Contents

Preface XXIII List of Contributors XXV

Volume 1

1

Part One Upstream Technologies 1

v

1	Strategies for Plasmid DNA Production in Escherichia coli 3
	Eva Brand, Kathrin Ralla, and Peter Neubauer
1.1	Introduction 3
1.2	Requirements for a Plasmid DNA Production Process 4
1.3	Structure of a DNA Vaccine Production Process 6
1.4	Choice of Antigen 7
1.5	Vector DNA Construct 8
1.5.1	Popular Amplification Systems 8
1.5.2	Intrinsic Factors 9
1.6	Host Strains 11
1.6.1	endA and recA 12
1.6.2	relA 12
1.6.3	Nucleoside Pathway 14
1.6.4	gyrA 15
1.6.5	Strains for Production Processes 15
1.7	Cultivation Medium and Process Conditions 16
1.8	Lysis/Extraction of Plasmid DNA 19
1.9	Purification 20
1.9.1	Clarification of the Lysate and Intermediate Purification 21
1.9.2	Purification by Chromatography 23
1.9.2.1	Anion-Exchange Chromatography 23
1.9.2.2	Hydrophobic Interaction Chromatography 24
1.9.2.3	Gel Filtration 24
1.9.2.4	Membrane Chromatography 24
1.9.2.5	Chromatography on Porous Monolithic Supports 25

- Contents
 - Formulation 26 1.10
 - Lipoplexes 27 1.10.1
 - 1.10.2 Polyplexes 27
 - Inorganic Nanoparticles 28 1.10.3
 - Conclusions 28 1.11
 - References 28

2 Advances in Protein Production Technologies 43

Linda H.L. Lua and Yap Pang Chuan

- 2.1 Introduction 43
- 2.2 Glycoengineering for Homogenous Human-Like Glycoproteins 45
- 2.3 Bacteria as Protein Factories 47
- 2.4 Mammalian Cell Technology 50
- 2.5 Yeast Protein Production 53
- 2.6 Baculovirus–Insect Cell Technology 55
- 2.7 Transgenic Animal Protein Production 57
- 2.8 Plant Molecular Farming 59
- 2.9 Cell-Free Protein Production 62
- 2.10 Future Prospects 65 References 66

Part Two Protein Recovery 79

3 Releasing Biopharmaceutical Products from Cells 81 Anton P.J. Middelberg

- 3.1 Introduction 81
- Cell Structure and Strategies for Disruption 83 3.2
- 3.3 Cell Mechanical Strength 85
- 3.4 Homogenization 89
- 3.4.1 Mechanisms 90
- 3.4.2 Modeling 91
- 3.5 Bead Milling 95
- 3.5.1 Modeling 96
- 3.6 Chemical Treatment 98
- 3.7 Cellular Debris 100
- 3.7.1 Modeling 102
- 3.8 Conclusions 103 References 104

4 Continuous Chromatography (Multicolumn Countercurrent Solvent Gradient Purification) for Protein Purification 107

Guido Ströhlein, Thomas Müller-Späth, and Lars Aumann

- 4.1 Introduction 107
- 4.1.1 Overview of the Biopharmaceutical Market 107
- 4.1.2 Overview of Purification of Biopharmaceuticals 108
- 4.1.3 Introduction to Continuous Chromatographic Processes 108

- 4.2 Overview of Continuous Chromatographic Processes 110
- 4.2.1 SMB and Its Derivatives 110
- 4.2.1.1 Applications of SMB in the Pharmaceutical Industry: Small Molecules 111
- 4.2.1.2 Limitations of SMB 112
- 4.2.2 MCSGP Goes Beyond SMB and Makes Continuous Chromatography Possible for Bioseparations 112
- 4.3 Principles of MCSGP 113
- 4.3.1 Tasks in Batch Chromatogram 113
- 4.3.1.1 Generic Purification Problem 114
- 4.3.2 Six-Column MCSGP Principle 115
- 4.3.3 Three-Column MCSGP Principle 115
- 4.3.4 Four-Column MCSGP with Separate CIP Position 116
- 4.3.5 Four-Column MCSGP with a Separate Position for Continuous Feed *118*
- 4.3.6 MCSGP Process for Separations with More Than Three Fractions 119
- 4.4 Application Examples of MCSGP 120
- 4.4.1 Polypeptide Purification with Reversed-Phase Chromatography 120
- 4.4.2 mAb Charge Variant Separation 125
- 4.4.3 mAb Capture and Polish from Supernatant 127
- 4.4.4 Size-Exclusion Chromatographic Purification with MCSGP 129
- 4.5 Enabling Features and Economic Impact of MCSGP 134
- 4.6 Annex 1: Chromatographic Process Decision Tree 135 References 136
- 5 Virus-Like Particle Bioprocessing 139

Yap Pang Chuan, Linda H.L. Lua, and Anton P.J. Middelberg

- 5.1 Introduction 139
- 5.2 Upstream Processing 143
- 5.2.1 Intracellular Expression and Assembly 143
- 5.2.2 Cell-Free Approaches 147
- 5.3 Downstream Processing 147
- 5.3.1 Gardasil Downstream Processing 148
- 5.3.2 VLP Aggregation 149
- 5.3.3 Purification of Cell-Assembled VLPs 150
- 5.3.4 Purification for *In Vitro* Assembly 152
- 5.4 Analysis 154
- 5.5 Conclusions 157
- 5.6 Nomenclature 158
 - Acknowledgments 158 References 158
- **6 Therapeutic Protein Stability and Formulation** 165 Robert Falconar
- 6.1 Introduction 165
- 6.2 Protein Stability 167

VIII Contents

6.2.1	Structural Stability 167
6.2.2	Thermal Stability 168
6.2.3	Chaotropes, Solvents, and pH 168
6.2.4	Shear 169
6.2.5	Freezing 169
6.2.6	Drying 170
6.2.7	Air–Liquid and Solid–Liquid Interfaces 170
6.2.8	Chemical Stability 171
6.2.9	Precipitation, Aggregation, and Fibril Formation 173
6.2.10	Leachables 174
6.3	Formulation and Materials 175
6.3.1	Liquid Formulations 175
6.3.2	pH 176
6.3.3	Amino Acids and Other Organic Buffers 177
6.3.4	Sugars and Polyols 177
6.3.5	Salts 177
6.3.6	Surfactants 178
6.3.7	Specific Binding 178
6.3.8	Chelating Agents 178
6.3.9	Redox Potential 179
6.3.10	Containers and Closures 179
6.3.11	Frozen Formulations 179
6.3.12	Freeze-Dried Formulations 180
6.4	Screening Methods 185
6.4.1	DSC 185
6.4.2	Thermal Scanning with Spectroscopic Detection of Protein
	Unfolding 187
6.5	Accelerated and Long-Term Stability Testing 188
6.5.1	Regulatory Perspective 188
6.5.2	Accelerated Stability Testing 189
6.6	Analytical Techniques for Stability Testing 189
6.6.1	Cell-Based Bioassays and In Vitro Binding Assays 190
6.6.2	High-Performance Liquid Chromatography and Capillary Zone
	Electrophoresis 191
6.6.3	Mass Spectrometry-Based Analysis 192
6.6.4	Detection of Protein Aggregates 192
6.6.5	Crude Analytical Assays: PAGE, IEF, Blotting, FTIR, CD, and UV
	Fluorescence 193
6.7	Conclusions 194
	References 195
7	Production of PEGylated Proteins 199
	Conan J. Fee and Vinod B. Damodaran
7.1	Introduction 199
7.2	General Considerations 200

- 7.2.1 Efficiency of PEG Conjugation 200
- 7.2.2 Control of Positional Isomerism 201
- 7.2.3 Control of the Number of PEG Adducts 202
- 7.2.4 Purification of Target Products 203
- 7.3 PEGylation Chemistry 204
- 7.3.1 Amine Conjugation 204
- 7.3.2 Thiol Conjugation 206
- 7.3.3 Oxidized Carbohydrate or N-Terminal Conjugation 208
- 7.3.4 Transglutaminase-Mediated Enzymatic Conjugation 208
- 7.3.5 Miscellaneous Conjugation Chemistries 209
- 7.3.6 Reversible PEGylation 209
- 7.4 PEGylated Protein Purification 210
- 7.4.1 Removal of Low-Molecular-Weight Contaminants 210
- 7.4.2 Removal of Free PEG 212
- 7.4.3 Separation of PEGylated and Native Protein Forms 213
- 7.4.4 Separation of PEGylated Species 215
- 7.5 Conclusions 217 References 218

Part Three Advances in Process Development 223

- 8 Affinity Chromatography: Historical and Prospective Overview 225 Laura Rowe, Graziella El Khoury, and Christopher R. Lowe
- 8.1 History and Role of Affinity Chromatography in the Separation Sciences 225
- 8.1.1 Introduction 225
- 8.1.2 Early History 226
- 8.1.3 Biological Ligands 226
- 8.1.4 Synthetic and Designed Ligands 228
- 8.1.5 Alternative Ligands 229
- 8.1.6 Role of Affinity Chromatography in the Separation Sciences 229
- 8.2 Overview of Affinity Chromatography: Theory and Methods 230
- 8.2.1 Basic Chromatographic Theory 230
- 8.2.2 Matrix Selection and Immobilization of an Affinity Ligand 232
- 8.2.3 Other Considerations 237
- 8.3 Affinity Ligands 239
- 8.3.1 Biological Ligands 239
- 8.3.1.1 Immunoaffinity Adsorbents 239
- 8.3.1.2 Bacterial Proteins 242
- 8.3.1.3 Lectins 246
- 8.3.1.4 Heparin 247
- 8.3.1.5 Glutathione 248
- 8.3.1.6 Avidin and Streptavidin 248
- 8.3.1.7 Vitamins and Hormones 249

- Contents
 - Nucleic Acids 249 8.3.1.8
 - Alternative Affinity Methods 250 8.3.1.9
 - 8.3.2 Synthetic and Designed Ligands 251
 - Immobilized Metals 252 8.3.2.1
 - 8.3.2.2 Hydrophobic Ligands 253
 - 8.3.2.3 Thiophilic Ligands 253
 - 8.3.2.4 Histidine 254
 - 8.3.2.5 Mixed-Mode Adsorbents 255
 - 8.3.2.6 Boronate 256
 - Benzhydroxamic Acid 256 8.3.2.7
 - 8.3.2.8 Dye Ligands 257
 - 8.3.2.9 **Biomimetics** 258
 - 8.4 Affinity Ligands in Practice: Biopharmaceutical Production 269
 - 8.5 Conclusions and Future Perspectives 271 References 272

Hydroxyapatite in Bioprocessing 9 283

- Frank Hilbrig and Ruth Freitag
- 9.1 Introduction 283
- 9.2 Materials and Interaction Mechanisms 285
- 9.2.1 Apatites for Chromatography 285
- 9.2.2 Structure-Function Relationship 289
- 9.2.3 Retention Mechanisms in Apatite Chromatography 294
- 9.3 Setting up a Separation 301
- 9.3.1 General Considerations 301
- 9.3.2 Elution Mode 305
- 9.3.3 Displacement Mode 309
- 9.4 Separation Examples 313
- 9.4.1 Proteins in General 313
- 9.4.2 Antibodies 313
- 9.4.3 Polynucleotides 322
- 9.4.4 Others 323
- 9.5 Conclusions 323
 - References 324

10 Monoliths in Bioprocessing 333

Aleš Podgornik, Miloš Barut, Matjaž Peterka, and Aleš Štrancar

- 10.1 Introduction 333
- 10.2 Properties of Chromatographic Monoliths 333
- 10.3 Monolithic Analytical Columns for Process Analytical Technology Applications 338
- 10.3.1 Upstream Applications 339
- 10.3.2 Downstream Applications 340
- 10.3.2.1 HPLC Analysis of IgG Proteins 340
- 10.3.2.2 HPLC Analysis of the IgM Samples 341

x

Contents XI

- 10.3.2.3 HPLC Anion-Exchange Analysis of the PEGylated Proteins 342
- 10.3.2.4 Viruses 344
- 10.4 Monoliths for Preparative Chromatography 348
- Protein Purification 349 10.4.1
- Purification of Viruses 351 10.4.2
- 10.4.3 Plasmid DNA Purification 354
- 10.4.4 Negative Chromatography 357
- 10.5 Enzyme Reactors 358
- Proteome Analysis 358 10.5.1
- Biosensors 360 10.5.2
- 10.5.3 Bioconversion of Target Molecules 360
- Study of Enzyme-Intrinsic Properties 362 10.5.4
- 10.6 Conclusions 364 References 364

11 Membrane Chromatography for Biopharmaceutical Manufacturing 377 Omar M. Wahah

- Membrane Adsorbers-Introduction and Technical 11.1 Specifications 377
- Introduction 377 11.1.1
- 11.1.2 Membrane Adsorber Construction 380
- Types of Available Ligands 382 11.1.3
- Use and Scaling-Up with Membrane Adsorbers 384 11.1.4
- 11.2 Comparing Resins and Membrane Adsorbers 387
- 11.2.1 Flow-Through Polishing Applications 389
- 11.2.2 Bind-and-Elute Applications 390
- 11.2.3 Economical Modeling and Case Studies 391
- 11.3 Membrane Chromatography Applications and Case Studies 393
- 11.3.1 Validation of Membranes into a Purification Process 393
- 11.3.2 Virus Purification and Vaccine Manufacture 395
- 11.3.3 Virus Removal 396
- 11.3.4 Endotoxin Removal 399
- 11.3.5 HCP Removal 402
- 11.3.6 DNA Removal 404
- Aggregate Reduction 404 11.3.7
- Conclusions 406 11.4 References 407

12 Modeling and Experimental Model Parameter Determination with Quality by Design for Bioprocesses 409

- Christoph Helling and Jochen Strube
- 12.1 Introduction 409
- ObD Fundamentals 410 12.2
- 12.3 Process Modeling and Experimental Model Parameter Determination 411

XII Contents

12.3.1	Modeling 413
12.3.2	Experimental Model Parameter Determination 414
12.3.2.1	Isotherm Parameters 414
12.3.2.2	Fluid Dynamics 416
12.3.2.3	Mass Transfer Kinetics 417
12.4	Process Robustness Study 425
12.4.1	Model Error 425
12.4.2	Model Parameter Determination Error 426
12.4.3	Variation of Process Conditions 431
12.5	Conclusions 439
12.6	Nomenclature 440
	Acknowledgments 441
	References 442

Volume 2

Part Four Analytical Technologies 445

13	Biosensors in the Processing and Analysis of
	Biopharmaceuticals 447
	Sriram Kumaraswamy
13.1	Introduction 447
13.2	Principles and Commercial Applications of Biosensors 448
13.2.1	Labeled versus Label-Free Biosensors 449
13.2.2	Label-Free Biosensors 451
13.2.2.1	Label-Free Biosensors in Commercial Use 451
13.2.2.2	Introduction to BLI 453
13.2.2.3	Introduction to SPR 453
13.2.2.4	Introduction to RWG 455
13.2.3	Sample Handling Considerations 455
13.2.3.1	Sample Handling by BLI 456
13.2.3.2	Sample Handling by SPR 456
13.2.3.3	Sample Handling by RWG 458
13.2.4	Comparison of Biosensor Chips 458
13.2.4.1	Octet Dip and Read Biosensors 459
13.2.4.2	Biacore Chips 459
13.2.4.3	Epic Microplates 462
13.2.5	Comparison of Throughput 462
13.3	Use of Biosensors in Biopharmaceutical Production and
	Processing 464
13.3.1	Quantification of Therapeutics and Other Minor Impurities 464
13.3.2	Purification on Chromatography Columns in Downstream Process
	Development 465
13.3.3	Kinetic Analysis for Characterization of Biopharmaceuticals 466

- 13.3.4 Vaccine Design and Efficacy 468
- 13.4 Conclusions 469 References 470
- 14 Proteomics Toolkit: Applications in Protein Biological Production and Method Development 473

Glenwyn Kemp and Achim Treumann

- 14.1 Introduction 473
- 14.1.1 Problem of Availability 474
- 14.1.2 What Is Proteomics? 474
- 14.2 Applications of Proteomics 475
- 14.2.1 Protein Identification and Characterization 475
- 14.2.2 Protein Modifications 476
- 14.2.3 Protein Interactions 476
- 14.2.4 Protein Quantitation 477
- 14.3 Myths and Misconceptions-Perceived Drawbacks of Proteomics 477
- 14.3.1 High Set-Up Cost 477
- 14.3.2 Time-Consuming/Low Throughput 478
- 14.3.3 Expertise and Training 478
- 14.3.4 Reproducibility 479
- 14.4 Critical Factors for Industrialization of Proteomics 480
- 14.4.1 Quality Control 480
- 14.4.2 Robustness and Reliability 481
- 14.5 Case Studies 481
- 14.5.1 Two-Dimensional PAGE 481
- 14.5.2 Mass Spectrometry as a Process Development Tool 482
- 14.5.2.1 Matrix-Assisted Laser Desorption Ionization Biotyping 483
- 14.5.3 Quantitative Proteomics 484
- 14.5.3.1 Stable Isotope Labeling 484
- 14.5.3.2 Isobaric Labeling 485
- 14.6 Conclusions 486 References 487
- 15 Science of Proteomics: Historical Perspectives and Possible Role in Human Healthcare 489

Nawin Mishra

- 15.1 Science of "Omics" 489
- 15.2 Major Advances in Biology That Led to the Sciences of "Omics" 489
- 15.3 Mendel's Principles of Inheritance 490
- 15.4 One Gene/One Enzyme Concept of Beadle and Tatum 490
- 15.5 Watson–Crick Structure of DNA 490
- 15.6 Development of Different Technologies Responsible for the Emergence of Genomics and Proteomics 491
- 15.6.1 Genomics-Specific Technologies 491

- XIV Contents
 - Protein Separation, Protein Sequencing, and Their Throughput 15.6.2 Technologies 492
 - 15.7 Genomics 492
 - 15.8 Proteomics 493
 - 15.8.1 Start of Proteomics 496
 - 15.8.2 Development of Proteomics 498
 - 15.8.2.1 Two-Dimensional Gel Electrophoresis 498
 - 15.8.2.2 Mass Spectrometry 499
 - 15.8.2.3 X-Ray Crystallography and Nuclear Magnetic Resonance Spectroscopy 501
 - Proteomics as a Basis for Differentiation 501 15.8.3
 - 15.9 Interactomics: Complexity of an Organism Based on the Interactions of Proteins 501
 - 15.10 Relation between Diseases, Genes, and Proteins: Diseasome Concept 503
 - 15.11 Proteins as Biomarkers of Human Diseases 503
 - 15.11.1 Modification of Proteins 503
 - 15.12 Metabolomics 505
 - 15.13 Proteomics and Drug Discovery 506
 - 15 14 Current and Future Benefits of Proteomics in Human Healthcare 506
 - 15.14.1 Understanding Complex Diseases and Possibility of Personalized Medicine 506
 - 15.14.2 Better Drugs for Human Diseases 507
 - 15.14.3 Identification of Protein Biomarkers 507
 - 15.14.4 Drug Development 507
 - 15.14.5 Discovery of New Proteins as Drugs 507
 - 15.14.6 Proteins Linked to Brain Diseases 508 References 508

Part Five Quality Control 511

16	Consistency of Scale-Up from Bioprocess Development to
	Production 513
	Stefan Junne, Arne Klingner, Dirk Itzeck, Eva Brand, and Peter Neubauer
16.1	Inhomogeneities in Industrial Fed-Batch Processes 513
16.2	Effects of Conditions in Industrial-Scale Fed-Batch Processes on the
	Main Carbon Metabolism 515
16.3	Effects of Conditions in Industrial-Scale Fed-Batch Processes on Amino
	Acid Synthesis 518
16.4	Scale-Down Reactors for Imitating Large-Scale Fed-Batch Process
	Conditions at the Laboratory Scale 520

16.5 Improved Two-Compartment Reactor System to Imitate Large-Scale Conditions at the Laboratory Scale 523

Contents XV

- Description of the Hydrodynamic Conditions in the PFR Part of the 16.6 Presented Two-Compartment Reactor 526
- 16.7 Description of Oxygen Transfer in the PFR Part of the Two-Compartment Reactor 529
- *E. coli* Fed-Batch Cultivations in the Two-Compartment Reactor 16.8 System 531
- 16.9 Future Perspectives for the Application of a Two-Compartment Reactor 537 References 538
- 17 Systematic Approach to Optimization and Comparability of **Biopharmaceutical Glycosylation Throughout the Drug Life Cycle** 545 Darvl L. Fernandes
- 17.1 Costs of Inconsistent, Unoptimized Drug Glycosylation 545
- Scheme 1: Traditional Approach to Comparability of Drug 17.2 Glycosylation 547
- Incomparable Glycosylation During Scale-Up of Myozyme[®] 548 17.2.1
- Why Incomparable Glycosylation Occurs with Traditional Drug 17.2.2 Scale-Up 549
- 17.3 Scheme 2: Comparability of Drug Glycosylation Using QbD DS 551
- 17.3.1 QbD Approach to Glycosylation in the A-MAb Case Study 552
- Scheme 3: Enhanced QbD Approach to Comparability of Drug 17.4 Glycosylation 554
- 17.4.1 Informatics Tools for Enhancing QbD for Glycoprotein Drugs 554
- Case for a Population Model for Comparability of Glycoprotein 17.4.2 Therapeutics 555
- 17.4.3 Domain Ontology Model for Drug Realization 557
- 17.4.4 Ontology Map 557
- 17.4.5 Elements View of the Ontology Map 560
- Building a Population Comparability Model for Drug 17.4.6 Glycosylation 561
- 17.4.6.1 SE Board 562
- 17.4.6.2 Step 1: Categorize the Biological Behaviors of the Drug in Terms of Safety and Efficacy 563
- 17.4.6.3 Step 2: Determine and Prioritize the Glycosylation Critical Quality Attributes 563
- 17.4.6.4 Step 3: Develop a Tuned Glycoprofiling System to Measure the GCQAs 571
- 17.4.6.5 Step 4: Describing and Optimizing the Glycosylation QTPP by Glycoform Activity Modeling 573
- 17.4.6.6 Using Glycan Activity Modeling in Glycosylation Optimization and Comparability Studies 577
- Conclusions 580 17.5 Acknowledgments 581 References 581

XVI Contents

18	Quality and Risk Management in Ensuring the Virus Safety of
	Biopharmaceuticals 585
	Andy Bailey
18.1	Introduction 585
18.2	QRM and Virus Safety 586
18.2.1	Product Complexity and Risk 587
18.3	Pillars of Safety 590
18.3.1	Sourcing–Defining the Baseline Risk 590
18.3.1.1	Epidemiology–A Powerful Tool for Reducing Risk for Human- and Animal-Derived Components 592
18.3.1.2	Additional Measures for Controlling Animal-Derived Materials 596
18.3.2	Testing-Reducing Further the Baseline Risk 596
18.3.2.1	<i>In Vitro</i> and <i>In Vivo</i> Adventitious Agent Tests-Advantages and Disadvantages 597
18.3.2.2	Infectivity Tests for Endogenous Retroviruses 597
18.3.2.3	Electron Microscopy Tests for Retroviruses 598
18.3.2.4	Reverse Transcriptase Assays 598
18.3.2.5	PCR Testing-Advantages and Disadvantages 599
18.3.3	Sourcing and Testing–Is It Enough? 599
18.3.4	Pathogen Clearance–Controlling the Residual Risk 600
18.3.5	Controlling Suppliers of Media and Other Active Pharmaceutical
	Ingredients 601
18.4	Committee for Proprietary Medicinal Products Guidelines for
	Investigational Medicinal Products–Risk Management in Practice 602
18.4.1	Using Generic Data to Reduce Virus Safety Testing 603
18.4.2	Experience with Well-Characterized Cell Lines 603
18.4.3	Reducing Virus Validation Requirements for IMPs 604
18.4.4	Platform Purification Processes 605
18.5	Developing a Robust Risk Minimization Strategy–what is the Correct
	Paradigm? 60/
	References 609
19	Ensuring Quality and Efficiency of Bioprocesses by the
	Tailored Application of Process Analytical Technology and
	Quality by Design 613
10.1	Introduction 612
19.1	PAT and ObD in Bioprocessing Engineering Meets Biology 614
19.2	PAT and QbD in bioprocessing – Engineering Meets biology 014
19.2.1	Engineering Meets Biology 616
19.2.2	Aspects of Biological Demands, Selected Examples, 617
10.3.1	Basic Patterns of Nutrient Metabolism: Clucose and Clutamine as
10 2 4 4	Complementary Major Carbon and Energy Sources 618
19.3.1.1	Chitemine Metabolism 625
19.3.1.2	Guitamine Metabolism 025 Chusese and Chitemine Concentrations in Patch Cultures (25
19.3.1.3	Giucose and Giutamine Concentrations in Batch Cultures 625

- 19.3.2 Effect of Culture States on Glycosylation 626
- 19.3.2.1 Dissolved Oxygen Partial Pressure and pH 627
- 19.3.2.2 Concentrations of Nutrients 629
- 19.3.2.3 Concentrations of Metabolic Byproducts: Lactate and Ammonia 629
- 19.3.2.4 Supplementing Suitable Precursors 632
- 19.3.2.5 Effects on Secreted Glycoproteins in the Medium 632
- 19.3.3 Cell–Cell Adhesion and Aggregation: Influence on the Growth Behavior of CHO Cells 632
- 19.3.3.1 Conclusions 637
- 19.4 Technical and Engineering Solutions 638
- 19.4.1 PAT and QbD Compliant Process Understanding and Process Control: From Data to Information and Knowledge, and Its Transfer from Bioprocess Development to Manufacturing 639
- 19.4.1.1 Acquisition of Primary Data 640
- 19.4.1.2 Gaining/Deriving Information from Data 644
- 19.4.1.3 Process Understanding Based on Knowledge 646
- 19.4.1.4 Demonstration of Process Understanding and Proof-of-Concept 647
- 19.4.1.5 Process Control 648
- 19.4.2 Challenge of Speed and Quality in Bioprocess Development 649
- 19.5 Conclusions 653 Acknowledgments 653 References 654

Part Six Process Design and Management 657

- **20 Bioprocess Design and Production Technology for the Future** 659 Jochen Strube, Florian Grote, and Reinhard Ditz
- 20.1 Introduction 659
- 20.2 Analysis of Biomanufacturing Technologies 662
- 20.2.1 Process Concepts in Biomanufacturing 663
- 20.2.2 Total Process Analysis 666
- 20.2.2.1 mAbs 667
- 20.2.3 Batch to Continuous Manufacturing 672
- 20.2.3.1 Discussion 677
- 20.3 AAC: Anything and Chromatography 679
- 20.3.1 Expanded-Bed Chromatography 679
- 20.3.2 Membrane Chromatography 681
- 20.3.3 Liquid–Liquid Extraction 682
- 20.3.4 Crystallization/Precipitation 684
- 20.4 Process Integration 685
- 20.5 Process Design and QbD 689
- 20.6 Package Unit Engineering and Standardization 691
- 20.7 Downstream of Downstream Processing 694
- 20.7.1 Human Insulin 695

XVIII Contents

20.7.2	Antibiotics (Penicillin) 69	6
20.8	Conclusions 699	
	Acknowledgments 699	
	References 700	

- 21 Integrated Process Design: Characterization of Process and Product Definition of Design Spaces 707 Richard Francis
- 21.1 Introductory Principles 707
- 21.2 Original Process Development Paradigm 707
- 21.3 The Essential QbD Concepts 710
- 21.4 Conclusion 715 References 715
- 22 Evaluating and Visualizing the Cost-Effectiveness and Robustness of Biopharmaceutical Manufacturing Strategies 717 Suzanne S. Farid
- Suzurine S. Furia
- 22.1 Introduction 717
- 22.2 Scope of Research on Decision-Support Tools for the Biotech Sector 719
- 22.2.1 Challenges 720
- 22.2.2 Typical Stages of Analysis and Approaches 722
- 22.3 Capturing Process Robustness Under Uncertainty 723
- 22.3.1 Fed-Batch versus Perfusion Culture Strategies 723
- 22.3.2 Robustness of Legacy Purification Facilities to Higher Titer Processes 725
- 22.4 Reconciling Multiple Conflicting Outputs Under Uncertainty 728
- 22.4.1 Stainless Steel versus Single-Use Facilities for Clinical Trials 728
- 22.5 Searching Large Decision Spaces Efficiently 731
- 22.5.1 Portfolio Management: Portfolio Selection and Capacity Sourcing 731
- 22.5.2 Chromatography Sizing Optimization for Future Facilities 735
- 22.6 Integrating Stochastic Simulation with Multivariate Analysis 736
- 22.6.1 Predicting Short-Term Facility Fit Upon Tech Transfer to Larger Facilities 737
- 22.7 Conclusions 737 Acknowledgments 739 References 740

Part Seven Changing Face of Processing 743

- 23 Full Plastics: Consequent Evolution in Pharmaceutical Biomanufacturing from Vial to Warehouse 745 Roland Wagner and Dethardt Müller
- 23.1 Increased Demand, Reduced Volumes, and Maximum Flexibility–Driving Force to Plastic Devices 745

- 23.2 Plastic The Flexible All-Round Replacer: From Material to Function 747
- 23.3 Pollution with Plastics: Leachables and Extractables 753
- 23.4 Plastics for Storage: Vial and Bag 755
- 23.4.1 Vial 755
- 23.4.2 Bag 755
- 23.5 Plastics for Cultivation: Flask, Tube, and Unstirred and Stirred Bioreactor 757
- 23.5.1 Flasks 757
- 23.5.2 Tubes 757
- 23.5.3 Bioreactors 757
- 23.6 Plastics for Purification: Column and Membrane 760
- 23.6.1 Column 760
- 23.6.2 Membrane 761
- 23.7 Case Study: Comparability of Plastic Bag-Based Bioreactors in Cultivation Processes 761
- 23.8 Conclusions and Prospects 763 References 765
- 24 BioSMB[™] Technology: Continuous Countercurrent Chromatography Enabling a Fully Disposable Process 769 Marc Bisschops
- 24.1 Introduction 769
- 24.1.1 Evolution of Continuous Countercurrent Chromatography 769
- 24.1.2 Continuous Chromatography Systems 773
- 24.1.3 Industrial Applications of Continuous Chromatography 774
- 24.1.3.1 Fractionation Chromatography 774
- 24.1.3.2 Continuous Ion-Exchange Chromatography 775
- 24.2 Continuous Chromatography in Biopharmaceutical Industries 776
- 24.2.1 Industry Drivers 776
- 24.2.2 Potential Application Areas 778
- 24.2.3 Key Challenges 779
- 24.2.4 BioSMB[™] Technology 780
- 24.2.4.1 Disposable Format 780
- 24.2.4.2 Prepacked Columns 780
- 24.2.4.3 Alternative Chromatography Formats 781
- 24.3 Process Design Principles 781
- 24.3.1 Process Design Fundamentals 781
- 24.3.1.1 Thermodynamic Equilibrium 781
- 24.3.1.2 Mass Transfer Kinetics 782
- 24.3.1.3 Other Phenomena 783
- 24.3.1.4 Performance Prediction 783
- 24.3.2 Process Design Features 783
- 24.3.2.1 Fractionation Chromatography 784
- 24.3.2.2 Capture Chromatography 785

XX Contents

24.4	Case Studies 786
24.4.1	Protein A Chromatography 786
24.4.2	Aggregate Removal Using Hydrophobic Interaction
	Chromatography /8/
24.4.3	Vaccine Purification Using Size-Exclusion Chromatography 788
24.5	Conclusions 789
	References 790
25	Single-Use Technology: Opportunities in Biopharmaceutical
	Processes 793
	Maik W. Jornitz, Detlev Szarafinski, and Thorsten Peuker
25.1	Current Single-Use Technologies 793
25.1.1	Liquid Hold Bags 794
25.1.2	Mixing 795
25.1.3	Product and Component Transfer 797
25.1.4	Purification 798
25.1.5	Filtration 800
25.1.6	Sterile Connections 801
25.1.7	Filling 802
25.2	Future Single-Use Operations 802
25.2.1	Upstream Opportunities 803
25.2.2	Downstream Opportunities 804
25.2.3	Single-Use Process Engineering 804
25.3	Automation Requirements in Single-Use Manufacturing 806
25.3.1	Data Acquisition 808
25.3.2	Monitoring and Control 808
25.3.3	Facility-Wide Automation Structure 808
25.4	Qualification and Validation Expectations 809
25.4.1	Equipment Qualification 809
25.4.2	Process Validation 811
25.5	Operator Training 815
	References 815
26	Single-Use Biotechnologies and Modular Manufacturing Environments
	Invite Paradigm Shifts in Bioprocess Development and
	Biopharmaceutical Manufacturing 817
	Alfred Luitjens, John Lewis, and Alain Pralong
26.1	Introduction 817
26.2	Paradigm Shift at Crucell 819
26.2.1	Introduction to Crucell 819
26.2.2	Evolution of Single-Use Biotechnology 821
26.2.2.1	Phase I: Single-Use Technology Development-Success with
	Small-Scale Plastic Cell Culture Units 821
26.2.2.2	Phase II: Single-Use Biotechnologies Development-Scale-Up,
	Capsules, and Coupling 824

- 26.2.2.3 Phase III: Single-Use Biotechnologies Development-Industrialization and Simplification 829
- 26.2.2.4 Crucell Manufacturing of mAbs with the PER.C6® Cell Line: A Completely Single-Use Fed-Batch Process 835
- 26.2.2.5 Missing Elements and Outlook 839
- 26.2.3 Adaptation of Facility Layout to Single-Use Technology 842
- Process Development Value Stream 849 26.2.4
- 26.2.5 Assessment of the Crucell Paradigm Shift 854
- 26.3 Conclusions and General Outlook 856 References 857

Index 859