PROCESS SCALE PURIFICATION OF ANTIBODIES

Edited by

UWE GOTTSCHALK

Group Vice President
Purification Technologies
Sartorius Stedim Biotech

A JOHN WILEY & SONS, INC., PUBLICATION

PROCESS SCALE PURIFICATION OF ANTIBODIES

PROCESS SCALE PURIFICATION OF ANTIBODIES

Edited by

UWE GOTTSCHALK

Group Vice President
Purification Technologies
Sartorius Stedim Biotech

A JOHN WILEY & SONS, INC., PUBLICATION

Copyright © 2009 by John Wiley & Sons, Inc. All rights reserved.

Published by John Wiley & Sons, Inc., Hoboken, New Jersey.
Published simultaneously in Canada.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, scanning, or otherwise, except as permitted under Section 107 or 108 of the 1976 United States Copyright Act, without either the prior written permission of the Publisher, or authorization through payment of the appropriate per-copy fee to the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, (978) 750-8400, fax (978) 750-4470, or on the web at www.copyright.com. Requests to the Publisher for permission should be addressed to the Permissions Department, John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, (201) 748-6011, fax (201) 748-6008, or online at http://www.wiley.com/go/permission.

Limit of Liability/Disclaimer of Warranty: While the publisher and author have used their best efforts in preparing this book, they make no representations or warranties with respect to the accuracy or completeness of the contents of this book and specifically disclaim any implied warranties of merchantability or fitness for a particular purpose. No warranty may be created or extended by sales representatives or written sales materials. The advice and strategies contained herein may not be suitable for your situation. You should consult with a professional where appropriate. Neither the publisher nor author shall be liable for any loss of profit or any other commercial damages, including but not limited to special, incidental, consequential, or other damages.

For general information on our other products and services or for technical support, please contact our Customer Care Department within the United States at (800) 762-2974, outside the United States at (317) 572-3993 or fax (317) 572-4002.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic formats. For more information about Wiley products, visit our web site at www.wiley.com.

Library of Congress Cataloging-in-Publication Data
Process scale purification of antibodies / [edited by] Uwe Gottschalk.
p. ; cm.
Includes bibliographical references and index.
ISBN 978-0-470-20962-2 (cloth)
1. Monoclonal antibodies—Purification. 2. Biochemical engineering. I. Gottschalk, Uwe.
[DNLM: 1. Antibodies, Monoclonal—isolation & purification. 2. Antibodies, Monoclonal—therapeutic use. 3. Chromatography—methods. 4. Technology, Pharmaceutical—methods. QW 575.5.A6 P963 2009]
TP248.65.M65P76 2009
615′.19—dc22

2008038592

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

CONTENTS

Preface **xvii**

About the Author **xxi**

Contributors **xxiii**

1 Downstream Processing of Monoclonal Antibodies: Current Practices and Future Opportunities **1**

Brian Kelley, Greg Blank, and Ann Lee

1.1 Introduction, 1
1.2 A Brief History of cGMP mAb and Intravenous Immunoglobulin (IgIV) Purification, 2
1.3 Current Approaches in Purification Process Development: Impact of Platform Processes, 4
1.4 Typical Unit Operations and Processing Alternatives, 8
1.5 VLS Processes: Ton-Scale Production and Beyond, 11
1.6 Process Validation, 13
1.7 Product Life Cycle Management, 15
1.8 Future Opportunities, 17
1.9 Conclusions, 20
1.10 Acknowledgments, 21
1.11 References, 21

2 The Development of Antibody Purification Technologies **25**
John Curling

2.1 Introduction, 25
2.2 Chromatographic Purification of Antibodies before Protein A, 27
2.3 Antibody Purification after 1975, 31
2.4 Additional Technologies for Antibody Purification, 32
2.5 Purification of mAbS Approved in North America and in Europe, 37
2.6 Acknowledgments, 44
2.7 References, 45

3 Harvest and Recovery of Monoclonal Antibodies: Cell Removal and Clarification **53**
Abhinav A. Shukla and Jagannadha Rao Kandula

3.1 Introduction, 53
3.2 Centrifugation, 56
3.3 Microfiltration, 58
3.4 Depth Filtration, 64
3.5 Flocculation, 68
3.6 Absolute Filtration, 70
3.7 Expanded-Bed Chromatography, 72
3.8 Comparison of Harvest and Clarification Unit Operations, 73
3.9 Acknowledgments, 74
3.10 References, 75

4 Protein A-Based Affinity Chromatography **79**
Suresh Vunnum, Ganesh Vedantham, and Brian Hubbard

4.1 Introduction, 79
4.2 Properties of Protein A and Commercially Available Protein A Resins, 80
4.2.1 Protein A Structure, 80
4.2.2 Protein A–Immunoglobulin G (IgG) Interaction, 80
4.2.3 Stoichiometry of Protein A–IgG Binding, 81
4.2.4 Protein A Stability, 81
4.2.5 Commercial Protein A Resins, 81
4.2.6 Static Capacity, 83
4.2.7 DBC, 83
4.2.8 Leaching, 84
4.2.9 Production Rates, 85
4.3 Protein A Chromatography Step Development, 85
4.3.1 Loading/Binding, 86
4.3.2 Wash Development, 87

4.3.3 Elution, 88
4.3.4 Stripping, 89
4.3.5 Regeneration and CIP, 89
4.4 Additional Considerations During Development and Scale-Up, 90
4.4.1 Controlling HMW Formation, 91
4.4.2 Removal of Soluble HMW Contaminants, 91
4.4.3 Turbidity, 92
4.5 Virus Removal/Inactivation, 94
4.5.1 Virus Removal, 95
4.5.2 Low-pH Inactivation, 95
4.5.3 Bovine/Transmissible Spongiform Encephalopathy (BSE/TSE) Clearance, 96
4.6 Validation and Robustness, 96
4.6.1 Validation, 96
4.6.2 Robustness, 97
4.7 Conclusions, 97
4.8 Acknowledgments, 98
4.9 References, 99

5 Purification of Human Monoclonal Antibodies: Non-Protein A Strategies 103

Alahari Arunakumari and Jue Wang

5.1 Introduction, 103
5.2 Integrated Process Designs for Human Monoclonal Antibody (HuMab) Production, 105
5.3 Purification Process Designs for HuMabs, 106
5.3.1 Protein A Purification Schemes, 106
5.3.2 Non-Protein A Purification Schemes, 107
5.3.3 Host Cell Protein (HCP) Exclusion Approach for Ion-Exchange Purification Schemes, 108
5.4 Conclusions, 119
5.5 Acknowledgments, 121
5.6 References, 121

6 Purification of Monoclonal Antibodies by Mixed-Mode Chromatography 125

Pete Gagnon

6.1 Introduction, 125
6.2 A Brief History, 126
6.3 Prerequisites for Industrial Implementation, 127
6.4 Mechanisms, Screening, and Method Development, 129
6.5 Capture Applications, 137

6.6 Polishing Applications, 138
6.7 Sequential Capture/Polishing Applications, 139
6.8 The Future, 139
6.9 Acknowledgments, 140
6.10 References, 140

7 Integrated Polishing Steps for Monoclonal Antibody Purification 145
Sanchayita Ghose, Mi Jin, Jia Liu, and John Hickey

7.1 Introduction, 145
7.2 Polishing Steps in Antibody Purification, 146
7.2.1 Ion-Exchange (IEX) Chromatography, 146
7.2.2 HIC, 151
7.2.3 HA Chromatography, 155
7.2.4 Mixed-Mode and Other Modes of Chromatography, 156
7.2.5 Dedicated Virus Removal Steps, 158
7.3 Integration of Polishing Steps, 159
7.3.1 Case Study I: Selection and Placement of Polishing Steps, 159
7.3.2 Case Study II: Selection of Operational Mode and Influence of the Previous Polishing Step, 161
7.4 Conclusions, 163
7.5 Acknowledgments, 163
7.6 References, 164

8 Orthogonal Virus Clearance Applications in Monoclonal Antibody Production 169
Joe X. Zhou

8.1 Introduction, 169
8.2 Model Viruses and Virus Assays, 170
8.3 Virus Clearance Strategies from First in Human (FIH) to Biological License Application (BLA) Filing, 172
8.4 Orthogonal Viral Clearance in mAb Production, 173
8.4.1 Major Viral Clearance Steps: Capture, Low-pH Viral Inactivation, and Polishing, 173
8.4.2 Disposable Systems, 173
8.5 Conclusions and Future Perspectives, 183
8.6 Acknowledgments, 184
8.7 References, 184

9 Development of A Platform Process for the Purification of Therapeutic Monoclonal Antibodies 187

Yuling Li, David W. Kahn, Olga Galperina, Erich Blatter, Robert Luo, Yaling Wu , and Guihang Zhang

9.1 Introduction, 187
9.2 Chromatography Steps in the Platform Process, 189
9.2.1 Capture Step: General Considerations, 189
9.2.2 Intermediate/Polishing Steps, 191
9.3 Virus Inactivation, 193
9.4 UF/DF Platform Development, 194
9.4.1 Use in Platform Processes, 194
9.4.2 Challenges, 194
9.4.3 Application Examples, 195
9.5 Platform Development: Virus Filtration and Bulk Fill, 195
9.5.1 Virus Filtration in Platform Processes, 195
9.5.2 Bulk Fill in Platform Processes, 196
9.6 Examples of Platform Processes, 196
9.6.1 Example 1: Three-Column Protein A-Based Process, 196
9.6.2 Example 2: Three-Column HCIC-Based Process, 197
9.6.3 Example 3: Two- or One-Column Process without Protein A, 198
9.7 Developing a Viral Clearance Database Using a Platform Process, 199
9.8 Summary, 200
9.9 References, 201

10 Advances in Technology and Process Development for Industrial-Scale Monoclonal Antibody Purification 203

Nuno Fontes and Robert van Reis

10.1 Introduction, 203
10.2 Affinity Purification Platform, 204
10.2.1 Overview, 204
10.2.2 Standard Purification Sequence, 205
10.2.3 Challenges and Opportunities, 205
10.3 Advances in CEX Purification of mAbs, 206
10.3.1 Overview, 206
10.3.2 High-Capacity CEX, 207
10.3.3 An Exclusion Mechanism in Ion-Exchange Chromatography, 207
10.3.4 Factors Affecting the Critical Conductivity, 210
10.3.5 Advances in mAb CEX Process Development, 213

10.4 HPTFF, 215
10.4.1 Overview, 215
10.4.2 Advances in HPTFF, 217
10.5 A New Nonaffinity Platform, 218
10.6 References, 219

11 Continuous Chromatography for the Purification of Monoclonal Antibodies 223

Thomas Müller-Späth and Massimo Morbidelli

11.1 Introduction, 223
11.2 Product Variants and the Separation Problem, 224
11.3 Definition of Performance Parameters, 225
11.4 Gradient Chromatography for Biomolecules, 225
11.5 Continuous Chromatography to Increase Productivity, 226
11.5.1 The Concept of Continuous Chromatography, 226
11.5.2 The Simulated Moving Bed (SMB) Process, 226
11.5.3 Advantages and Disadvantages of Batch and SMB Chromatography, 227
11.6 The MCSGP Process, 229
11.6.1 MCSGP Process Design, 229
11.6.2 MCSGP for the Capture of mAbs from Clarified Cell Culture Supernatants, 230
11.6.3 MCSGP for the Separation of mAb Variants, 232
11.7 Upgrades for Continuous Processes to Improve Stability, 233
11.8 Impact of Increasing Fermentation Titers, 233
11.9 Outlook, 236
11.10 Acknowledgments, 237
11.11 References, 237

12 Process Economic Drivers in Industrial Monoclonal Antibody Manufacture 239

Suzanne S. Farid

12.1 Introduction, 239
12.2 Challenges When Striving for the Cost-Effective Manufacture of mAbs, 240
12.2.1 Constraints, 240
12.2.2 Uncertainties, 241
12.3 Cost Definitions and Benchmark Values, 242
12.3.1 Capital Investment, 242
12.3.2 Cost of Goods (COG) per Gram, 243
12.4 Economies of Scale, 246
12.5 Overall Process Economic Drivers, 247
12.5.1 Titer, 247
12.5.2 Overall DSP Yield, 248

12.5.3 Batch Duration, 250
12.5.4 Batch Success Rate, 250
12.5.5 Logistics, 251
12.6 DSP Drivers at High Titers, 251
12.6.1 Material Reuse and Lifetime, 252
12.6.2 Buffer/WFI Demands, 253
12.6.3 Chromatography Capacity, 254
12.7 Process Economic Trade-Offs for DSP Bottlenecks, 254
12.7.1 Chromatography Resin Dynamic Binding Capacity, 254
12.7.2 Chromatography Flow Rates, 255
12.7.3 Chromatography Resin Cycle Limits, 255
12.7.4 Platform Processes, 255
12.7.5 Alternatives to Chromatography, 256
12.8 Summary and Outlook, 256
12.9 References, 257

13 Design and Optimization of Manufacturing **263**

Andrew Sinclair

13.1 Introduction, 263
13.2 Process Design and Optimization, 265
13.3 Modeling Approaches, 267
13.3.1 Cost Modeling for mAb Manufacture, 267
13.3.2 Process Simulation for mAb Manufacture, 274
13.4 Cost Models in Practice, 279
13.4.1 Manufacturing Strategies—Disposables vs. Continuous, 280
13.4.2 Manufacturing Technologies—Single-Use Systems, 281
13.5 Simulation in Practice, 286
13.5.1 Managing Multiproduct Manufacture, 286
13.5.2 Resource Management, 288
13.6 Acknowledgments, 290
13.7 References, 290

14 Alternatives to Packed-Bed Chromatography for Antibody Extraction and Purification **293**

Jörg Thömmes and Uwe Gottschalk

14.1 Introduction, 293
14.2 Increasing the Selectivity of Harvest Procedures: Flocculation of Particulate and Nonparticulate Impurities, 294
14.3 Solutions for Antibody Extraction, Concentration, and Purification, 295

14.3.1 Extraction and Concentration by Precipitation, 295
14.3.2 Extraction and Concentration by Liquid-Phase Partitioning, 297
14.3.3 Concentration by Evaporation, 298
14.4 Nonchromatographic Solutions for Antibody Purification and Formulation, 298
14.4.1 Crystallization, 298
14.4.2 Controlled Freeze–Thaw, 300
14.4.3 Lyophilization, 301
14.5 Membrane Adsorbers, 302
14.6 Conclusions, 304
14.7 Acknowledgments, 305
14.8 References, 305

15 Process-Scale Precipitation of Impurities in Mammalian Cell Culture Broth 309

Judy Glynn

15.1 Introduction, 309
15.2 Precipitation of DNA and Protein—Other Applications, 311
15.3 A Comprehensive Evaluation of Potential Precipitants for Impurity Removal, 312
15.3.1 Protocol, 312
15.3.2 Ammonium Sulfate Precipitation, 314
15.3.3 Polymer Precipitation, 315
15.3.4 Precipitation with Ionic Liquids, 315
15.3.5 Precipitation with Cationic Detergents, 315
15.3.6 Ethacridine Precipitation, 316
15.3.7 Caprylic Acid Precipitation, 317
15.4 Industrial-Scale Precipitation, 319
15.5 Cost of Goods Comparison, 320
15.6 Summary, 321
15.7 Acknowledgments, 321
15.8 References, 322

16 Charged Ultrafiltration and Microfiltration Membranes in Antibody Purification 325

Mark R. Etzel

16.1 Introduction, 325
16.2 Charged Ultrafiltration Membranes, 326
16.3 Concentration Polarization and Permeate Flux, 327
16.4 Stagnant Film Model (SFM), 328
16.5 Osmotic Pressure Model, 328

16.6 Mass Transfer Coefficient, 329
16.7 Sieving Coefficient, 329
16.8 Diffusion–Convection Model, 330
16.9 Scale-up Strategies and the Constant Wall Concentration (C_w) Approach, 331
16.10 Protein Fractionation Using Charged Ultrafiltration Membranes, 333
16.11 Case Study, 333
16.11.1 Methods, 334
16.11.2 Results, 334
16.11.3 Discussion, 337
16.12 Membrane Cascades, 337
16.13 Charged Microfiltration Membranes, 339
16.14 Virus Clearance, 339
16.15 Salt Tolerance, 341
16.16 Conclusions, 343
16.17 Acknowledgments, 344
16.18 References, 345

17 Downstream Processing of Monoclonal Antibody Fragments 349
Mariangela Spitali

17.1 Introduction, 349
17.2 Production of Antibody Fragments for Therapeutic Use, 351
17.3 Downstream Processing, 351
17.3.1 Primary Recovery, 352
17.3.2 Capture, 356
17.3.3 Expanded-Bed Chromatography, 361
17.3.4 Further Purification and Polishing, 362
17.4 Improving the Pharmacological Characteristics of Antibody Fragments, 365
17.5 Conclusions, 367
17.6 Acknowledgments, 368
17.7 References, 369

18 Purification of Antibodies Other Than IgG: The Case of IgM and IgA 373
Charlotte Cabanne and Xavier Santarelli

18.1 Introduction, 373
18.2 Purification of Immunoglobulin M (IgM), 374
18.2.1 IgM Structure and Properties, 374
18.2.2 IgM Purification Technologies, 374
18.2.3 Affinity and Pseudoaffinity Matrices, 376

18.3 Purification of IgA, 378
18.3.1 IgA Structure and Properties, 378
18.3.2 Affinity and Pseudoaffinity Matrices, 378
18.4 Conclusion, 380
18.5 Acknowledgments, 380
18.6 References, 380

19 Purification of Antibodies From Transgenic Plants 387
Zivko L. Nikolov, Jeffrey T. Regan, Lynn F. Dickey, and Susan L. Woodard

19.1 Introduction, 387
19.2 Antibody Production in Transgenic Plants, 388
19.2.1 Subcellular Localization and Glycosylation, 388
19.2.2 Other Factors Affecting mAb Accumulation, 390
19.3 Downstream Processing of Antibodies Produced in Transgenic Plants, 391
19.3.1 Tissue Disintegration, 393
19.3.2 Solid–Liquid Separation, 394
19.3.3 Clarification and Pretreatment of Crude Extracts, 395
19.4 Purification of Plant-Derived mAbs Using Protein A, 396
19.5 Purification of Plant-Derived mAbs Using Nonprotein A Media, 397
19.6 Polishing Steps, 398
19.7 Conclusions, 399
19.8 Acknowledgments, 401
19.9 References, 401

20 Antibody Purification: Drivers of Change 407
Narahari Pujar, Duncan Low, and Rhona O'Leary

20.1 Introduction, 407
20.2 The Changing Regulatory Environment—Pharmaceutical Manufacturing for the Twenty-First Century, 410
20.2.1 Using Design Space to Enable Change, 412
20.2.2 High-Throughput and Microscale Approaches to Process Development and Characterization, 415
20.3 Technology Drivers—Advances and Innovations, 416
20.3.1 PAT, 416
20.3.2 Process Control Technology, 417
20.4 Economic Drivers, 418
20.4.1 Cost of Goods (COG), 418
20.4.2 Single-Use Disposable Components, 418
20.4.3 Globalization, 419
20.4.4 Follow-on Biologics (FOBs) or Biosimilars, 420

20.5 Conclusions, 421
20.6 Acknowledgments, 422
20.7 References, 422

Index **427**

PREFACE

Monoclonal antibodies are an important component of the biopharmaceutical industry. Within the burgeoning market of protein-based therapeutics, they are the market leaders in terms of volume sales and the most common class of product. Almost all commercial antibodies are produced in cultured mammalian cells, and an entire subindustry has grown up around downstream processing to ensure that manufacturing processes generate safe and pure products suitable for administration to humans. This is an industry in which I have been involved for many years, and one that is facing exciting and difficult challenges.

I first came into contact with the world of monoclonal antibodies in 1986, when their production in cultured mammalian cells was still in its infancy. This was at the Cancer Research Campaign Laboratories in Nottingham, UK. I was a PhD student from Germany, and one of my main tasks was to purify antibodies from mouse ascites, a horrible process for obvious reasons. A milligram of antibodies was worth far, far more than its weight in gold.

At the time, my colleagues and I had visions of curing cancer by drug targeting, and we linked all sorts of cytotoxic agents to the antibodies we produced. Unfortunately, some of the expectations surrounding the medical use of antibodies turned out to be premature and unrealistic. Our awareness of this coincided with the first real downturn in the biotechnology sector, but antibodies survived in niche markets for diagnostics and research reagents. Years later, new life has been breathed into therapeutic antibodies and they are now back, stronger than ever. Indeed, they represent the fastest-growing area in biotherapeutics with 21 products on the US market (as of 2007) and hundreds in clinical and preclinical development (1).

At the end of the 1980s, antibodies were produced commercially using mammalian cells cultured in perfusion fermenters, but yields rarely exceeded

100 mg/L. Huge volumes of culture broth needed to be processed, and the easiest way to bring the volume down was polyethylene glycol (PEG) precipitation with tons of material and endless centrifugation cycles. The yields were poor and difficult to reproduce, but there were no alternatives. Since that time, the productivity of cell cultures has increased significantly, with 1–5 g/L titers now routine and the real prospect of 10–20 g/L yields in the next decade. How far we have come since the early days of biomanufacturing!

The increase in titers has heaped pressure on the downstream processes that we use to extract and purify antibodies from cell culture broth, and the technologies used in downstream processing have been forced to modernize and improve in the face of this increasing challenge. There is little doubt that packed-bed chromatography is the workhorse of current downstream processing, its high resolution and relative simplicity making it the central enabling technology in modern bioseparation processes (2). As productivity increases, however, doubts have been cast on the ability of column chromatography to cope with the dramatically increasing product titers in fermentation (3). Unlike fermentation, capturing steps in downstream processing have hardly any economy of scale. Bind-and-elute cycles in chromatography are driven by mass rather than by volume, and this means that increasing batch sizes translate into increasing costs in a near linear fashion. This phenomenon particularly affects the first column, where all of the product must be captured. This initial recovery step has therefore been identified as the most serious potential bottleneck, with knock-on effects throughout the processing facility, e.g., in terms of column sizes, buffer preparation, and hold. However, this opinion is not shared by everyone, and debate continues as to whether or not packed-bed chromatography is here to stay (4, 5).

These challenges and their surrounding issues set the scene for this exciting book, in which I have compiled a selection of chapters from top-tier industrial and academic experts providing up-to-date accounts of current best practice in the manufacture of monoclonal antibodies. Opinions on the suitability of packed-bed chromatography in today's manufacturing environment differ, particularly in the light of emerging competitive technologies, and the first chapter by Ann Lee and colleagues captures that debate and puts the case for and against the continuing reliance on traditional chromatography methods. The second chapter by John Curling provides an informative historical overview of the development of antibody purification technologies, providing the basis for the next five chapters, which consider some of today's major steps in antibody processing—harvesting and recovery (Abhinav Shukla and colleagues), Protein A chromatography (Suresh Vunnum and colleagues), non-Protein A strategies (Alahari Arunakumari and Jue Wang), mixed mode chromatography (Pete Gagnon), and integrated polishing (Sanchayita Ghose and colleagues).

Looking closer, the pace at which fermentation is guiding the way is not the only challenge for modern downstream processing. The regulatory frame-

work, particularly current good manufacturing practice (cGMP) is a moving target, and quality requirements are constantly leading to tighter specifications and higher safety margins, e.g., with regard to small, nonenveloped viruses. The chapter by Joe Zhou therefore deals with orthogonal methods for virus removal, before we consider platform technologies that integrate virus clearance with capture and purification (Yuling Li and colleagues). Nuno Fontes and Robert van Reis then consider the important aspects of scaling up antibody purification to industrial levels with a platform of methods that offer the potential to set a new standard in antibody manufacture. Finally in this section, Thomas Müller-Späth and Massimo Morbidelli consider the use of continuous chromatography for the high-resolution separation of antibodies, based on a laboratory-scale strategy they developed.

The next two chapters look at the economic perspectives of antibody manufacture, one from the standpoint of process economics (Suzanne Farid) and the other from the standpoint of process design and optimization (Andrew Sinclair). We then turn to the consideration of emerging technologies, which may replace, augment, or supplement traditional chromatography: flocculation, precipitation, and membrane adsorbers for antibody purification (Jörg Thömmes and Uwe Gottschalk); precipitation for the elimination of impurities (Judy Glynn); and charged filtration membranes (Mark Etzel).

While most of the book focuses on the purification of typical, full-size IgG molecules produced in fermenters, the final section deals with noncanonical antibody varieties and novel sources. There are chapters dealing with the purification of antibody fragments (Mariangela Spitali) and non-IgG monoclonals (IgM and IgA; Charlotte Cabanne and Xavier Santarelli), followed by a chapter considering the promising use of plant-based systems for antibody manufacture, and the particular challenges faced when isolating antibodies and other biopharmaceuticals from plant sources (Zivko Nikolov and colleagues).

The final chapter wraps up the book by looking to the future and considering what drives change in the industry, particularly what factors are likely to influence the techniques and technologies that will be adopted for antibody purification in the decade to come. This concluding chapter is written by Hari Pujar, Duncan Low, and Rhona O'Leary, three distinguished authors representing the top-tier companies in the sector.

In all likelihood, we will not see a revolution in downstream processing like the one that has galvanized upstream process development over the last 20 years. The chapters in this book are, however, evidence that the future of antibody purification holds great promise, underlining the progress that has been made in closing the performance gap between upstream production and downstream processing.

All the contributors to this book live and die for the production of antibodies. Some of us have been there from the very first day, while others have joined more recently, but we all passionately believe that technological advances and innovation can help to break through the current ceiling in

antibody processing and can lead to affordable, high-quality pharmaceutical products in the future.

Uwe Gottschalk

REFERENCES

1. Frost & Sullivan (2008). *US Biotechnology—Therapeutic Monoclonal Antibodies Market*. Frost & Sullivan, Palo Alto, CA.
2. Curling, J., Gottschalk, U. (2007). Process chromatography: Five decades of innovation. *BioPharm International* 20, 70–94.
3. Langer, E.S. (2008). *Fifth Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production*. BioPlan Associates Inc., Rockville MD.
4. Kelley, B. (2007). Very large scale monoclonal antibody purification: The case for conventional unit operations. *Biotechnology Progress* 23, 995–1008.
5. Low, D., O'Leary, R., Pujar, N.S. (2007). Future of antibody purification. *Journal of Chromatography B* 848, 48–63.

ABOUT THE AUTHOR

Dr. Uwe Gottschalk
Group Vice President
Purification Technologies
Sartorius Stedim Biotech

D-37079 Göttingen
Tel: (0551) 308-2016
Telefax: (0551) 308-3705
Email: Uwe.Gottschalk@Sartorius-Stedim.com

Dr. Gottschalk is Group Vice President, Purification Technologies, at Sartorius Stedim Biotech. He worked in different capacities for Bayer Health Care from 1991 to 2004, overseeing the purification of monoclonal antibodies and recombinant proteins produced in various expression systems.

Dr. Gottschalk holds a PhD in Chemistry from the University of Münster. He is a member of BioPharm International's editorial advisory board and has written extensively in the areas of biotechnology and somatic gene therapy. In academia, Dr. Gottschalk is Head Lecturer at the University of Duisburg–Essen (Germany) and also lectures at the Ecole Polytechnique Fédérale de Lausanne (EPFL) in Lausanne (Switzerland).

Dr. Gottschalk is a board member of the VBU Association of German Biotech Companies.

CONTRIBUTORS

Alahari Arunakumari, PhD, Senior Director, Process Development, Medarex, Inc., 519 Route 173 West, Bloomsbury, NJ 08804; Email: aarunakumari@medarex.com

Greg Blank, PhD, Director, Late Stage Purification, Genentech, Inc.

Erich Blatter, PhD, Senior Scientist, Purification Sciences-BioPharmaceutical Development, Human Genome Sciences, Inc.

Charlotte Cabanne, PhD, Associate Professor, EA 4135, ESTBB, Université Victor Segalen Bordeaux 2

John Curling, CEO, John Curling Consulting AB, Swedenborgsgatan 3-5, SE-753 34 Uppsala, Sweden; Email: john@consultcurling.se

Lynn F. Dickey, PhD, VP of Research and Technology Development, Biolex Therapeutics

Mark R. Etzel, PhD, Professor, University of Wisconsin, 1605 Linden Drive, Madison, WI 53706; Email: etzel@engr.wisc.edu

Suzanne S. Farid, PhD, Senior Lecturer/Associate Professor, The Advanced Centre for Biochemical Engineering, Department of Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE, UK; Email: s.farid@ucl.ac.uk

Nuno Fontes, PhD, Late Stage Purification—Process Research & Development, Genentech, Inc., 1 DNA Way South, San Francisco, CA 94080; Email: fontes.nuno@gene.com

Pete Gagnon, Chief Scientific Officer, Validated Biosystems, 240 Av Vista Montana, Ste 7F, San Clemente, CA 92672; Email: pete@validated.com

Olga Galperina, PhD, Associate Director, Purification Sciences-BioPharmaceutical Development, Human Genome Sciences, Inc.

Sanchayita Ghose, PhD, Manager, Process Sciences—Downstream, Bristol-Myers Squibb, Co., East Syracuse NY 13057; Email: sanchayita.ghose@bms.com

Judy Glynn, PhD, Senior Principal Scientist, Pfizer, Inc., 700 Chesterfield Parkway West, Chesterfield, MO, 63017; Email: judy.k.glynn@pfizer.com

Uwe Gottschalk, PhD, Group Vice President Purification Technologies, Sartorius Stedim Biotech, Göttingen, Germany; Email: uwe.gottschalk@sartorius-stedim.com

John Hickey, Associate Director, Process Sciences—Downstream & Project Management, Bristol-Myers Squibb, Co.

Brian Hubbard, PhD, Executive Scientific Director, Process & Product Development, Amgen Inc.

Mi Jin, PhD, Scientist, Process Sciences—Downstream, Bristol-Myers Squibb, Co.

David W. Kahn, PhD, Director, Purification Sciences-BioPharmaceutical Development, Human Genome Sciences, Inc.

Jagannadha Rao Kandula, Manufacturing Sciences, Bristol-Myers Squibb, Co.

Brian Kelley, PhD, Senior Director, Bioprocess Development, Genentech, Inc., Inc., 1 DNA Way, South San Francisco, CA 94080; Email: kelley.brian@gene.com

Ann Lee, PhD, Vice President, Process Research & Development, Genentech, Inc.

Yuling Li, PhD, Senior Director, Purification Sciences-BioPharmaceutical Development, Human Genome Sciences, Inc., 14200 Shady Grove Road, Rockville, MD 20850; Email: Yuling_Li@hgsi.com

Jia Liu, PhD, Engineer, Process Sciences—Downstream, Bristol-Myers Squibb, Co.

Duncan Low, PhD, Process Development, Amgen

Robert Luo, PhD, Senior Scientist, Purification Sciences-BioPharmaceutical Development, Human Genome Sciences, Inc.

Massimo Morbidelli, PhD, Institute for Chemical and Bioengineering, ETH Zürich, Wolfgang-Pauli-Str. 10, 8093 Zurich, Switzerland; Email: massimo.morbidelli@chem.ethz.ch

Thomas Müller-Späth, Dipl.-Ing., Institute for Chemical and Bioengineering, ETH Zürich

Zivko L. Nikolov, PhD, PE, Dow Professor, Biological and Agricultural Engineering, Texas A&M University, College Station, Texas 77843-2117; Email: znikolov@tamu.edu

Rhona O'Leary, PhD, Early Stage Purification, Genentech

Narahari Pujar, PhD, Bioprocess R&D, Merck & Co., Inc., West Point, PA; Email: hari_pujar@merck.com

Jeffrey T. Regan, Biolex Therapeutics

Xavier Santarelli, PhD, Professor, EA 4135, ESTBB, Université Victor Segalen Bordeaux 2, 146 rue Léo Saignat, Bordeaux cedex, France; Email: xavier.santarelli@estbb.u-bordeaux2.fr

Abhinav A. Shukla, Manufacturing Sciences, Bristol-Myers Squibb, Co., East Syracuse, NY 13057; Email: abhinav.shukla@bms.com

Andrew Sinclair, CEO and Founder, BioPharm Services, Lancer House, Chesham, HP5 1RD, UK; Email: a.sinclair@biopharmservices.com

Mariangela Spitali, PhD, Senior Group Leader, Protein Purification Research, Bioprocess R&D, UCB Celltech, UCB Pharma Ltd., 208 Bath Road, Slough, Berkshire, SL1 3WE, UK; Email: Mari.Spitali@celltech.UCB-group.com

Jörg Thömmes, PhD, Senior Director Purification Process Development, Biogen Idec, San Diego, USA

Robert van Reis, Late Stage Purification—Process Research & Development, Genentech, Inc.

Ganesh Vedantham, PhD, Director, Process & Product Development, Amgen Inc.

Suresh Vunnum, PhD, Scientific Director, Process & Product Development, Amgen Inc., 1201 Amgen Court West, Seattle, WA 98006; Email: vvunnum@amgen.com

Jue Wang, PhD, Senior Manager, Purification Process Development, Medarex, Inc.

Susan L. Woodard, PhD, Department of Biological and Agricultural Engineering, Borlaug Center/TAMU 2123, Texas A&M University

Yaling Wu, PhD, Senior Scientist, Purification Sciences-BioPharmaceutical Development, Human Genome Sciences, Inc.

Guihang Zhang, PhD, Senior Scientist, Purification Sciences-BioPharmaceutical Development, Human Genome Sciences, Inc.

Joe X. Zhou, PhD, Scientific Director, Process Development Department, Amgen, Inc., CEO of Wison Bio-engineering Company, 1399 Zhangheng Road, Zhangjiang, Pudong, Shanghai 201203, China; Email: xhz_1999@yahoo.com or joezhou@wison.com

1

DOWNSTREAM PROCESSING OF MONOCLONAL ANTIBODIES: CURRENT PRACTICES AND FUTURE OPPORTUNITIES

Brian Kelley, Greg Blank, and Ann Lee

1.1 INTRODUCTION

Monoclonal antibodies (mAbs) are now established as the most prevalent class of recombinant protein therapeutics. They can be expressed at high levels in cell culture, are typically very soluble, and are relatively stable during processing. The nearly universal use of mammalian cell expression systems for mAb synthesis, combined with the selection of homologous, humanized mAb framework protein sequences, provides opportunities to harmonize manufacturing processes around base platforms that can then be used with only slight variations from product to product. In addition, by using a platform process, manufacturing plants designed for the production of one mAb can usually be readily adapted to produce others.

For these reasons, mAbs represent a unique group of biological products. They accommodate rapid process development time lines, can be produced in large quantities, and may be manufactured in multiple facilities during their lifecycle as a result of their common process flowsheets. As a result, they have relatively low manufacturing costs and benefit from the flexibility of production at either in-house or contract production facilities. Although mAbs are not commodity products that are substitutable in the clinical setting, they have

Process Scale Purification of Antibodies, Edited by Uwe Gottschalk
Copyright © 2009 John Wiley & Sons, Inc.

distinct advantages in production scale and cost, as well as in product development speed and convenience, when compared to other recombinant protein therapeutics.

This introductory chapter attempts to set the context for the following chapters, which cover many aspects of mAb purification in detail. A typical mAb purification process flow sheet is described and used to illustrate the impact of purification platforms on mAb production. Factors to consider with respect to the various process alternatives or new technologies described in upcoming chapters are addressed, emphasizing the integration of unit operations and process design principles into an optimized, holistic process. Both current practices and controversial topics are introduced, among them the challenges of very large-scale (VLS) production, issues related to facility fit, the maturation of process purification technology for mAb processing, the need for innovations in mAb downstream processing, and the impact of the evolving regulatory environment. It is hoped that this backdrop will stimulate critical thinking and comprehensive analysis when the processing options described in the following chapters are being considered.

1.2 A BRIEF HISTORY OF cGMP mAb AND INTRAVENOUS IMMUNOGLOBULIN (IgIV) PURIFICATION

The processes used for production of IgIV from human plasma differ from those used for recombinant mAbs. Figure 1.1 shows a consensus processing scheme, based on many published process flow sheets, for the purification of IgIV. Most IgIV processes lack chromatographic steps and instead rely on multiple fractional precipitation steps based on the Cohn process developed in the 1950s (1). Some recently developed processes include chromatographic

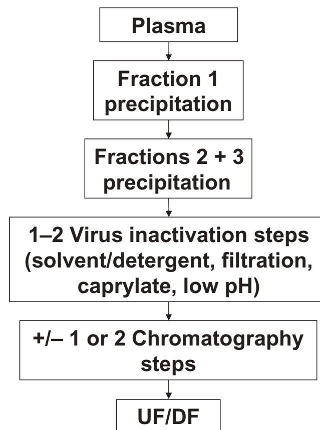

FIGURE 1.1 Cohn-based IgIV consensus process.