell Concentration Through Bleed Rate Control 169
8.2.2.5 Effect of Total Dilution Rate on Culture Viability 170
8.2.2.6 Development of the Perfusion Rate Profile 170
8.2.2.7 Initial Testing of Robustness of Upstream Process in 1.5 l Fermentations 170
8.2.2.8 Scaling Up and Consistency in 4.5 l Fermentations 171
8.2.2.9 Process Scale-Up 174
8.2.3 Conclusions for Case Study 1 174
8.3 Case Study 2: ATF-Based Cell Retention 176
8.3.1 ATF Technology 176
8.3.2 Methods 177
8.3.3 Results 177
8.3.3.1 Cell Counts Achieved Using Perfusion Technology 177
8.3.3.2 Effect of Feed Strategy 178
8.3.3.3 Yield Improvements Achieved Using Fed-Batch and Concentrated Perfusion 179
8.3.3.4 Protein Stability 179
8.3.4 Conclusions for Case Study 2 180
8.4 Final Remarks 181

9 Single-Use Systems Support Continuous Bioprocessing by Perfusion Culture  
*William G. Whitford*

9.1 Introduction 183
9.2 Potential Advantages in Continuous Processing 187
9.2.1 Improved Product Quality 187
9.2.2 Ease in Process Development 188
11 Monoclonal Antibody Continuous Processing Enabled by Single Use
Mark Brower, Ying Hou, and David Pollard

11.1 Introduction

11.1.1 Single-Use Revolution to Enable Process Intensification and Continuous Processing

11.1.2 Principles of Continuous Multicolumn Chromatography for Biological Production (BioSMB)

11.2 Continuous Downstream Processing for Monoclonal Antibodies Unit Operation Development

11.2.1 Surge Vessels and Balancing Flows

11.2.2 Primary Recovery: Centrifugation and Depth Filtration

11.2.3 Bulk Purification: Continuous Multicolumn Chromatography – BioSMB Protein A Capture and Viral Inactivation

11.2.3.1 Protein A Loading Zone Optimization

11.2.3.2 Protein A Elution Zone Considerations

11.2.3.3 Viral Inactivation

11.2.4 Fine Purification: Flow-Through Anion Exchange Chromatography (AEX)

11.2.4.1 Effects of Sample Flow Rate on AEX Membrane Chromatography

11.2.4.2 Effect of Sample Loading Amount on AEX Membrane Chromatography

11.2.4.3 Scaling-Up Membrane Chromatography for Continuous Processing

11.2.5 Fine Purification: Continuous Multicolumn Chromatography – BioSMB Cation Exchange Chromatography

11.2.5.1 Cation Exchange Loading Zone Optimization

11.2.5.2 Cation Exchange Elution Zone Considerations

11.2.6 Formulation: Continuous Ultrafiltration

11.3 Pilot-Scale Demonstration of the Integrated Continuous Process

11.4 Summary

References

12 Continuous Production of Bacteriophages
Aleš Podgornik, Nika Janež, Franc Smrekar, and Matjaž Peterka

12.1 Bacteriophages

12.1.1 Life Cycle

12.1.2 Determination of Bacteriophage Properties

12.2 Bacteriophage Cultivation

12.2.1 Chemostat

12.2.2 Cellstat

12.2.3 Cellstat Productivity

12.2.4 Bacteriophage Selection

12.2.5 Technical Challenges
12.3 Continuous Purification of Bacteriophages 325
12.3.1 Centrifugation 326
12.3.2 Precipitation and Flocculation 326
12.3.3 Filtration 327
12.3.4 Chromatographic and Other Adsorption Methods 328
12.4 Conclusions 329
References 329

13 Very High Cell Density in Perfusion of CHO Cells by ATF, TFF, Wave Bioreactor, and/or CellTank Technologies – Impact of Cell Density and Applications 339
Véronique Chotteau, Ye Zhang, and Marie-Francoise Clincke
13.1 Introduction 339
13.2 Equipment 340
13.3 Results and Discussion 342
13.3.1 Perfusion Using ATF or TFF in Wave-Induced Bioreactor 342
13.3.1.1 Cell Growth 342
13.3.1.2 IgG Production 344
13.3.2 Perfusion Using CellTank 346
13.3.2.1 Cell Growth 346
13.3.2.2 IgG Production 347
13.3.3 Very High Cell Density 347
13.3.4 Cryopreservation from Very High Cell Density Perfusion 350
13.4 Conclusions 353
Acknowledgments 354
References 354

14 Implementation of CQA (Critical Quality Attribute) Based Approach for Development of Biosimilars 357
Sanjeev K. Gupta
14.1 Background 357
14.2 Biosimilar Product Development 358
14.3 Attributes/Parameters in Biopharmaceuticals 359
14.3.1 Critical Quality Attributes 359
14.3.2 Critical Process Parameters (CPP) 359
14.3.3 The ICH Q8 “Minimal Approach” to Pharmaceutical Development 359
14.3.4 Quality-by-Design 360
14.4 Quality Attributes and Biosimilars Development 361
14.5 Quality, Safety, and Efficacy of Biosimilars 362
14.6 Implementing CQA Approach for Biosimilar Development 364
14.6.1 Identification of the CQA 364
14.6.2 CQA-Based Clone Selection and Upstream Process Development 365
14.6.3 Factors Affecting CQAs of the Biologics 366
16.3 Todays Facility Designs 418
16.3.1 Construction and Design Types 418
16.3.2 Process Location and Flow 422
16.4 Future Processing and Facility Requirements 424
16.4.1 Upstream Technologies 424
16.4.2 Downstream Technologies 426
16.4.3 Single-Use Engineering and Design 427
16.4.4 Facilities and Process Design 428
References 431

17 Evaluating the Economic and Operational Feasibility of Continuous Processes for Monoclonal Antibodies 433
Suzanne S. Farid, James Pollock, and Sa V. Ho
17.1 Introduction 433
17.2 Background on Continuous Processing 434
17.2.1 Perfusion Culture 434
17.2.2 Semicontinuous Chromatography 436
17.3 Tool Description 438
17.4 Case Study 1: Fed-batch Versus Perfusion Culture for Commercial mAb Production 440
17.5 Case Study 2: Semicontinuous Affinity Chromatography for Clinical and Commercial Manufacture 446
17.6 Case Study 3: Integrated Continuous Processing Flowsheets 450
17.7 Conclusions 452
Acknowledgments 452
References 453

18 Opportunities and Challenges for the Implementation of Continuous Processing in Biomanufacturing 457
Sadettin S. Ozturk
18.1 Introduction 457
18.2 A Brief History of Continuous Processing in Biomanufacturing 458
18.3 Opportunities for Continuous Processing in Biomanufacturing 459
18.3.1 Higher Process Yields 459
18.3.2 Higher Process Efficiencies 461
18.3.3 Compact and Flexible Facilities 461
18.3.4 Stable and Consistent Production 462
18.3.5 Better Product Quality 462
18.4 Challenges for Implementing Continuous Processing in Biomanufacturing 462
18.4.1 Process Complexity 463
18.4.1.1 Cell Retention 463
18.4.1.2 High Cell Density 466
18.4.1.3 Longer Run Times 467
18.4.2 Process Scalability in a Continuous Perfusion Process 470
18.4.2.1 Scale and Capacity Limitations 470
18.4.2.2 Process Scale-up 471
18.4.3 Process Consistency and Control 473
18.4.4 Process Characterization and Validation 474
18.4.4.1 Complexity of a Scale-down Model for a Perfusion Process 474
18.4.4.2 Process Optimization and Characterization for a Perfusion Process 475
18.4.4.3 Process Validation 475
18.5 Conclusions 476
Acknowledgment 476
References 476

19 The Potential Impact of Continuous Processing on the Practice and Economics of Biopharmaceutical Manufacturing 479
   L. Richard Stock, Marc Bisschops, and Thomas C. Ransohoff
19.1 Introduction 479
19.2 Background (Review of Status Quo – How We Make Biopharmaceutical Products Today) 480
19.3 The Rationale for Continuous Processing 483
19.4 The Obstacles for Implementation of Continuous Processing for Biopharmaceuticals 485
19.5 The Potential Impact of Continuous Manufacturing on Process Economics 487
19.6 The Potential Impact of Continuous Processing on Biopharmaceutical Manufacturing Practices 490
19.7 Summary 492
References 492

Index 495
List of Contributors

Marc Bisschops
Tarpon Biosystems, Inc.
Worcester, MA
USA

and

Tarpon Biosystems Europe B.V.
BioScience Park
Archimedesweg 17
2333 CM Leiden
The Netherlands

William Cataldo
EMD Millipore
80 Ashby Rd.
Bedford, MA 01730
USA

Véronique Chotteau
KTH (Royal Institute of Technology)
School of Biotechnology
Cell Technology Group
Roslagstullsbacken 21
106 91 Stockholm
Sweden

Marie-Francoise Clincke
KTH (Royal Institute of Technology)
School of Biotechnology
Cell Technology Group
Roslagstullsbacken 21
106 91 Stockholm
Sweden

and

UCB Pharma S.A.
Allée de la Recherche, 60
1070 Brussels
Belgium

Mark Brower
Merck & Co., Inc.
Merck Research Labs
BioProcess Development
2000 Galloping Hill Road
Kenilworth, NJ 07033
USA

Leda R. Castilho
Federal University of Rio de Janeiro (UFRJ)
Cell Culture Engineering Laboratory
COPPE, Chemical Engineering Program
21941-972 Rio de Janeiro
Brazil

and

Véronique Chotteau
KTH (Royal Institute of Technology)
School of Biotechnology
Cell Technology Group
Roslagstullsbacken 21
106 91 Stockholm
Sweden

and

UCB Pharma S.A.
Allée de la Recherche, 60
1070 Brussels
Belgium
List of Contributors

Aloke Das
Supply chain Management
Senior Analyst at Dell Inc.
Ireland

Willem A. de Jongh
ExpreS2ion Biotechnologies
DTU Science Park
Ager Allé 1
2970 Horsholm
Denmark

Alison Dupont
EMD Millipore
80 Ashby Rd.
Bedford, MA 01730
USA

Suzanne S. Farid
University College London
Department of Biochemical Engineering
The Advanced Centre for Biochemical Engineering
Torrington Place
London WC1E 7JE
UK

Pedro Ferreira Gomes
University of Porto
Faculty of Engineering
Laboratory of Separation and Reaction Engineering (LSRE)
Associate Laboratory LSRE/LCM
Department of Chemical Engineering
Rua Dr. Roberto Frias, s/n
4200-465 Porto
Portugal

Christopher Gillespie
EMD Millipore
80 Ashby Rd.
Bedford, MA 01730
USA

Sanjeev K. Gupta
Ipca Laboratories Ltd.
Department of Biotechnology (R&D)
Plot #125, Kandivali Industrial Estate, Kandivali (W)
Mumbai 460007
Maharashtra
India

Sa V. Ho
Pfizer
Biotherapeutics Pharmaceutical Sciences
1 Burtt Road
Andover, MA 01810
USA

Ying Hou
Merck & Co., Inc.
Merck Research Labs
BioProcess Development
2000 Galloping Hill Road
Kenilworth, NJ 07033
USA

Jad Jaber
EMD Millipore
80 Ashby Rd.
Bedford, MA 01730
USA
List of Contributors

Nika Janež
The Centre of Excellence for Biosensors, Instrumentation and Process Control – COBIK
Center for Biotechnology
Tovarniška 26
5270 Ajdovščina
Slovenia

Maik W. Jornitz
G-CON Manufacturing Inc.
6161 Imperial Loop
College Station, TX 77845
USA

Mark-Henry Kamga
University of Massachusetts Lowell
Department of Chemical Engineering
1 University Avenue
Lowell, MA 01854
USA

Namjoon Kim
University of Massachusetts Lowell
Department of Chemical Engineering
1 University Avenue
Lowell, MA 01854
USA

Mikhail Kozlov
EMD Millipore
80 Ashby Rd.
Bedford, MA 01730
USA

Hae Woo Lee
Clinical Manufacturing Center
Daegu-Gyeongbuk Medical Innovation Foundation
Cheombok-ro 80
Dong-gu, Daegu
South Korea

Ping Li
East China University of Science and Technology
State Key Laboratory of Chemical Engineering
College of Chemical Engineering
130 Meilong Road, Xuhui
Shanghai 200237
China

José M. Loureiro
University of Porto
Faculty of Engineering
Laboratory of Separation and Reaction Engineering (LSRE)
Associate Laboratory LSRE/LCM
Department of Chemical Engineering
Rua Dr. Roberto Frias, s/n
4200-465 Porto
Portugal

Sunil Mehta
kSep Systems
1101 Hamlin Road
Durham, NC 27704
USA

Massimo Morbidelli
ETH Zurich
Institute for Chemical and Bioengineering
Vladimir-Prelog-Weg 1
HCI F 129
8093 Zurich
Switzerland
and

Chairman Dept. of Chemistry &
Applied Biosciences
Institute for Chemical and
Bioengineering
ETH Zurich
Vladimir-Prelog-Weg 1/HCI F129
CH-8093 Zurich-Hoenggerberg

Thomas Müller-Späth
ETH Zurich
Institute for Chemical and
Bioengineering
Vladimir-Prelog-Weg 1
HCI F 137
8093 Zurich
Switzerland

Nico M.G. Oosterhuis
Easthouse Biotech Solutions BV
Landschrijverlaan 35
9451KT Rolde
The Netherlands

Sadettin S. Ozturk
MassBiologics of the University of
Massachusetts Medical School
Process and Analytical
Development
460 Walk Hill Street
Mattapan, MA 02126
USA

Matjaž Peterka
The Centre of Excellence for
Biosensors, Instrumentation and
Process Control – COBIK
Center for Biotechnology
Tovarniška 26
5270 Ajdovščina
Slovenia

Michael Phillips
EMD Millipore
80 Ashby Rd.
Bedford, MA 01730
USA

Aleš Podgornik
The Centre of Excellence for
Biosensors, Instrumentation and
Process Control – COBIK
Center for Biotechnology
Tovarniška 26
5270 Ajdovščina
Slovenia

and

Faculty of Chemistry and Chemical
Technology
Ljubljana University
Večna pot 113
1000 Ljubljana
Slovenia

David Pollard
Merck & Co., Inc.
Merck Research Labs
BioProcess Development
2000 Galloping Hill Road
Kenilworth, NJ 07033
USA

James Pollock
University College London
Department of Biochemical
Engineering
The Advanced Centre for
Biochemical Engineering
Torrington Place
London WC1E 7JE
UK
Ajish Potty
EMD Millipore
80 Ashby Rd.
Bedford, MA 01730
USA

Lars Poulsen
ExpreS2ion Biotechnologies
DTU Science Park
Agern Allé 1
2970 Horsholm
Denmark

Thomas C. Ransohoff
BioProcess Technology Consultants, Inc.
12 Gill Street
Woburn, MA 01801-1728
USA

Alirio E. Rodrigues
University of Porto
Faculty of Engineering
Laboratory of Separation and Reaction Engineering (LSRE)
Associate Laboratory LSRE/LCM
Department of Chemical Engineering
Rua Dr. Roberto Frias, s/n
4200-465 Porto
Portugal

Romas Skudas
Merck Millipore
Frankfurter Str. 250
64293 Darmstadt
Germany

Franc Smrekar
Jafral d.o.o.
Koprska ulica 94
1000 Ljubljana
Slovenia

L. Richard Stock
BioProcess Technology Consultants, Inc.
12 Gill Street
Woburn, MA 01801-1728
USA

Matthew Stone
EMD Millipore
80 Ashby Rd.
Bedford, MA 01730
USA

William G. Whitford
GE Healthcare
HyClone Cell Culture
925 West 1800 South
Logan, UT 84321
USA

Alex Xenopoulos
EMD Millipore
80 Ashby Rd.
Bedford, MA 01730
USA

Seongkyu Yoon
University of Massachusetts Lowell
Department of Chemical Engineering
1 University Avenue
Lowell, MA 01854
USA

Ye Zhang
KTH (Royal Institute of Technology)
School of Biotechnology
Cell Technology Group
Roslagstullsbacken 21
106 91 Stockholm
Sweden
Preface

A continuous process requires the ability to think laterally and have a proactive mindset across the entire team from lab development through to production. Continuous manufacturing process is not new. It has been in use by the chemical, food, and beverage industries successfully. The biopharmaceutical industries are reluctant to engage in applying advanced technology on continuous processes, and are still using the batch process, which has been in use since the nineteenth century. The batch process is an archaic process that progresses sequentially step by step, creating a specified and fixed amount of therapeutic product, which in modern times is not state-of-the-art. Several reviews and articles have shown that considerable advances have been made by technologist in offering systems for continuous processes. It has been established that continuous processing promises efficiency because it is a well controlled and flexible process, and there is less waste and produces higher quality products. There is considerable economic benefit in applying the continuous process in manufacturing.

Momentum is gathering pace behind the implementation of continuous manufacturing in the pharmaceutical industry. The regulatory bodies are now encouraging companies to move toward continuous manufacturing. Consequently, leading biopharma industries seem to be in the mend of thinking that the time is right for a major effort in the development of continuous processes in their organizations. As more companies look at the practical evidence from pilot and demonstration units, the adoption and commercialization of the new technology is picking up speed and currently several leading global biopharmaceutical industries are moving to implement continuous manufacturing processes in collaboration with technologist and suppliers. It will not be far away that industries will apply the continuous manufacturing process and thus we are setting up a Gold standard for the future, maybe in 10 years or more.

This book presents the most recent scientific and technological advances of continuous processing, as well as methods and applications in the field of biomanufacturing. Each chapter provides introductory material with an overview of the topic of interest; a description of the technology and methods, protocols, instrumentation, and application, and a collection of published data with an extensive list of references for further details.
It is our hope that this book will stimulate a greater appreciation of the usefulness, efficiency, and the potential of single-use systems in continuous processing of biopharmaceuticals, and that it will stimulate further progress and advances in the field of continuous processing to meet the ever-increasing demands and challenges in the manufacturing of therapeutic products.

The completion of this book has been made possible with the help and encouragements of many friends and colleagues. It is a great pleasure for me to acknowledge, with deep gratitude, the contribution of 19 authors of the chapters in this book. Their outstanding work and thoughtful advice throughout the project have been important in achieving the breadth and depth of this book.

I would be most grateful for any suggestions that could serve to improve future editions of this volume.

Finally, my deep appreciation to Dr Frank Weinreich of Wiley-VCH for inviting me to edit the volume and also to Lesley Fenske and her colleagues for their sustained encouragement and help.

Maidenhead, UK
June 2014

G. Subramanian
Proteins Separation and Puriﬁcation by Expanded Bed Adsorption and Simulated Moving Bed Technology
Ping Li, Pedro Ferreira Gomes, José M. Loureiro, and Alirio E. Rodrigues

1.1 Introduction

Proteins not only play an important role in biology, but also have large potential applications in pharmaceuticals and therapeutics, food processing, textiles and leather goods, detergents, and paper manufacturing. With the development of molecular biology technologies, various kinds of proteins can be prepared from upstream processes and from biological raw materials. However, there exist various proteins and contaminants in these source feedstocks, and the key issue is that proteins can be separated and puriﬁed efﬁciently from the source materials, in order to reduce the production cost of the high-purity protein. The development of techniques and methods for proteins separation and puriﬁcation has been an essential prerequisite for many of the advancements made in biotechnology.

Most separation and puriﬁcation protocols require more than one step to achieve the desired level of protein purity. Usually, a three-step separation and puriﬁcation strategy is presented, which includes capture, intermediate separation and puriﬁcation, and ﬁnal polishing during a downstream protein separation and puriﬁcation process. In the capture step the objectives are to isolate, concentrate, and stabilize the target proteins. During the intermediate separation and puriﬁcation step the objectives are to remove most of the bulk impurities, such as other proteins and nucleic acids, endotoxins, and viruses. In the polishing step most impurities have already been removed except for trace amounts or closely related substances. The objective is to achieve ﬁnal purity of protein.

In the capture step, as the primary recovery of proteins, the expanded bed adsorption (EBA) technology has been widely applied to capture proteins directly from crude unclaried source materials, such as, Escherichia coli homogenate, yeast, fermentation, mammalian cell culture, milk, and animal tissue extracts [1,2]. The expanded bed is designed in a way that the suspended adsorbent particles capture target protein molecules, while cells, cell debris,
particulate matter, and contaminants pass through the column unhindered. After loading and washing, the bound proteins can be eluted by elution buffer and be concentrated in a small amount of elution solution, apart from the bulk impurities and contaminants in source materials. With specially designed adsorbents and columns, the adsorption behavior in expanded beds is comparable to that in fixed beds. Various applications of EBA technology have been reported from laboratory-scale to pilot-plant and large-scale production [1–9].

During the intermediate purification and final polishing steps, the techniques of the conventional elution chromatography have been applied successfully. A new challenge should be the application of simulated moving bed (SMB) to the separation and purification of proteins. SMB chromatography is a continuous process, which for preparative purposes can replace the discontinuous regime of elution chromatography. Furthermore, the counter-current contact between fluid and solid phases used in SMB chromatography maximizes the mass transfer driving force, leading to a significant reduction in mobile and stationary phase consumption when compared with elution chromatography [10–14]. Examples of products that are considered for SMB separation and purification are therapeutic proteins, antibodies, nucleosides, and plasmid DNA [15–23].

When the binding capacities of proteins on adsorbent are close to each other, an isocratic SMB mode may be used to separate and purify the proteins, where the adsorbents have the same affinity capacity to proteins in all sections in SMB chromatography. However, usually the binding capacities of proteins are so different that we cannot separate them by the isocratic mode with a reasonable retention time. In conventional elution chromatography, a gradient mode should be used for the separation of proteins. It is most commonly applied in reversed-phase and ion exchange chromatography (IEC), by changing the concentration of the organic solvent and salt in a stepwise gradient or with a linear gradient, respectively. For SMB chromatography, only a stepwise gradient can be formed by introducing a solvent mixture with a lower strength at the feed inlet port compared with the solvent mixture introduced at the desorbent port; then the adsorbents have a lower binding capacity to proteins in sections I and II to improve the desorption, and have a stronger binding capacity in sections III and IV to increase adsorption in SMB chromatography. Some authors state that the solvent consumption by gradient mode can be decreased significantly when compared with isocratic SMB chromatography [17–19,24–29]. Moreover, when a given feed is applied to gradient SMB chromatography, the protein obtained from the extract stream can be enriched if protein has a medium or high solubility in the solution with the stronger solvent strength, while the raffinate protein is not diluted at all [24].

In this chapter, we shall describe the developments made at the Laboratory of Separation and Reaction Engineering (LSRE) for proteins separation and purification by expanded bed chromatography and salt gradient ion exchange simulated moving bed technology.


1.2 Protein Capture by Expanded Bed Technology

1.2.1 Adsorbent Materials

The design of a special adsorbent is a key factor to enhance the efficiency of expanded bed adsorption. The EBA process will be more effective for those adsorbents that have both high-density base matrix and salt-tolerant ligand. The high-density matrix means minimizing dilution arising from biomass or viscosity in feedstock and reducing dilution buffer consumption; the lack of sensitivity of the ligand to ionic strength and salt concentration means there is no need for dilution of feedstock [30–32].

“Homemade” adsorbents are commonly used for research purposes. Agarose and cellulose are the major components utilized on the tailoring of the adsorbents. Table 1.1 shows a list of such adsorbents.

Table 1.1 “Homemade” adsorbents.

<table>
<thead>
<tr>
<th>Year</th>
<th>Core</th>
<th>Adsorbent</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>Crystalline quartz</td>
<td>6% Agarose</td>
<td>[33]</td>
</tr>
<tr>
<td>1994</td>
<td>Perfluorocarbon</td>
<td>Polyvinyl alcohol – perfluorodecalin</td>
<td>[34]</td>
</tr>
<tr>
<td>1995</td>
<td>Crystalline quartz - Red H-E7B</td>
<td>6% Agarose</td>
<td>[35]</td>
</tr>
<tr>
<td>1995</td>
<td>Perfluorocarbon</td>
<td>Polyvinyl alcohol – perfluoropolymer</td>
<td>[36]</td>
</tr>
<tr>
<td>1996</td>
<td>Crystalline quartz - Cibacron blue (3GA)</td>
<td>6% Agarose</td>
<td>[37]</td>
</tr>
<tr>
<td>1997</td>
<td>Fluoride-modified porous zirconium oxide</td>
<td></td>
<td>[38]</td>
</tr>
<tr>
<td>1999</td>
<td>Polycrlyamide gel</td>
<td>Silica</td>
<td>[39]</td>
</tr>
<tr>
<td>1999</td>
<td>Glass</td>
<td>Agarose</td>
<td>[40]</td>
</tr>
<tr>
<td>2000</td>
<td>Celbeads</td>
<td>Cellulose</td>
<td>[41]</td>
</tr>
<tr>
<td>2000</td>
<td>Stainless steel</td>
<td>Agarose</td>
<td>[30]</td>
</tr>
<tr>
<td>2001</td>
<td>Celbeads</td>
<td>Cellulose</td>
<td>[42]</td>
</tr>
<tr>
<td>2001</td>
<td>Nd–Fe–B alloy powder</td>
<td>Agarose</td>
<td>[43]</td>
</tr>
<tr>
<td>2002</td>
<td>Stainless steel</td>
<td>6% Agarose</td>
<td>[44]</td>
</tr>
<tr>
<td>2002</td>
<td>Stainless steel</td>
<td>6% Agarose</td>
<td>[45]</td>
</tr>
<tr>
<td>2002</td>
<td>Crystalline quartz</td>
<td>6% Agarose (Streamline DEAE) modified with a layer of polyacrylic acid (PAA)</td>
<td>[46]</td>
</tr>
<tr>
<td>2002</td>
<td>Nd–Fe–B with Cibacro Blue 3GA (CB)</td>
<td>4% Agarose</td>
<td>[47]</td>
</tr>
<tr>
<td>2002</td>
<td>Zirconia-silica (ZSA)</td>
<td>4% Agarose</td>
<td>[9]</td>
</tr>
<tr>
<td>2002</td>
<td>ZSA - Cibacro Blue (CB)</td>
<td>4% Agarose</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>Zirconia-silica (ZSA)</td>
<td>Agarose</td>
<td>[48]</td>
</tr>
<tr>
<td>2003</td>
<td>CB-6AS</td>
<td>Cellulose</td>
<td>[49]</td>
</tr>
<tr>
<td>2003</td>
<td>Titanium oxide</td>
<td>Cellulose</td>
<td>[50]</td>
</tr>
</tbody>
</table>

(continued)
The drawback of agarose/cellulose-based adsorbents is their low density. Therefore, EBA adsorbents were developed by incorporating a dense solid material in the beads. Table 1.2 shows a list of commercial adsorbents.

Adsorbents used in EBA have been developed by some major companies as shown in Table 1.3. The name of the adsorbents are influenced by the ligand used, for example, diethylaminoethyl (DEAE), sulphopropyl (SP), quaternary amine (Q), recombinant protein A (r-Protein A), imino diacetic acid (Chelating), multimodal function (Direct CST I), carboxymethyl (CM), sulfopropyl (S) and polyethyleneimine (PEI) [3,30,71].

The trend is to use a dense solid core material to allow processing of higher flow rates and therefore reach a better productivity [30–32].

Streamline DEAE and Streamline SP (specially designed for an expanded bed), are classical ion exchangers, in which binding proteins are primarily based on interactions between charged amino acids on the protein surface and oppositely charged immobilized ligands. Protein retention on an ionic surface of adsorbent can be simply explained by the pI-value (isoelectric point) of a protein. But in practical applications, it is found that these ion exchangers have a lower binding capacity to proteins in high ionic strength and salt concentration feedstock. Streamline Direct CST I is a cation exchanger with multimodal functional groups, which not only takes advantage of electrostatic interaction, but also takes advantage of hydrogen bond interaction and hydrophobic interaction to tightly bind proteins. In other words, the new type of