Biopharmaceutical Manufacturing, Volume 2

Unit processes

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To Dr Sarah Yim, whose humility, scientific wisdom and vision help grow many minds at the FDA and in the biosimilars community.

—SKN

I am grateful to my father for encouraging me to be a curious scientist and to my husband for supporting me to practice my passion.

—SL

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Preface

Overview

The evolution of humanity owes much to the resilience of *Homo sapiens* against the challenges of Nature through changing lifestyles as knowledge became widespread. In prehistoric times, from about 3.5 million years ago until writing emerged around 5500 years ago, there was less spread of knowledge because there was little long-distance travel. Writing did not become a widespread tool for disseminating knowledge until the nineteenth century when education became more accessible.

The end of the era of prehistory occurred at very different dates in different places. It came earlier in regions such as Mesopotamia, the Indus River Valley, and Egypt, where the development of medicines was recorded. However, myths were also believed widely, such as osteoarthritis being caused by lifting heavy objects, which was routine work for most.

Throughout human history, it has proven to be advantageous to utilize other life forms as resources. The use of genetic engineering to transform yeast, bacteria, mammalian cells, plants, and viruses into recombinant therapeutic proteins showcases a continuous timeline of discovery and exploration, beginning with hunting and gathering, then the domestication of animals and crops, ultimately leading to the production of therapeutic proteins.

In prehistoric cultures, plant products (herbs and chemicals derived from natural sources) were among the oldest cures for illness. Earths and clays may also have offered some of the first medicines of prehistoric peoples. Moving from prehistoric times to ancient civilizations, the Egyptian Imhotep describes the diagnosis and treatment of 200 diseases in 2600 BC. The birth of Hippocrates in 460 BC created much of the concept of medicine, and Galen brought much to the science of medicine in 130 AD. Pedanius Dioscorides wrote *De Materia Medica* in around 60 AD. Vaccination against communicable diseases occupies a large portion of medicine's history, starting with the Persian physician Rhazes, who identified smallpox in 910 (although Pylarini gave the first smallpox inoculations in 1701). Avicenna wrote *The Book of Healing* and *The Canon of Medicine* in 1010. Anton van Leeuwenhoek observed bacteria under a microscope in 1683. However, it was not until 1857 when Louis Pasteur identified germs as the cause of disease and developed vaccines for anthrax, rabies, and tuberculosis. Many vaccines have followed.

The first chemically synthesized drug was chloral hydrate, in 1832, and aspirin was first produced in 1899. Since then millions of new molecules have been synthesized. A large number have ended up as effective drugs to treat just about every type of ailment, from modulating immune systems to inactivating viruses.

The discovery of insulin in 1922 revolutionized biological medicine. Insulin was also the first biopharmaceutical drug developed by recombinant technology. Penicillin became available in 1928 thanks to Fleming and streptomycin thanks to Waksman.

Individual enzymatic transformation phases with micro-organisms in chemical manufacturing pathways, such as the biotransformation of steroids in 1950, expanded the scope of biotechnological pharmaceutical manufacturing. Growing

knowledge of the regulation of primary and secondary metabolite production, as well as expertise with microbes as biological agents brought significant advances in the field of biotechnology drugs.

In 2006 the first vaccine directed against a specific cause of cancer was approved. The first mRNA vaccine was approved in 2021 to treat the coronavirus COVID-19. This is a significant milestone that has opened the door to many new and novel biopharmaceutical products.

Definitions

Biopharmaceuticals

In writing this book, we need to define clearly what a 'biopharmaceutical product' is. This is necessary to bring a focus to the scope of the book. Chapter 1 describes how the definition of biopharmaceutical products varies between regulatory agencies and within the scientific literature, and why we created a new definition to encompass the concept that is described in this book:

> A biopharmaceutical drug product, a biologic(al) medical product, or a therapeutic biologic, is any nonendogenous macromolecule manufactured in, extracted from, or semi-synthesized from an engineered living entity.

Biotechnology

A similar dilemma arose in defining 'biotechnology', which is the critical technology for the manufacturing of biopharmaceuticals. The term 'biotechnology' has only been defined recently. It is important to look at how it is understood in different regions since this affects how regulatory controls on biotechnology-derived items are assessed. The definitions of the world's principal regulatory regions are listed in table P.1.

The use of biological entities to manufacture industrial products is known as biotechnology. The term 'biotechnology' defines numerous areas of application, including alcohol brewing, antibiotic production, and dairy processing.

For hundreds of years, brewers have used yeast to ferment grain into alcohol. Similarly, farmers and breeders use 'genetic engineering' to develop crops and livestock by selecting desirable traits in plants and animals. Scientists have only recently altered an organism's genetic material at the cellular or molecular level thanks to 'new' biotechnology techniques. Although these approaches are more accurate, the findings are comparable to those obtained using traditional genetic techniques that include whole species. The word 'biotechnology-derived products' (BDPs) refers to things produced using modern biotechnology techniques. The development of BDPs presents several obstacles, including the convergence of numerous sciences and an almost artful application of technology to produce consistent results.

However, the current interest in biotechnology is primarily the result of two major advances:

- Gene transplantation made possible by recombinant DNA (rDNA) technology (gene coding). For example, a desired protein could be inserted into a prokaryotic or eukaryotic cell, and that cell could express the desired protein.

Table P.1. Global definitions of biotechnology.

Jurisdiction	Definition	Comments
Britain	A strategy for applying biological systems, structures, or processes to both manufacturing and service industries.	Manufacturing and services industries are non-committal, broad, technology-driven, and bureaucratic.
European	Biotechnological applications applied to the manufacturing and service industries.	Changes in the definition of 'components and targets,' with the word 'integrated' playing a prominent role in defining biotechnology.
Japan	The integration of biochemistry, microbiology, and engineering sciences for the technological application of microbiological, biotechnological, and engineering sciences.	The complete utilization of technological means, such as copying and various other types, can be deemed significant because it is helping to produce 'useful' substances.
USA	Under the supervision of a licensed specialist, the use of biological agents such as bacteria or cellular components.	Harmful effects of using micro-organisms are evident, and it is also apparent that control and concerns about the technology being used unlawfully are also important.

- The spike in the production of monoclonal antibodies during times of increasing demand (i.e. antibodies arising from a single lymphocyte).

According to the Biotechnology Industry Organization, '[b]iotechnology [is] the combination of biology and technology, includ[ing] biologic applications, diagnostic tools, and businesses that improve everyday life by providing solutions to some of life's most vexing problems'.

We are adopting a simple definition of biotechnology as:

> Any nonendogenous macromolecule made in, extracted from, or semi-synthesized from an engineered living entity produced by any technology and labeled as a biopharmaceutical product.

Biopharmaceuticals are macromolecules produced in a genetically modified organism, excluding naturally derived macromolecules. There are several conflicting definitions of biopharmaceuticals; for example, the FDA considers proteins produced by a recombinant process as a biological product subject to a biological license application (BLA) filing. Protein molecules with less than 40 amino acids are not considered proteins, regardless of how they are produced. These proteins are called peptides, some of which are produced using the same technology as used for recombinant antibodies; in this context, biopharmaceuticals are products such as liraglutide with fewer than 40 amino acids produced by recombinant technology with multiple unit processes.

The impact of biopharmaceuticals

Approximately 400 biotechnology medications and vaccines have aided hundreds of millions of people since 1982. Even more are in development, treating illnesses that were once thought to be incurable, such as AIDS, Alzheimer's disease, and stroke prevention. Diseases such as cancer tend to be preventable in a limited period. For example, many enzymes used in the food industry are likely to cause cancer.

Most biopharmaceuticals are recombinant proteins manufactured by expression in genetically modified biological entities such as mammalian cell lines, bacteria, insects, fungi, and plants. The productivity of these entities continues to improve as we learn more about the ways to change their genomes. These technological advances have led to well-characterized genomes, plasmid vector versatility, the availability of different host strains, and cost-effectiveness compared to other expression systems. Biopharmaceuticals are expected to account for up to 50% of all drugs in production over the next five to ten years.

The total number of biopharmaceutical entities approved by the FDA is 218, with multiple BLAs for some. The highest number of BLAs assigned was for somatropin, followed by albumin, which was also the first BLA approved. Trend analysis shows that the trend for new biological entity approvals is becoming significantly higher, albeit slower (figure P.1).

Moreover, while injections have been the primary delivery method for the first biopharmaceuticals, future products will be supplied through, for example, oral and dermatological routes, with inhibited formulations using a range of encapsulation approaches to reduce the biological instability resulting from protein aggregation and denaturation, due to physicochemical changes such as deamination.

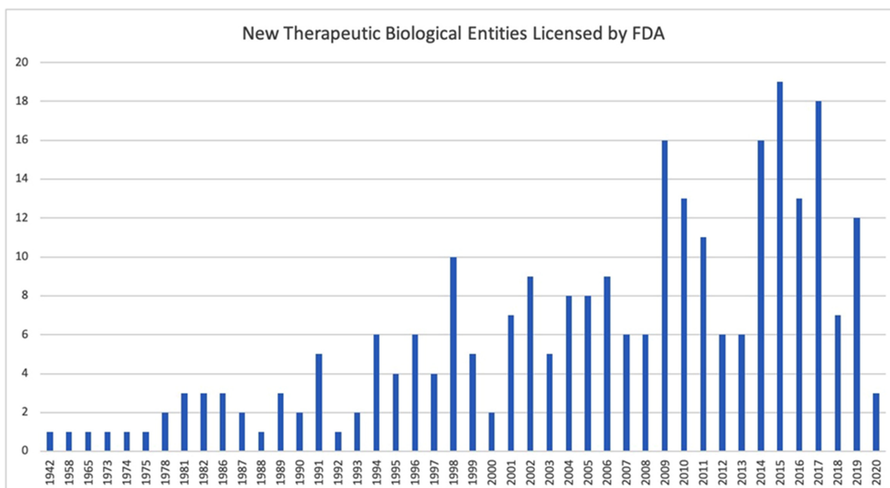

Figure P.1. BLAs licensed by the FDA from 1942 to March 2020. (Source: *FDA Purple Book*, https://www.fda.gov/drugs/therapeutic-biologics-applications-bla/purple-book-lists-licensed-biological-products-reference-product-exclusivity-and-biosimilarity-or).

Another derivative of organic medicines has been specialist products, a new classification of medicinal products for advanced treatment based on 'genes, cells or tissue engineering', including medicines for gene therapy, somatic cell therapy drugs, and combinations thereof. Advanced therapy medical products (ATMPs) are the most recent classification of pharmaceuticals for advanced therapy products. In European Medicines Agency contexts the term 'advanced therapy' specifically refers to ATMPs, although outside these contexts this term is relatively unspecific.

For example, gene-based and cellular biologics are often at the forefront of biomedical science to treat several medical conditions for which no other therapies exist. The DNA and RNA vaccines will fall in the category of gene therapy, as described in this book.

The FDA has approved several cellular and gene therapy products as of early 2020. Cancer, genetic disorders, and infectious diseases are among the diseases for which gene therapy drugs are being researched. Plasmid DNA is one of the many forms of gene therapy products available. Therapeutic genes may be genetically modified into circular DNA molecules and delivered to human cells. In addition, several cord blood products are permitted for use only in the 'hematopoietic stem cell transplant' procedure in patients with blood-forming (hematopoietic) system disorders. Cord blood contains blood-forming blood cells, which can be used to treat blood cancer patients with conditions such as leukemia and lymphomas, and certain blood-forming and immune system disorders, such as sickle cells and Wiskott–Aldrich syndrome.

Biopharmaceutical development

Drug development is a time-consuming and costly procedure. The costs for a new medicine range from $314 million to $2.8 billion on average, with a 15 year regulatory clearance process. To support the return on investment, research companies have embarked on extensive intellectual property protection that goes well beyond protein identity and gene sequence expression; the patents now include protection of formulations, manufacturing process, cell lines, indications, delivery devices, and many more. Figure P.2 shows the top 20 biopharmaceuticals and their associated patents. A complete list is provided in chapter 8.

The technology for the development and manufacturing of biopharmaceuticals has transformed significantly over the past couple of decades, with the introduction of single-use systems and novel concepts such as continuous manufacturing. The drivers for new technology include the regulatory constraints for assuring product safety in addition to the cost of goods. For example, not long ago many companies were manufacturing blood products across the globe. However, when the FDA brought in new guidelines in the 1980s on the virus clearance of animal products, the entire industry almost collapsed, leaving only a few ventures into this complex product that requires extensive testing to assure safety.

Automation is becoming a norm for most industries, the biopharmaceutical industry being no exception, but the regulatory constraints make the adoption of such modifications cumbersome and expensive.

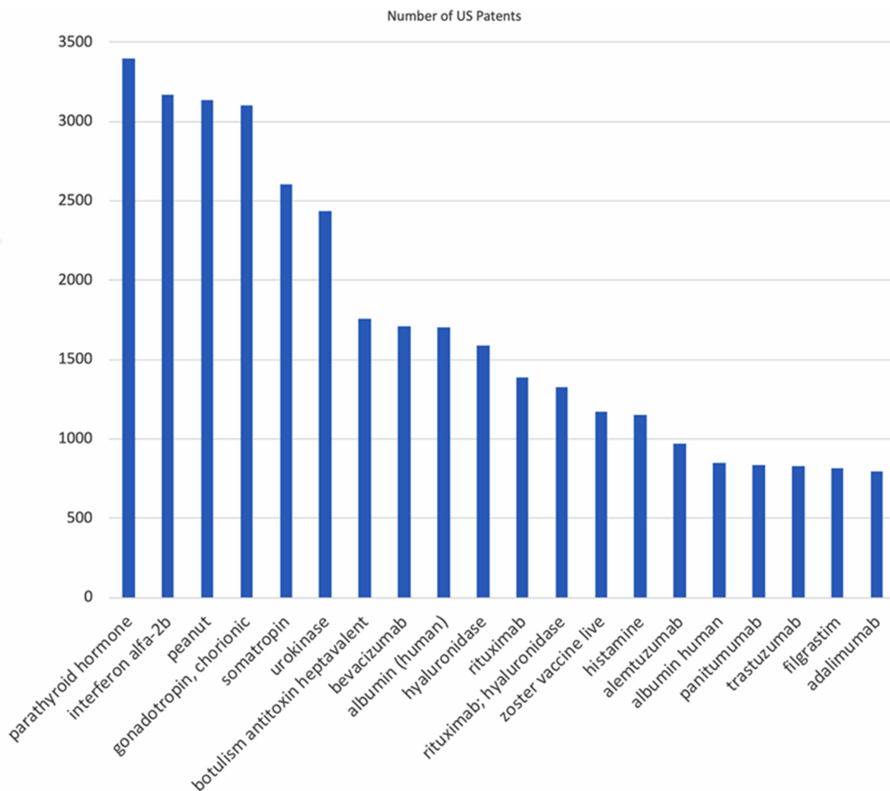

Figure P.2 The number of patents awarded to biological molecules—top candidates.

Global markets

The global pharmaceutical market amounted to $1.2 trillion in 2018, up from $100 billion in 2017, and will rise to $1.5 trillion (based on invoice pricing) in 2023 with a composite annual growth rate (CAGR) of 4%–5%. This is below 6.3% of the CAGR for 2014–2018. In 2018 expenditure in the United States amounted to $485 billion, 5.2% above the previous year, and spending in 2023 will rise to $625 billion.

The top-ten selling products in 2019 included seven biotherapeutic products (figure P.1). All except three, Xarelto, Revlimid, and Eliquis, are biopharmaceuticals. In 2019 biopharmaceutical products had a total market of about $300 billion, growing at a CAGR of 12%. With more than 50% of all new drug applications now constituting biological drugs, biological medicines will soon reach 50% of the global market. In addition, a significant push in the biopharmaceutical industry comes from biosimilars as more patents expire (figure P.3).

About this book

This book on biopharmaceutical manufacturing is divided into two volumes. The first volume, *Regulatory Aspects*, provides an overview of the science behind biopharmaceuticals pivotal to securing regulatory approval for commercial

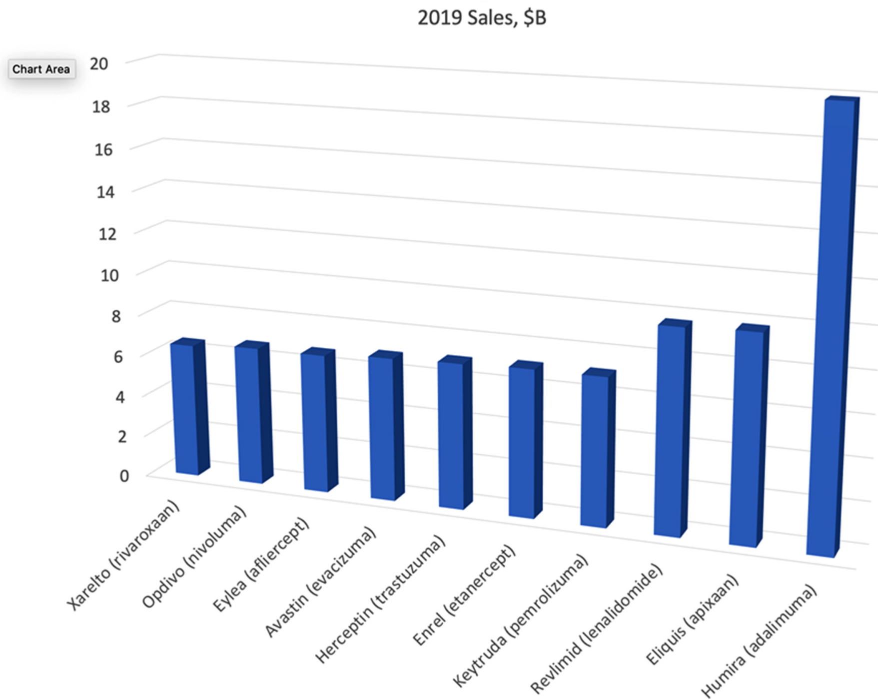

Figure P. 3. Sales of the top-ten drugs in 2019 (in billions of dollars). All except three are biopharmaceuticals.

biopharmaceutical products. The second volume, *Unit Processes*, is a detailed review of the manufacturing steps, technology implementation, and systems analysis leading to the commercial production of biopharmaceuticals.

The leading technology used in the manufacture of biopharmaceuticals is relatively new, and we foresee many spin-offs of the current recombinant engineering to manufacture many products in the future. Therefore, to expand the utility of this book we have included descriptions of a few emerging technologies that have yet to be approved for human use.

In writing this book we focused on the emerging role of recombinant technology in the manufacture of biological products, evaluated the scientific changes that are arriving rapidly in biotechnology, examined the fast expansion of the industry, and realized that there is a need to combine multiple disciplines to achieve successful commercial manufacturing. The workforce needed for this fast-expanding industry is in extremely short supply, while higher qualification requirements continue to increase as newer concepts and applications come to the market. This treatise should serve well the students who intend to adopt this industry as their career, graduate students who are specializing in biotechnology, and above all those who are actively engaged in the development, regulatory filing, manufacture, and assuring regulatory compliance to secure the market authorization of BLA for these products.

One indication of how fast the field of biopharmaceutical manufacturing is expanding can be gauged from the publications listed under the heading of

'biopharmaceutical manufacturing', with over 5000 publications over the last five years and 10 000 publications over ten years[1]. A search for 'biopharmaceuticals' shows more than 1.4 million hits in ten years and a half as many over five years.

We have developed and manufactured biopharmaceutical products, and this experience is offered in writing these chapters. SKN has written several books on this subject, including the first book on the topic, *Handbook of Biogeneric Therapeutic Proteins*, followed by the two-volume *Handbook of Biosimilars and Interchangeable Products*, the book *Disposable Manufacturing*, a book on regulatory aspects, *FDA Perspective of Biosimilarity*, and the textbook *Modern Bioprocessing*. Each of these books presented a different view of the industry and technology, with a focus on biopharmaceuticals. While we have focused on the scientific principles, we have merged the learning with practical examples. The diversity of topics in this book is intentional. In our experience, the scientists and engineers involved in development and manufacturing need to be fully aware of the patent systems and regulatory compliance needs, and stay informed on the fast-emerging trends in the art and science of biopharmaceuticals. Given below is a summary of the chapters in each volume.

Volume 1. *Biopharmaceutical Manufacturing: Regulatory Processes*

The commercial manufacture of biopharmaceuticals is challenging because of the highly rigorous regulatory compliance requirements that may not be as restrictive for other types of drugs. The reason for this is that any change in the structure of biopharmaceuticals during the manufacturing process can result in severely altered responses and side effects. The impact of these changes cannot be readily established since many effects of these drugs appear in the long term, such as in the form of altered immune response that may create new diseases such as diabetes, multiple sclerosis, and other autoimmune disorders. Understanding the regulatory concerns requires a deep understanding of the nature of the products, the strategic and tactical challenges in their manufacture, and a keen sense of their development process.

Volume 1 encompasses all the necessary details intended to teach and train the scientists and technicians involved in commercial manufacturing. Their role extends to assuring the quality of the product and the manufacturing facility's compliance. In all types of pharmaceutical manufacturing it is important that all those involved in any stage of the manufacturing be fully engaged since it is impossible to maintain current good manufacturing practice (cGMP) compliance without the collaboration of all teams—this is the focus of this volume.

Chapter 1. Introduction to biopharmaceuticals

This introductory chapter provides a clear definition of biopharmaceuticals, including biosimilars, commercial production, and global regulatory compliance requirements. Detailed descriptions of the nature and properties of biopharmaceuticals and how these macromolecules have shifted the arena of drug therapy are provided. A classification of the drug types that constitute biopharmaceuticals is given and examples are provided. There is extensive discussion of protein structure and the properties of hormones,

[1] https://pubmed.ncbi.nlm.nih.gov/?term=biopharmaceutical+manufacturing&sort=pubdate&sort_order=asc.

peptides, enzymes, protein scaffolds, antibodies, and others, and the structural modifications that can change the disposition characteristics of biological molecules are described in detail. The critical concern in using biological drugs is their immunogenicity, which we cover in detail to understand the structural elements that can produce an immune response. The factors that affect the pharmacokinetics of therapeutic proteins are essential for understanding in developing a safe and effective product. The manufacturing of biopharmaceuticals involves recombinant technology, and detailed analyses of the expression systems and their relative advantages and disadvantages are provided. This chapter's main objective is to educate scientists and technicians engaged in the manufacture of biopharmaceuticals about the essential science elements required to develop and manufacture these products and secure their regulatory approval.

Chapter 2. Antibody biopharmaceuticals
Antibodies form the most significant commercial category of biopharmaceutical products; this chapter describes in detail their structure and mode of action, with a listing of 83 FDA approved monoclonal antibodies, types of antibodies, and their targets including bispecific, multi-specific, antibody fragments, single chain, humanized, chimeric, and fully human monoclonal antibodies. Related topics include affinity maturation, antigenized antibodies, IgG1 fusion proteins, and drug or toxin conjugates. The development technologies of mouse hybridoma, transgenic animals, phage display, and single B-cells are detailed and compared for clinical and cost advantages. The commercial production of monoclonal antibodies is summarized, and an extensive list of online databases to find antibody properties is provided.

Chapter 3. Gene and cell therapy biopharmaceuticals
The newer technologies of gene and cell therapy and gene editing are described in a summary form in chapter 3. Gene and cell therapy constitute recent advances in the field of biopharmaceutical products. An overview of the diseases, risks, development, and ethical issues is provided, along with a comprehensive list of currently approved products. Gene therapy and cell therapy, including DNA and mRNA vaccines and CAR-T (T-cell therapy using chimeric antigen receptor (CAR)) techniques, are introduced. Gene editing technologies define the methodologies and their relative advantages. Upstream and downstream technologies for gene and cell therapy products and allogenic products are described, including regulatory controls, characterization of the cell population, release testing, and radioisotope tagging. Issues related to vectors and vector preparation are discussed. Finally, preclinical evaluation methods of evaluation and challenges in commercializing gene and cell therapy products are described.

Chapter 4. Formulation of biopharmaceuticals
The delivery of biopharmaceuticals is highly complex due to their structural instability, quick physical degradation, and complex chemical interactions with excipients and degradation that can lead to immunogenicity and other side effects. Extensive

descriptions of chemical degradation and the methods to obtain stability in the formulated products are provided with examples of commercial formulations. While the current methodology for the delivery biopharmaceuticals remains parenteral, a large number of new dosage forms have emerged, including oral, nasal, transdermal, pulmonary, ocular polymer-based, hydrogels, lipid-based emulsions, liposomes, and nanoparticle systems. Also included is a description of nontraditional dosage forms.

Chapter 5. Drug development cycle
Regulatory approval of biopharmaceutical products is subject to strict compliance and the creation of a registration dossier. In this chapter we describe in sufficient detail all the steps related to the filing of a BLA in the US or a marketing authorization application (MAA) in the EU. The topics include early discovery documentation, pharmacopeia, preclinical research, and IND steps to phase 4. The expectations of regulatory inspections (audits) are presented based on the authors' long experience in both the US and EU systems. This chapter also details the stepwise approach taken in the US, EU, and Japan to evaluate a regulatory dossier. Biopharmaceutical manufacturing has changed significantly since the 1980s, primarily because of the regulatory authorities' stricter controls on therapeutic products. This chapter will provide a global view of how the agencies approve products and facilities to manufacture these products. A comprehensive approach requires a complete understanding of these steps to make the large-scale commercial manufacturing of biopharmaceuticals possible.

Chapter 6. Biosimilar biopharmaceuticals
Biosimilars represent the fastest growing category of biopharmaceuticals, where a copy of the originator's biological drug is developed as a low-cost competitor to the first BLA product. The regulatory agencies have placed extreme caution on safety, and the development process takes a stepwise approach, described in detail in this chapter. Regulations across the globe are compared, and advice is provided on cost optimization of development. The key elements to establish biosimilarity, including analytical assessment, nonclinical testing, clinical pharmacology testing, and clinical efficacy testing, are described to focus on expediting the development process. A summary of FDA licensed products with more information on the studies submitted and the status of biosimilars in the EU is also provided.

Chapter 7. Intellectual property considerations
The development of biopharmaceuticals is an expensive exercise and the burden of reducing the risk of litigation for the infringement of other patents and protecting one's own intellectual property requires close collaboration between scientists and legal teams. This chapter describes the protection of intellectual property and walks the reader through a process to avoid infringing on that of others. The finer points of the definition of the vocabulary used in a patent application and the legal language are described in simple language. All types of patents, differences in global patent laws, and a detailed description of patents related to biological drugs are provided.

Details on writing freedom-to-operate documents are provided, along with a comprehensive list of the patent expiry of biological drugs and the approved BLAs and their patent expiry.

Volume 2. *Biopharmaceutical Manufacturing: Unit Processes*
In recent years, most biopharmaceutical manufacturing operations have narrowed down to upstream processes. A product is produced (expressed) and separated from a culture medium and purified (downstream) to a quality suitable for human use. Commercial manufacturing differs from a laboratory-scale set-up where the cost and time for production are often not pivotal. Today antibodies are made in quantities of thousands of kilograms, and some cytokines only into the hundreds of grams; both scales require different scopes of the same unit operations and processes. Volume 2 is geared toward providing a practical plan for deploying recombinant manufacturing technology, starting with the creation of a productive cell line and finishing with packaging a product for human use. Economy and safety of the process may not always be mutually exclusive; the key is to assure a consistent high yield while maintaining the quality standards assured in the regulatory filing. This volume provides practical training for technicians and advises scientists on modifying the process and maintaining cGMP compliance.

Chapter 1. Understanding bioprocessing
Biopharmaceutical manufacturing is complex due to the multiple steps that must integrate well within meeting cGMP compliance requirements. This chapter summarizes the manufacturing process, which is discussed in greater detail in the following chapters of this volume. This chapter further includes sections that do not fit into other unit processes, such as cost containment, documentation, and cGMP compliance assurance. The personnel involved in biopharmaceutical manufacturing must understand the entire process regardless of their specific role, which may be limited to a specific unit process, to enable smooth integration. A biopharmaceutical manufacturing chain's strength is only as strong as its weakest link, making it essential that the entire team understands each step. This chapter provides a stepwise description of clearly distinct unit processes or actions, a discussion of how they are connected, and the routine practices and development choices that make a manufacturing process commercially feasible. We strongly urge the reader to read this chapter with great scrutiny to make best use of the rest of the book.

Chapter 2. Recombinant manufacturing system
The manufacturing engine for biopharmaceutical products is a living entity—a bacterium, a yeast, a mammalian cell, or transgenic cells and species. The DNA is modified to combine with a foreign gene of expression entity. The genetically modified entity can express proteins of interest. This chapter discusses recombinant DNA technology, the types of entities available for recombination of their DNA, and a detailed comparison of each of these expression systems' advantages and

disadvantages. Understanding the fundamentals governing these processes is extremely important to process design. This chapter focuses on the fundamentals of protein expression, the availability of various host systems, and the selection basis for the intended application.

Chapter 3. Cell line development

The cell line expressing the desired target protein is the core of the recombinant therapeutic process. Given the various choices of expression system available, the simplicity of microbial systems (*Escherichia coli*) and the popular mammalian systems (Chinese hamster ovary cells) are preferred. The advantages of microbial systems lie in their fast growth and easy manipulation, but the lack of post-translational modifications (glycosylation) is a major drawback. In contrast, mammalian cell cultures are more complex than microbial cultures. They have a longer doubling time, but this is compensated for in their mechanism to allow posttranslational modifications essential for protein functionality. Stable cell line development, a time-consuming process, entails incorporating linearized plasmid DNA encoding the therapeutic protein into the host genome. When the cells divide the transgenes are transferred to the subsequent generations. Screening for recombinants is performed by including a selectable marker on the plasmid. This can be an antibiotic resistance gene or a metabolic gene. The recombinants are heterogeneous concerning their growth characteristics, expression (productivity), and product quality. Therefore it is essential to screen the potential clones to establish the right production cell line based on these features. A necessary consideration for mammalian cell lines is to ensure that the cell line is clonally derived from a single cell, confirming the homogeneity (genotypic and phenotypic). The chosen cell line (production) must demonstrate proper growth, productivity, and product quality over the generations required to run the manufacturing process.

Chapter 4. Upstream equipment and systems

The technology for the upstream unit process has evolved significantly over the past few decades through new uses of systems initially developed for fermentation to allow the growth of many different cell cultures to yield a target protein molecule. The biological process of cell growth and expression when a genetically modified organism is involved is more sensitive to bioprocess conditions. To overcome these shortcomings, bioreactors are now designed with high-level technology to enable reproducible batch yields. This chapter describes many types of equipment and operational processes that now find their place at the core of biopharmaceutical production. While the science of bioprocessing requires a greater depth of learning about the physics and chemistry of bioprocess, in this chapter the most commonly used equipment and how it is made operational in a commercial setting is the focus.

Chapter 5. Upstream process

The upstream unit process yields a crude product either as an inclusion body in bacteria or a secreted product from a bacterial or a mammalian cell. Optimization of the upstream process is pivotal in producing a cost-effective product at a commercial scale. This

requires making an optimal choice of the cell line, culture media, and types of operation, and then, once the product is expressed, removing it from the bioreactors and separating cell debris and other impurities to prepare the yield for downstream purification. If the upstream process is not optimized the downstream process will not achieve the desired product safety and efficacy. This chapter describes the upstream systems and the materials used in achieving a highly productive yield of a recombinant biopharmaceutical.

Chapter 6. Downstream process
The target protein synthesized by the cells, either by secretion in the medium (mammalian cells) or intracellularly as inclusion bodies, must be isolated from the host cells and purified to remove or reduce product-related and process-related impurities to produce a final product with sufficient purity. Downstream processing involves several unit operations, including but not limited to centrifugation, filtration, chromatography purification, precipitation, and concentration ultrafiltration/diafiltration (UF/DF). The impurities vary in their nature. The overall downstream process design should be robust and consistent in ensuring that these impurities are removed or maintained within established limits that do not pose a safety concern. In the case of mammalian processes or processes that use raw materials of animal or human origin, adventitious viruses should be addressed in the process design to ensure there is no risk of viral contamination in the final product. Downstream processes may include more than one orthogonal step for impurity removal. Platform processes allow for a starting point for the process design of molecules that belong to the same class, but further optimization specific to the target protein is necessary.

Chapter 7. Process and product lifecycle development
Production starts with a batch record for a commercial product that is primed for economically viable manufacturing. The process evolves from a development laboratory setting step-by-step procedures into a final and mature manufacturing process. Focusing solely on qualification efforts without recognizing the development constraints is an imperfect exercise. A good process design and building quality into the process are of primary importance for ensuring a robust process. The process development is initiated with process design, wherein the target product is identified. The development of the product profile is followed by identifying the process steps, characterization, and validation using a risk assessment, prior knowledge, and process and product understanding to establish a robust system that allows consistent manufacturing of the desired product quality. These activities help establish a well-defined control strategy. Manufacturers must maintain the process in a state of control for the product's lifecycle and manufacture after defining and verifying the process, even as the environment, raw materials, equipment, personnel, and manufacturing process shift.

Chapter 8. Quality and compliance systems
Commercial manufacturing mandates compliance with regulatory requirements that are determined by the quality system in place. While scientists and technicians are familiar with the technology aspect, cGMP and good laboratory practice (GLP) compliance require a different level of understanding that is documentation based

and also continued compliance with the systems. This chapter describes how to create a cGMP and GLP compliant system starting with the responsibility of the management, resource allocation, design and qualification of manufacturing systems, and continuous monitoring of quality attributes. The quality assurance system emphasizes the need for a validation master plan that includes elements specific to biopharmaceuticals, including virus validation, virus inactivation, and analytical methods validation. Details of process validation from a statutory and analytical perspective are presented. The discussion of good laboratory practice covers the current regulatory requirements, creating a plan and documentation to stay compliant, and specific analytical methodologies issues. A valuable database of the regulatory inspection results from over 20 years of FDA audits is included to provide a self-audit analysis to assure full compliance.

Chapter 9. Single-use technology
Single-use technology has long been part of biopharmaceutical manufacturing but remained limited to a few components until a couple of decades ago when the regulator imposed requirements to isolate contamination, at a higher capital cost of establishing traditional manufacturing facilities. Improved yield and quality become evident, resulting in the fast growth of single-use components that now range from starting a cell culture to upstream, downstream, and fill and finish. Since big pharma had already invested in fixed-pipe technology, single-use technology remains favored by smaller or newer companies. This chapter presents a detailed outlook of the present and the future of single-use technology, that is expected to become the primary technology in the next couple of decades. This chapter provides introductions to single-use technology companies' sources, the regulatory compliance details, and suggestions for creating a comprehensive single-use train.

Chapter 10. Advancements and trends in biomanufacturing
Recombinant protein therapeutics is the single most important product group driving today's pharmaceutical development with a broad range of indications—oncology to cardiovascular and other infectious diseases. With several developments and blockbuster drugs on the market, there is an increasing need to enable technologies and other supporting functionalities to keep pace with this demand. Monoclonal antibodies have emerged as top-class in the recombinant protein group. Since the first licensed product, manufacturing processes have evolved over the years, starting with improving low productivity processes, expanding the use of host cell systems, and engineering these systems to their full potential to express both simple and complex proteins. These upstream advancements have shifted the process constraints to the downstream processes, mainly due to a lack of flexibility in handling high productivity processes. The projected future demand for recombinant therapeutics will probably shift the emphasis onto developing processes that can accommodate multiple products in a flexible production facility. However, this is likely to be limited in the current operation state due to the current plant designs and a lack of flexibility with facilities. The next-generation facilities will maximize facility utilization, have a reduced footprint, ease scalability, and reduce the downtime between batches or

even products in a multiproduct facility, but most importantly, reduce processing time and cost, and improve efficiencies. Single-use technologies, process intensification strategies for upstream and downstream operations, and modular facilities enable technologies to be widely integrated into bioprocessing.

Additionally, a glossary of terms is included for quick reference and teaching of terminologies and concepts.

Acknowledgments

We owe our gratitude to our scientific and professional colleagues, particularly those we only know from the field's seminal literature but have never met. We may have subconsciously quoted their work, assuming it was all in the public domain; I hope they would forgive us for doing so, as it would be impossible to recognize them. An extensive bibliography is not required.

Finally, we would like to admit our mistakes, and we could not find a better statement than that which appeared in the first edition of *Encyclopedia Britannica* (1786):

> WITH regard to errors, in general, whether falling under the denomination of mental, typographical, or accidental, we are conscious of being able to point out a greater number than any critic whatever. Men who are acquainted with the innumerable difficulties attending the execution of a work of such an extensive nature will make proper allowances. To these, we appeal and shall rest satisfied with the judgment they pronounce.

We will appreciate receiving your comments to improve this treatise in the future, and most kindly, if you find any mistakes.

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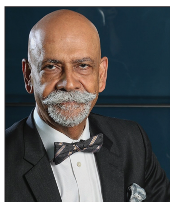

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Biopharmaceutical Manufacturing, Volume 2

Unit processes

Sarfaraz Niazi and Sunitha Lokesh

Chapter 1

Understanding bioprocessing

Biopharmaceutical manufacturing is complex due to the multiple steps that must integrate well while meeting the cGMP compliance requirements. This chapter provides an overview of the manufacturing process as detailed in the rest of the chapters in this volume. This chapter further includes sections that do not fit in other unit processes, such as cost containment, documentation, and cGMP compliance assurance. The personnel involved in biopharmaceutical manufacturing must understand the entire process regardless of their specific role,which may be limited to a particular unit process, to enable a smooth integration. A biopharmaceutical manufacturing chain's strength is only as strong as its weakest link, making it necessary for the the entire team understand each step. This chapter provides a stepwise description of clearly distinct unit processes or steps, a discussion of how they are connected, and the routine practices and development choices that make a manufacturing process commercially feasible. We strongly urge reading this chapter with great scrutiny to make best use of the rest of the book.

1.1 Overview

This chapter attempts to provide an overview of the fundamental concepts of biotherapeutic manufacturing methods, with a focus on recombinant proteins. Despite the modernization and adaptation of applicable technology, these concepts have remained constant over time. The manufacturing processes follow the same basic requirements for a cGMP compliant production process as other pharmaceutical products, such as process validation, environmental control, aseptic manufacturing, and quality control/quality assurance systems, but with a lot more complexity, because cell propagation, purification methods, and analytical controls are all important.

To obtain the required product from the starting components, product and process development necessitates many unit activities (figure 1.1).

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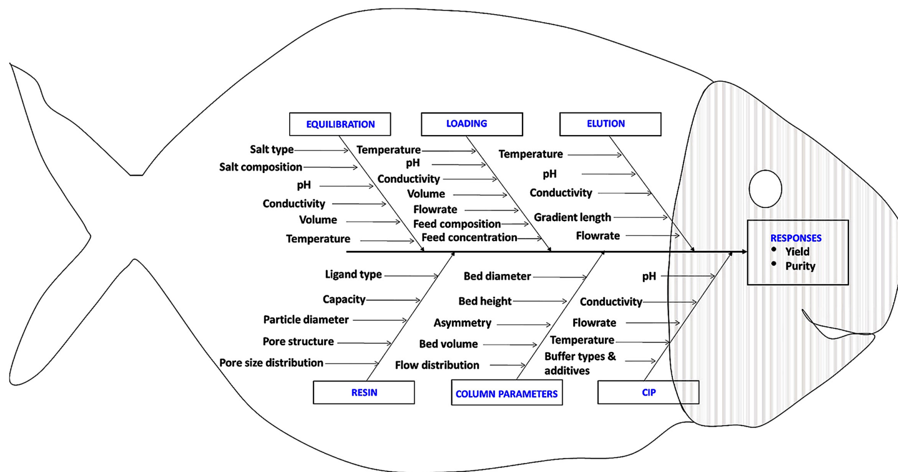

Figure 1.1. A recombinant manufacturing train's typical unit procedures. The target protein can be found in bacteria, mammalian cells, and insect cells, as well as transgenic animals and plants (upstream). The harvest is purified by a series of purification unit activities that include capture, intermediate purification, and polishing (downstream), resulting in pure bulk material (drug substance). Finally, the drug substance is converted into a human-friendly product (drug product). Upstream refers to protein expression and harvesting, while downstream refers to drug ingredient conversion, intermediate purification, polishing, and formulation. The primary strategic question in the upstream process is whether the cell culture should be done in batch, fed-batch, or continuous mode. Because of the greater yields in continuous processing, the latter is extremely appealing at low expression levels. The expression system utilized often determines the recombinant process outline, however, most recombinant processes follow a similar pattern.

1.1.1 Unit operation

To obtain the required result from the raw materials, product and process development necessitates several unit operations. The expression method, upstream, downstream, fill and finish, and packaging, for example, are all unit operations associated with building the overall process in recombinant manufacturing.

The unit operation of a process is a critical stage. Unit processes that include a physical change or chemical transformation include separation, crystallization, evaporation, filtration, polymerization, isomerization, and other reactions.

Upstream, downstream, and formulation processing are all part of the manufacturing process. Cell culture, which leads to fermentation, is referred to as upstream. The harvesting step separates the cells, followed by the separation of target proteins from the host and process-related contaminants, an intermediate purification step (or further separation from the host), and a polishing step to separate target proteins from impurities. At this point, the yield is referred to as a drug substance. By turning drug ingredients into drug products, the formulation phase creates a dosage form ready for human administration. This is an equally critical stage, as studies have shown that how the batch is handled can drastically alter protein structures.

1.2 Key considerations for the production of biotherapeutics

1.2.1 Cell-substrate

The beginning material for biopharmaceutical synthesis is bacterial, yeast, insect, or mammalian cell cultures. The cell seed lot system is used by manufacturers to assure the identification and purity of the initial raw material. A cell seed lot is made up of aliquots from a single culture. The master cell bank (MCB) has enough culture ampoules to supply the operational cell bank with source material. It is made from a single colony (bacteria, yeast) or a single eukaryotic cell and frozen to maintain genetic stability (WCB). The WCB is a collection of cells derived from one or more MCB ampoules that have been cryogenically frozen and used to kick off the production batch.

The most popular cellular expression systems used to generate biopharmaceuticals include bacteria (*E. coli*, *Bacillus subtilis*, *Lactococcus lactis*), yeast (*Saccharomyces cerevisiae*, *Pichia pastoris*), mammalian cells (Chinese hamster ovary (CHO), baby hamster kidney (BHK)), and insect cells. The baculovirus expression system, on the other hand, is in use to some extent and has the potential to become a future biopharmaceutical. A comprehensive list of cell lines and providers may be found at https://www.biocompare.com.

The choice of expression system is influenced by the type of target protein, post-translational changes, expression level, intellectual property rights, and manufacturing cost. While the *E. coli* expression system allows for quick and low-cost expression, it does not allow for complicated protein expression or the integration of *in vitro* folding and tag removal into the downstream process. Yeast generally expresses the target protein in its native form in the medium, but the levels of expression are very low. Many of the same advantages apply to insect cells as do to mammalian cells. Table 1.1 lists the advantages and disadvantages of each of these methods.

The origin and history of both the MCB and the WCB conversion passes are crucial to understand since the cell bank's genetic stability throughout storage and replication is a critical problem. A single MCB ampoule is used and then frozen or lyophilized. The new MCB must be extensively tested and characterized before being released. For biological products, a product licensing application or amendment must be submitted and accepted before a new MCB can be formed from a WCB. The information on the expression vector's fabrication, the fragment containing the genetic material that encodes the desired product, and the host cell's genotype and phenotype are all included in a product application(s). Biological systems are concerned about the genetic integrity of cell banks during development and storage, contaminating microbes, and endogenous viruses in particular mammalian cell lines. As part of the application document, manufacturers must include a list of all tests performed to describe and qualify a cell bank.

The tests needed to classify a cell bank are determined by the final product's intended use, the host and expression system, and the manufacturing process, including purification techniques. In addition, as technology progresses, the types of

Table 1.1. Comparison of various expression systems, advantages and disadvantages. (After Niazi S 2005 Manufacturing overview *Handbook of Biogeneric Therapeutic Proteins Regulatory, Manufacturing Testing and Patent Issues* (Boca Raton, FL: CRC Press).)

Qualifier	Bacteria	Yeast	Insect cells	Mammalian cells	Transgenic animals
Cost	Low	Low	High	High	Medium
Example	*E. coli*	*Saccharomyces cerevisiae, Pichia pastoris*	Lepidoptera	CHO	Cattle
Extracellular expression	No	Yes	Yes	Yes	Yes
Host cell protein expression	No	No	No	No	Yes
In vitro protein refolding	Yes	No	No	No	No
Level of expression	High	Medium	Medium	Medium	Very high
Major impurities	Endotoxins	Glycosylated products	Viruses	Viruses	Viruses and prions
Met-protein expression	Yes	No	No	No	No
Post-translation modifications	No	No	Yes	Yes	Yes
Regulatory track record	Good	Good	N/A	Good	N/A
Time to produce expression system	Fast: 5 days	Fast: 14 days	Medium: 4 weeks	Slow: 4–8 weeks	Very slow: 6–33 months
Unintended glycosylation	No	Possible	Possible	Possible	Possible

tests can change. The MCB is rigorously evaluated using the following tests, though they are not the only ones used:

- Detection assays for viral contamination.
- DNA fingerprinting is used to characterize genotypes.
- Nutrient requirements, isoenzyme analysis, development, and morphological characteristics are used to characterize phenotypes.
- Other microbial pollutants are detected using a sterility test and a mycoplasma test.
- Restriction enzyme mapping and sequence analysis characterize the vector/cloned fragment at the molecular level.
- Retrovirus detection using reverse transcriptase assay.
- The desired product can be generated in a repeatable manner.

The WCB does not need to be tested as fully as the MCB, although WCB characterization is required. The WCB is submitted to the following tests, albeit this is not a complete list:

- Characterization of phenotypes.
- Enzyme mapping for restriction.
- Testing for sterility and mycoplasma.
- Testing the red product's ability to be produced in a consistent manner.

The MCB and WCB must be processed in a way that maintains genetic stability. Cells stored in liquid nitrogen (or its vapor phase) are more stable than those kept at −70 °C. In the case of a freezer failure, it is also advised that the MCB and WCB be placed in multiple locations.

1.2.2 Media

The medium is chosen to offer the requisite growth rate and nutrients to the species producing the intended result. There should be no dangerous or poisonous components in component materials that could be passed to the finished product through cell culture, fermentation, or purification. There is a huge amount of water in the medium. The recombinant method utilized, the production phase, and the intended application of the result will all influence the consistency of the water. Before being used, raw materials must meet approval standards if they are supplied by a certain manufacturer. A small-scale pilot run followed by a full-scale production run is recommended when employing raw materials from a different manufacturer to confirm that growth parameters, yield, and final product purity stay the same.

For the growth of most mammalian cell cultures, serum is frequently required. Companies must take care to guarantee that the serum is sterile because it can be a source of contamination by unwanted species, particularly mycoplasma. It is also possible that bovine serum includes the agent that causes bovine spongiform encephalopathy (BSE). Because there is no sensitive *in vitro* test to detect the presence of this agent, it is vital that the makers know the source of the serum and receive verification that it is not from BSE-endemic areas. Proteases and other enzymes generated from bovine sources can also contain BSE. Biological product makers must declare the provenance of the components they employ in their production.

The medium must be sterilized using either sterilization *in situ* (SIP) or continuous sterilization systems (CSS). Any nutrients or chemicals introduced after this must be sterile. The air lines must have sterile filters and must meet the following criteria on a regular basis, although not exhaustively:

- Confirm that the serum source is compliant.
- Ensure that the medium is sterile, confirm that the sterilization cycle has been properly checked.
- Ensure that quality management has checked all raw materials.
- Determine whether the bovine source contains contaminates.
- Document any cases where the medium did not meet any of the requirements.

- Verify that no expired raw materials were used in the manufacturing process.
- Check to see if the medium and other additives were stored properly.

1.2.3 Process overview

Table 1.2 lists the processes that are specific to expression systems.

1.2.4 Upstream production process

Cell cultures can be operated in batch, fed-batch, or continuous mode, depending on the expression system employed. Continuous systems might take weeks to complete, resulting in multiple harvest pools and needing a detailed batch strategy definition. For bioreactor inoculation, transition, and harvesting, validated aseptic procedures must be applied. To add or remove materials from industrial bioreactors, steam-sterilized lines and steam-lock assemblies are frequently utilized. It may not be essential to turn off the steam supply if the heating of the line or bioreactor vessel wall will not affect the culture.

To achieve proper and effective expression of the desired product, a bioreactor device must be closely monitored and precisely regulated. Other fermentation process characteristics such as growth rate, pH, waste byproduct level, viscosity, chemical additives, density, mixing, aeration, foaming, and others must be established and monitored. The final product may be affected by shear pressures, process-generated heat, seals, and gasket effectiveness.

A number of growth factors regulate protein synthesis. Any of these parameters can alter deamidation, isopeptide synthesis, and host cell proteolytic processing. While nutrient-deficient media are frequently utilized as a selection mechanism, media low in certain amino acids can result in replacements. During development *E. coli* is depleted of methionine and leucine, causing it to manufacture norleucine and insert it into a site ordinarily held by methionine, yielding a wild-type protein homologue. Because these products are so closely linked, chromatographic separation can be challenging; this might affect how release requirements are implemented and how successful the product purification process is.

It is necessary to validate computer programs for fermentation control, data logging, data reduction, and analysis.

In bioreactor systems built for recombinant micro-organisms, a pure culture must be maintained, and the culture must be kept within the devices. Containment can be achieved by using a host-vector system that is less capable of surviving outside of a laboratory setting and, when necessary, physical methods.

1.2.5 Downstream process

The first chromatographic capture phase sample was prepared using centrifugation, filtration, and micro-filtration. In other circumstances, expanded bed technology was employed to integrate the harvest and capture phases. Because of the huge volumes treated, significant improvements in ionic strength or pH are not suggested.

Table 1.2. Process overview for different expression systems. (After Niazi S 2015 Upstream systems optimization *Biosimilars and Interchangeable Biologics* (Boca Raton,FL: CRC Press).)

General	Cell culture	Batch-fed or perfusion; 3–40 days.
	Harvest	Centrifugation/filtration; omit if using an expanded bed.
	Capture	Chromatographic unit operation is typically based on affinity or IEC. Remove major host and process-related impurities and water.
	Variable unit operation	The unit operation may be refolding (if *E. coli* is used) or virus inactivation if insect cells, mammalian cells, or transgenic animals are used.
	Intermediate purification	The chromatographic unit operation is typically based on HIC, IEC, or HAC stepwise gradient technology used to remove host and process-related impurities.
	Variable unit operation	This unit operation may include tag removal (if *E. coli* is used) or virus removal by filtration if insect cells, mammalian cells, or transgenic animals are used.
	Polishing	The chromatographic unit operation is typically based on HP-IEDC or HP-RPC stepwise/linear-gradient technology used to remove product-related impurities.
	Variable unit operation	SEC or ultrafiltration to assure proper drug substance formulation.
	Drug substance	The drug substance's conversion to drug product typically includes a change of buffer, precipitation, or crystallization.
	Formulation	Batch manufacturing often including stabilizers such as albumin.
	Finished drug product	Filling in appropriate containers such as vials or pre-filled syringes.
E. coli (gram-negative)	Fermentation	Expression of N-terminally extended target protein to overcome the formation of Met-protein.
	Harvest	Harvest of cells by centrifugation before cell disruption.
	Cell disruption	Disruption with a French press or similar; wash out inclusion bodies.
	Extraction	Extraction under reducing and denaturing conditions (e.g. 0.1 M cysteine, 7 M urea pH 8.5).
	Capture	Purification under reducing and denaturing conditions (e.g. IEC or IMAC if the protein is His-tagged).
	Renaturation	Controlled folding of the target protein using hollow fiber, SEC, dilution, or buffer exchange.
	Intermediate purification	Purification of the folded target protein (e.g. IEC, HIC, HAC).
	Enzyme cleavage	Cleavage of the N-terminal extension with exo- or endoproteases.
	Polishing 1	Purification of the target protein (e.g. HP-IEC, HP-RPC).

Table 1.2. (*Continued*)

	Polishing 2	Purification of the target protein by SEC (not always included).
	Drug substance	The purified bulk product.
	Formulation	Re-formulation of the drug substance preparing for administration to humans.
	Drug product	The final product.
Gram-positive bacteria	Fermentation	The expression of the target protein to the periplasmatic room or medium.
	Harvest	Harvest of cells by centrifugation before cell disruption. This step may be bypassed using expanded bed technology.
	Capture	Purification of the target protein from the supernatant.
	Intermediate purification	Purification of the target protein (e.g. IEC, HIC, HAC).
	Polishing 1	Purification of the target protein (e.g. HP-IEC, HP-RPC).
	Polishing 2	Purification of the target protein by SEC (not always included).
	Drug substance	The purified bulk product.
	Formulation	Re-formulation of the drug substance preparing for administration to humans.
	Drug substance	The final product.
Yeast	Fermentation	The expression of the target protein to the medium.
	Harvest	Harvest of cells by centrifugation before cell disruption. This step may be bypassed using expanded bed technology.
	Capture	Purification of the target protein from the supernatant or by expanded bed technology.
	Intermediate purification	Purification of the target protein (e.g. IEC, HIC, HAC).
	Polishing 1	Purification of the target protein (e.g. HP-IEC, HP-RPC).
	Polishing 2	Purification of the target protein by SEC (not always included).
	Drug substance	The purified bulk product.
	Formulation	Re-formulation of the drug substance preparing for administration to humans.
	Drug product	The final product.
Insect Cells	Cell culture	The expression of the target protein to the medium.
	Harvest	Harvest of cells by centrifugation before cell disruption. This step may be bypassed using expanded bed technology.
	Capture	Purification of the target protein from the supernatant or by expanded bed technology.

	Virus inactivation	Inactivation using low pH, high temperature, detergents, etc.
	Intermediate purification	Purification of the target protein (e.g. IEC, HIC, HAC).
	Virus filtration	Nano-filtration.
	Polishing	Purification of the target protein (e.g. HP-IEC, HP-RPC, SEC).
	Drug substance	The purified bulk product.
	Formulation	Re-formulation of the drug substance preparing for administration to humans.
	Drug product	The final product.
Mammalian cells	Cell culture	The expression of the target protein to the medium.
	Harvest	Harvest of cells by centrifugation before cell disruption. This step may be bypassed using expanded bed technology.
	Capture	Purification of the target protein from the supernatant or by expanded bed technology.
	Virus inactivation	Inactivation using low pH, high temperature, detergents, etc.
	Intermediate purification	Purification of the target protein (e.g. IEC, HIC, HAC).
	Virus filtration	Nano-filtration.
	Polishing	Purification of the target protein (e.g. HP-IEC, HP-RPC, SEC).
	Drug substance	The purified bulk product.
	Formulation	Re-formulation of the drug substance preparing for administration to humans.
	Drug product	The final product.
Transgenic animals	Raw milk	Milking of animals according to good agricultural practices.
	Skim milk	Centrifuged raw milk with low-fat content.
	Capture	Purification of the target protein from the skim milk.
	Virus inactivation	Inactivation using low pH, high temperature, detergents, etc.
	Intermediary purification	Purification of the target protein (e.g. IEC, HIC, HAC).
	Virus filtration	Nano-filtration.
	Polishing	Purification of the target protein (e.g. HP-IEC, HP-RPC, SEC).
	Drug substance	The purified bulk product.
	Formulation	Re-formulation of the drug substance preparing for administration to humans.
	Drug product	The final product.

Instead, a purification principle that is compatible with the features of the application sample should be adopted. The initial stage in the recovery procedure is to remove the target protein from the fermentation or cell culture media, which is often contaminated. In cell culture and yeast-derived products, many of these proteins are released directly into the medium, needing just cell separation to achieve considerable purification. Lysis of the bacteria is frequently required to recover the appropriate protein from *E. coli* derived products. Because the desired product can be cleaved by proteases generated by lysed organisms, fast purification of the desired protein is crucial in each situation. Because they are difficult to remove, complicate the recovery process, and have a considerable impact on the final product's stability, these trace proteases are a key source of worry in the purification of biotechnology-derived goods. The recovery process is usually designed to purify the final product to a high degree. The purity required for a product is determined by a variety of factors, but chronic use products will be required to have a significantly higher purity than single-use ones. Impurities in biotechnology products have been created to be removed or minimized throughout the recovery process. Impurities in the media include trace amounts of DNA, growth factors, residual host proteins, endotoxins, and residual cellular proteins.

1.2.5.1 Harvest and clarification

After the fermentation process is completed, the desired product is extracted and, if necessary, refolded to restore configuration integrity and purity. The first steps in the downstream process to retrieve the produced protein are harvest and clarity. Its goal is to remove significant impurities from the expressed protein (water, cell waste, lipoproteins, lipids, carbohydrates, proteases, glycosidases, colored compounds, adventitious agents, fermentation additives, and fermentation by-products) and prepare the sample for intermediate purification.

To retrieve intracellular proteins, cells must be disturbed during fermentation. This is accomplished through chemical, enzymatic, or physical methods. After the disruption, cellular debris can be recovered using centrifugation or filtration. To recover extracellular protein, centrifugation or membrane filtration are used to separate products from processing organisms. Initial separation procedures such as ammonium sulfate precipitation and aqueous two-phase separation can concentrate the components after centrifugation. Chromatographic procedures to eliminate contaminants and get the product closer to the final specifications are the most common additional purification processes. Extraction and isolation require filtration or centrifugation (table 1.3).

1.2.5.2 Capture

The target protein should be concentrated and stored in a way that will preserve its biological activity. The capture phase removes contaminants from the host and the process, serving as an initial purification step for the target molecule. Due to the enormous volumes handled and the nature of the application material, the first

Table 1.3. Key features of the harvest and recovery processes commonly used.

Harvest and clarification	Features
Centrifugation	Cells, cell debris, and residues are usually removed from the harvest using centrifugation. The primary way of elucidation.
Filtration	The separation of particles from the solution using high pore size filters is known as depth filtration. It is good for both primary and secondary clarification. For the removal of designated contaminants such as endotoxins, viruses, or prions, filtering techniques include conventional filtration, micro-filtration, ultrafiltration, and specialized filters.

chromatographic purification stage is based on the on/off principle based on selective binding of amino acids to the matrix. Large particle size materials are employed to prevent the column from clogging, and low resolution is expected. Because purity is not an issue at this point, one of the primary concepts of capture operations is to focus on the target protein features to accomplish a successful binding and pay less attention to impurities. If the affinity ligand stability does not impose excessive limits on the use of effective cleaning and sterilization protocols, affinity chromatography's high selectivity may be an interesting approach for capture. The most popular purification principles are ion-exchange chromatography, packed bed affinity, and extended bed affinity. With extended bed technology, direct application of the cell culture is feasible, reducing unit procedures. Simple technologies, broad parameter intervals, high flow rates, affordable chromatographic medium, and large particle sizes should be used in capture operations to provide process resilience, accuracy, and economy. The sample volume is reduced significantly, the quantity of contaminants is reduced, and the sample is ready for the next stage.

1.2.5.3 Intermediate purification process

The intermediate purification step is the first purification step where most principal contaminants are eliminated, including cellular proteins, culture media components, DNA, viruses, endotoxins, and others. Best results are obtained when medium-size particles are employed to alter the protein chemically and enzymatically and increasing the resolution of related molecules. Using desorption principles that are more selective, such as multi-step or continuous gradient elution processes.

The SEC (size exclusion chromatography) can be used as a final step to:

- Remove polymers
- Formulate bulk drug substances
- Do a buffer swap to any buffer
- Improve protein stability
- Make the bulk material's composition uniform.

1.2.5.4 Final purification process

Chromatography is one of the most effective methods for purifying proteins. Over the years this method has been turned into a commercial tool. The capture, intermediary, and polishing principles are used in most biopharmaceutical methods, and at least three separate chromatographic steps are used. The sample's composition determines the type of chromatographic medium to use. The co-solvents, in most cases, do not affect the protein's ability to bind to the column. Every chromatographic technique employs a balance of resolution, speed, power, and recovery. Choose rational chromatographic technique combinations based on the technique's key benefits and the state of the sample at the start or end of each step. Techniques may be mixed, but only when they are mutually beneficial. The purification method is mainly accomplished using one or more column chromatography techniques.

1.2.5.5 Virus removal

When using mammalian cells or transgenic animals as the expression system, virus inactivation and active filtration add an extra unit activity.

1.2.5.6 Product concentration and DS formulation

Ultrafiltration and diafiltration are two typical filtration operations for concentrating the final distilled product solution and, if necessary, performing a buffer exchange into the respective drug substance (DS) formulation components at the desired concentration. The low molecular weight impurities are pressed through the membrane with the aqueous liquid in the ultrafiltration phase, while the substance stays on the primary side. The particles do not settle on the membrane because of the crossflow direction.

Additional processing (e.g. the addition of surfactant) may be needed to achieve the final DS formulation, after which the DS is filtered through a 0.2 μm filter. Representative samples of the DS are collected and analyzed following specified guidelines. The bulk DS is often kept frozen (−20 °C), and the stability of the product is tested under various conditions to determine the best storage time and temperature. These conditions are validated to assure that the DS remains usable over time.

1.2.6 Formulation process

The drug product composition is critical; most biopharmaceuticals are packed in liquid or lyophilized form, which necessitates a certain minimum volume to contain the final drug product. Therefore, a size-exclusion or desalting phase might be required to reduce the volume. Although the upstream and downstream steps in deciding the final characterization of a product have received a lot of attention, the formulation phase can greatly impact the product's safety and efficacy. There are unlikely to be any additional ingredients in a lyophilized product, but a liquid formulation can contain many common ingredients. Albumin, sucrose, polysorbates, buffer salts, and other substances are examples.

1.3 Chromatography methods

1.3.1 Affinity chromatography (AC)

Identifying structural epitomes is how AC works. Low protein concentrations, vast application volumes, changing pH levels, and low to moderate ionic strength are all possible. AC is a selective technology that allows for large-scale impurity removal since the on/off concept is perfectly matched to the capture mode. Because proteins elute at low pH, the sample should be given at a neutral to slightly alkaline pH.

1.3.2 Anion exchange chromatography (AEC)

AEC is the most commonly utilized chromatographic technique. It is suitable for capture, intermediate purification, and polishing because it uses very specific media with predetermined particle sizes, spacers, and ligands. HIC has a poorer resolution than IEC, but when the proper ligand is found it can have a high selectivity.

1.3.3 Cation exchange chromatography (CEC)

CEC is suitable for low protein concentration, large application volumes and low ionic strength application. It is also tolerant of ethanol, urea, and non-ionic detergents.

1.3.4 Hydroxyapatite chromatography (HAC)

HAC is typically an effective answer when it comes to purification issues. The high-resolution reversed phase chromatography is mostly utilized for molecular weight proteins less than 25 KDa since the binding constant for larger molecular weight proteins is too high. Because of elution in organic solvents, protein stability in these buffers is a major issue. It is suitable for low protein concentration; large application volume; $pH > 7.0$; however, the matrix does not tolerate acidic pH.

1.3.5 Hydrophobic interaction chromatography (HIC)

At high salt concentrations, HIC is frequently employed in conjunction with ion exchange, binding proteins, and elution at low salt concentrations. If HIC is used in the capture step, the hydrophobic substances in the fermentation broth and cell cultures may reduce the binding capacity. If the feed volume is significant and salt is required to boost the ionic strength of the solution, it may be a less appealing capture strategy. Large quantities of salt would consequently be required, which increases manufacturing costs and creates a waste disposal issue.

1.3.6 Immobilized metal affinity chromatography (IMAC)

The International Metal-Ligand and Protein Complex Binding Center focuses on metal-ligand and protein complex binding. Low protein concentrations, large application volumes, neutral pH, strong ionic pressure, and denaturant, detergent,

and ethanol tolerance are all requirements. IMAC is rarely utilized in industrial downstream manufacturing. It is a versatile protein capture approach that is also a one-of-a-kind tool for histidine-tagged proteins. Given the ionic strength of biological starting materials, it is also a salt-tolerant process. The combined efforts of proteins' primary, secondary, and tertiary structures can result in exceptionally high selectivity.

1.3.7 Reversed-phase chromatography (RPC)

Alkaline pH is not tolerated by hydrophobic contact silica-based matrices. Low protein concentrations, high application sizes, low pH, and organic solvents are all well tolerated in this chromatography.

1.3.8 Size-exclusion chromatography (SEC)

High protein concentration; small application volumes; any pH; any ionic strength; any solvent; steric exclusion from the intraparticle volume; sample volume limited to a maximum of 5% of the column volume. It is suggested that SEC be used as the last purification step. Even though the sample volume rarely exceeds 5% of the column capacity, the approach allows for the removal of di- and polymeric compounds, transfer to a well-defined buffer, and increased protein stability in many circumstances. The relative value of each of the unit operations is shown in table 1.4.

The chemicals employed in chromatography operations might create contaminants in the end product, whether in the stationary (bonded) or mobile phases. Confirmation is the responsibility of the manufacturer (i.e. demonstrating the elimination of potentially harmful chemicals). Chemical leaching certification from a column material provider is insufficient because contamination is process and product based. Validation is required when isolating product monoclonal antibodies or when utilizing a technique that includes a monoclonal antibody purification phase. The procedure must demonstrate the removal of leaking antibody or antibody fragments. As a result, it is critical to make sure the cell line used to make monoclonal antibodies is devoid of infectious organisms such as viruses and mycoplasmas. The biggest concern is that the product could be tainted with an antigenic medication, posing a risk to patients. The process must be continuously monitored to prevent or limit contamination.

Antigenicity connected to the active drug component and host proteins is unique to biotechnology-derived medicines when compared to conventional drugs. Manufacturing processes that use specific solvents should be scrutinized to see if they can generate chemical reorganizations that alter the antigenic profile of the medicine. The manufacturer must also show that the output accuracy of the innovative chromatographic column is accurate. Because single-use goods, such as vaccines, are not provided on a regular basis, different concerns apply, and antigenicity is desired in this scenario.

Table 1.4. The relative time importance of various chromatographic methods in various downstream processes.

Principle	Capture	Intermediary purification	Polishing
Ultrafiltration	+++	+++	+++
SEC	+	+	+++
RPC	+	+	+++
Precipitation	+	+++	+++
Microfiltration	+++	+	+
IMAC	+++	++	+
IEC	+++	+++	+++
HIC	++	+++	+
HAC	+	+++	+
Filtration	+++	+++	+++
Crystallization	+	++	+++
Affinity	+++	+	+

On the other hand, validation of the removal of ligand or extraneous protein contamination is required. Biotechnology-produced goods, unlike those generated from natural sources, must have nucleic acid elimination verified during purification.

1.4 Process lifecycle

1.4.1 Maturity

As soon as a project enters the production phase, material delivery for preclinical and clinical studies becomes a big issue. The numbers necessary, according to cGMP rules, much exceed what can be generated in a production laboratory, hence clinical trial material must be manufactured. Process designers are faced with a dilemma: to what degree should process design be sacrificed in order to supply the desired material in the lowest amount of time? An immature process would demand process change late in the project, resulting in laborious analytical and biological testing repetitions. It is critical to recognize the magnitude of the situation. Consider developing drug substance and drug product stability testing, sources, toxicological research, and virus validation investigations on a mammalian pilot scale. Any data acquired using the old method would be thrown into doubt if a substantial process change occurred. While comparability studies can accomplish a lot, stability and viral validation investigations must be replicated, which will severely delay the project. Process economy, which should not compromise safety but can lead to a no-go decision, is another element that project managers must examine.

Until scaling up, the process maturity level must be defined, or, in other words, process maturity requirements must be defined *a priori*. Before tech transition, the

Table 1.5. Maturity of process.

Maturity criteria	Comments
Cell line	QA release of the MCB is required before transferring cells into the cGMP facility.
Drug product	Formulation to be flexible enough to change the composition of the bulk; short-term stability documented.
Drug substance	Short-term stability of the intermediary compounds to be documented.
In-process control	Parameters stated in intervals (validated and proven acceptable ranges) and monitored; analytical method description plus data for in-process analyses to be provided.
Intermediary compounds	Data from three small-scale batches should be provided; critical parameters and their interaction should be defined. Holding times of relevance to large-scale operations should be provided.
Process design	The design should be robust with provisions for removal of the host, process, and product-related impurities. Refolding is involved, including a well-defined renaturation step, where virus contamination can be an issue, integrate virus removal and inactivation steps with due consideration for the protein's denaturation.
Quality control	DS and DSP quality control plan defined and analytical method description plus typical data provided.
Raw materials	A list of raw materials should be provided; do not use materials not qualified to be used in cGMP manufacturing (see DMF discussion).
Specifications	Acceptance criteria for DS and DSP provided where possible.

conditions specified in the table below should ideally be met. Even the real world is not flawless, and some tasks can go unfinished during scale-up, resulting in significant changes and possibly process overhaul later on. As a balance between time to market and additional construction costs, some businesses intend to redesign processes between phases 2 and 3. Below are the maturity requirements for the technology transfer scale (table 1.5).

1.4.2 Scale-up

Except for the column diameter in chromatographic unit operations and the membrane region in filtration operations, the downstream approach is designed so that most parameters are adjusted (in a linear fashion). The most significant roadblock—the human factor—remains the eternal conflict between the pilot-scale mindset and commercial selling criteria. At the small-scale production level, the US FDA's Process and Analytical Technology (PAT) Initiative (http://www.fda.gov/cder/OPS/PAT.htm) recommends implementing the concept of 'build in', in which process protection, robustness, cGMP enforcement, facility constraints, economy, and time to market are all built into the process. The process is tested on a small

scale against predetermined process maturity factors, such as parameter intervals, in-process control points, essential parameters, interactions, robustness, yield, documentation standard, raw material certification, and analytical maturity procedures, before being transferred to a larger scale. The typical step-by-step scaling-up procedure is incompatible with this notion (a short-cut approach to accommodate unrealistic timelines). The cell culture and first purification processes (e.g. capture) are scaled up before the technology is established on a small scale. Stepwise scaling up creates a lot of uncertainty, takes a long time, and results in a late process overhaul. This level of work is also popularly outsourced, in particular if the organization intends to outsource manufacturing. Transferring an immature process into a pilot environment can delay phases 1–2 of clinical material manufacture because process modifications can put doubt on the stability, viral validation, and preclinical data. As a result, any symptoms of a developing mechanism should be handled as soon as possible. Immature process signs include the following:

- Many batches are discarded or fail to meet requirements.
- During production, the manufacturing process comes to a halt.
- Impurity profiles are changing.
- Unit processes need to be reworked.
- UV diagrams may not be superimposed over each other.
- Yields are poor or differ from batch to batch.
- Protein stability is a concern.
- The process is frequently redesigned.

1.4.3 Optimization

The process design is locked once the process has been scaled up, but there are room for improvement within the specified (proven acceptable range) parameter intervals. Adjusting the process to increase yields, process economy, process robustness, column lifespan, raw materials, and labor savings is part of the optimization process.

1.4.4 Tech transfer and documentation

Before moving the procedure from the production laboratory to the pilot plant, many considerations are taken into account. There are significant cultural variations between the two areas. The research team focuses on protein chemistry, aiming for the highest resolution and technically elegant solutions. On the other hand, the pilot worker is more concerned with engineering problems and making the best use of the equipment. As a result, good coordination between the two domains is essential, highlighting the need for 'design in'. The size of the tech transfer package reflects the efficiency and maturity of the process, as well as its supporting operations. The following sections discuss issues related to the technological shift from the production laboratory to the pilot facility.

The recombinant protein manufacturing technology transfer kit is a detailed document that discusses different issues in enough detail for the transferee to reproduce the procedure. The transfer package comprises:

- The rationale for the method, as well as a summary of the manufacturing schedule.
- Physical and chemical properties of the target protein molecule.
- Safety profile; reworking and propagation paperwork; cell line history; MCB vials, WCB vials, WCB release documentation.
- *Method*: Small batch data; key parameters; relevant impurities description; host, process, and product-related; process flow sheet; significant impurity removal processes; batch production plan are included.
- Any missing information in the master production document, batch record, formulation deviation criteria, creation history document, and compliance record.
- In-process materials, medication components, and drug products are all kept in controlled environments.
- *Shipping*: Terms and conditions for both the drug substance and the drug product, as well as packing requirements.
- *Controlling the process*: For pH, conductivity, redox potential, protein concentration, temperature, holding duration, load, transmembrane strain, linear flow, and other parameters, required tests and, if needed, parameter intervals (tolerances and requirements) are required.
- Acceptance criteria for appearance, identity, biological activity, purity, and quantity; pharmacopeial requirements, if applicable.
- For analytical techniques, there are descriptions, typical data, a method qualification standard, methods transfer procedures, and reference material.
- *Stability*: Real-time and extrapolated data are required for both drugs and drug products.
- *Virus validation*: documentation and policy, as well as a compliance analysis.
- *Validation*: The master plan, the employee training process, end-of-training goals, and follow-up support have all been modified.

1.4.5 Validation process

Purification techniques are usually tested on a small scale to see how effective the processing stage is. Many differences must be considered when comparing a laboratory-scale operation to a scale-up operation. Longer processing times may have a detrimental influence on product quality since the product is exposed to buffer and temperature conditions for longer periods of time. Product stability must be thoroughly documented under purifying circumstances. It is critical to understand a step's flaws and how successful it is. After process validation on the output size batch, the impact of scale-up should be compared. Although data on a small scale may improve validation, validation on production batch sizes is crucial. Validation protocols must be followed when columns are regenerated to allow for recurrent usage, and the process must be monitored for chemical and microbiological contamination on a regular basis.

When batches are rejected, it is vital to figure out what went wrong in the manufacturing and control procedures. To avoid the issue from occurring again, a proper action plan can be implemented.

1.5 Costing

Multiple factors influence the best scale-up and scale selection in an integrated model. The model for cost estimation is provided below.

The production process (facility depreciation, raw material pricing, quality control, and quality assurance) must match the intended results. As a general guideline, manufacturing costs should not exceed 15% of the price per dose (vial). The cost of labor, chromatographic media, filters and membranes, buffers, other raw materials, the amount and kind of in-process control analyses, and total output are all elements that affect the process economy. The expense of complying with environmental rules is increased. After they have been utilized, chemical solvents are more expensive to dispose of. The necessity that any material in contact with genetically modified cells be sterilized and disposed of properly adds a significant amount to the total production costs.

The expression system used, cultural needs, and the necessity for process robustness are all factors to consider in terms of cost. Thus this is part of the suggested 'design in' method. Outsourcing is strongly suggested for newcomers to the biopharmaceutical business because constructing a cGMP facility is costly. This advice is not only affordable, but it also exclusively offers logistical solutions. Few pharmaceutical companies understand how to treat animal farming and follow excellent cattle-raising methods when a transgenic animal is involved. Upstream, downstream, and quality control expenses are closely connected to differences in process design and batch sizes versus in-process monitoring and analytical control systems. Large batches can be processed in a methodical manner, reducing the workload on the workforce and the quantity of samples to be evaluated.

Table 1.6 can be used to calculate the expenses of processing a batch of recombinant-derived products. For cost estimates and individual costs not given in the table, an excel spreadsheet is advised.

Below are the expense contributions at different stages. Cost-cutting opportunities should be identified, particularly when determining the extent of scale-up. The relative contribution of each step should be the first criterion for deciding which step should be reworked to save money. In terms of long-term effects, the relative costs should be considered. Reduce a 1% annual expense, for example, and you will save a lot of money.

Upstream = $(B1 \times B2)/12 + B3 \times B4 + B5$

Downstream = $(C1 \times C2)/12 = (B3 \times A1 \times C5 \times C3)/(C4 \times C6 \times 1000) + (C7 \times C8) + C9 + C10\ C11$

Fill and pack = $D1 \times D2 + D3$

Total cost/batch = upstream + downstream + fill and pack + $E1 \times E2 + F1 \times F2 + G1 \times G2 + H1$

Yield/batch = $(B3 \times A1 \times A3)/100\,000$

Table 1.6. Input figures for cost calculations. (Source: Niazi S 2005 Manufacturing overview *Handbook of Bigeneric Therapeutic Proteins* (Boca Raton, FL: CRC Press).)

Category	Issue	Index	Comments
General	Expression level	A1	Amount in g/l expressed
	# of batches	A2	
	Process yield	A3	% of purified protein (drug product)
	Dose	A4	mg/dose
	Pack size	A5	mg/vial
	Vials needed	A6	# of vials/dose
Upstream	Facility	B1	Yearly cost ($) of using an upstream component of cGMP facility (including maintenance and workforce)
	Utilization	B2	#Months the upstream component is used for the given project
	Culture volume	B3	Volume in liters in a given batch
	Media cost	B4	Price in $/l of culture media
	Utensils	B5	Price in $ for utensils used (e.g. filters, bags, etc)
Downstream	Facility	C1	Yearly cost ($) for using a downstream component of the cGMP facility (including maintenance and workforce)
	Utilization	C2	# months the downstream component is used for a given project
	Chromatography steps	C3	Number of chromatography steps
	Binding capacity	C4	Average binding capacity in mg/ml
	Media cost	C5	Chromatography media cost in $/l
	Buffer volume	C7	Total consumption in l (on average, 15 column volumes are used/step)
	Buffer cost	C8	$/l
	Utensils	C9	Cost in $ for components used (filters, membranes, bags, etc)
	Raw materials	C10	Cost in $ for expensive reagents, enzymes, etc
	Formulation	C11	The cost in $ for the formulation of the drug substance
Fill and pack	Number of vials	D1	Vials/batch
	Price	D2	Price/vial
	Shipping cost	D3	
In-process control	# of analyses	E1	Total number per batch.
	Cost of analysis	E2	Average in $/IPC analysis
DS quality control	# of analyses	F1	The total number of drugs substance quality analysis per batch
	Cost	F2	$/ analysis
DP quality control	# of analyses	G1	Total number per batch of the drug product
	Cost	G2	$/analysis
QA release	Cost	H1	$/batch

Cost/G = total cost/yield (per batch)
Cost per vial = cost per batch/D1
Cost per dose = (cost per batch/D1) × A6

Based on this model we can study what components of the entire process can be altered to achieve the best possible production costs.

1.5.1 Batch

A batch is the amount of cell culture volume processed downstream as a cumulative amount of material resulting in the medicinal product. Batch definition is more challenging with continuous cell cultures or transgenic animal technology since the harvest is pooled in sub-fractions. Information on the flow, harvesting methods, pools, analytical in-process control systems, and intermediate compounds must all be included in the batch scheme. If numerous columns are used, or if parallel processing or processing separation is anticipated, details are frequently supplied. Because inclusion bodies from multiple fermentation batches may be mixed and processed together, the initial sub-batches must be specified.

The aggregate of each purification process' outputs is called yield. A ten unit downstream process with a 95% average recovery produces a 57% overall yield, which is sufficient. In most cases, however, an average recovery of 80% would result in a total yield of 11%, which is unacceptably low. In some circumstances, more than ten unit operations are required to ensure a healthy product, thus expect more than 95% recovery in most, if not all, unit operations. Pegylation, one of the most recent advances in molecular structure modification, has poorer yields. Due to the statistical nature of proportional reduction, small variations in step yields result in large changes; a 95% step yield with 15 steps yields 46% total yield; the same 15 steps in a 75% step yield just 1% total yield. The majority of the time, only five to ten steps are required. As a result, the total yield ranges from 60% to 6% in a 95% to 75% phase yield change.

Several factors influence batch size, all of which have an economic impact. Automated equipment is required for large batches, however, the number of samples to test is rather small. Insulin costs roughly \$50 g^{-1}, monoclonal antibodies cost \$150–200 g^{-1}, and certain cytokines cost \$1000 or more per gram, all of which are inversely proportionate to the quantity produced. A batch culture, also known as a fed-batch culture, lasts seven days and generates 100 g each day. With a 2× flow rate per 24 h and a one g/l expression stage, a 1000 l perfusion bioreactor produces 200 g day^{-1}. Assuming a daily milk volume of 20 l, a transgenic cow producing 20 g l^{-1} milk produces 400 g each day. In terms of productivity, the transgenic cow is far more successful than cell culture-based systems, but it is also possibly less dangerous. Animal-based expression systems should be actively investigated for large-scale activities, although this has not been successful.

The batch variations document describes variability and lack of repeatability; this could be due to a scale-up procedure that results in concentration gradients in big reactors or containers. The most prevalent reason of batch failure is contamination;

large-scale mammalian cell cultures with long cell growth periods, many bioreactors, and long time intervals have higher risk factors than other expression systems. Due to the high cost of cell culture media, the financial loss could be enormous. Consider a 100 000 l bioreactor—a single infection would necessitate discarding the entire batch.

Buffer planning on a large-scale needs automation and robotics management, resulting in a new set of validation requirements and techniques, including device validation (for automated systems, mechanized racks, and computers, see FDA CGMP section 211.68 (a, b)).

1.5.2 Upstream

Cell cultures offer the most opportunities as well as the most problems when it comes to scaling up. Large-scale animal cell cultures differ significantly from traditional microbial fermentation due to the fragility of mammalian cells. Mechanical stress causes the cells to be destroyed quickly, making high aeration and agitation problematic. Animal cells, on the other hand, expand slowly and at lower densities, and do not require the high oxygen inputs required by microbial cultures. The composition of the cell culture supernatant changes often as cell culture scales up, affecting the downstream process. Except for reactor capacity, all other parameters, including culture medium, pH, temperature, redox potential, osmolality, agitation rate, flow rate, ammonia, glucose, glutamine, lactate concentrations, pCO_2, and pO_2, remain constant within the given interval limits.

The most crucial decision early in the process is to select an expression system and, finally, a cell line. The target protein's nature (glycosylation, phosphorylation, acylation, size, and so on), expected expression levels, expression system development time, batch failure risk, safety considerations, the amount required, and regulatory history all factor into the decision.

The level of expression varies based on the type of target protein and the host organism. The yield in most hosts is less than 1 g l^{-1}. *E. coli* produces a better yield of 1–4 g l^{-1}, while mammalian cells produce much higher amounts when used for antibodies. Transgenic animals produce the highest yield of 5–40 g l^{-1}. The yield in transgenic plants is unknown and not commonly available for evaluation. The kind and consistency of the expressed protein have an impact on purification yield. Although *E. coli* expression levels are normally high, *in vitro* refolding and the production of N-terminal extended target proteins have a significant impact on total process output. Furthermore, stressed cells produce less stable proteins, resulting in large losses during the purification or processing of shelf-stable medicinal compounds.

The amount of time it takes for various expression systems to evolve varies significantly. Most hosts, on the other hand, would take 4–6 months to set up the system. The length of time required to achieve target protein expression is determined on the bioreactor technology employed. Still, it takes a few days for *E. coli* and other bacteria, two weeks for yeast, four weeks for epithelial cells, and two to sixteen weeks for mammalian cells, to name a few. Transgenic goats and cows have a longer development time due to the relatively high expression levels and quick access to target protein once the system has been optimized (18–24 months).

Microbial, insect, and mammalian cell culture media, as well as commercial-scale fermenter media, are all priced differently, with the former being the most expensive per liter. Unfortunately, mammalian cell cultures often have lower expression levels than microbial systems, making cultural medium a considerable cost consideration. In an expression system yielding 1000 g l^{-1} with a 40% yield, the media cost contribution is roughly \$25 g^{-1} of protein; when the expression level drops to 10 g l^{-1}, the media cost contribution rises to \$2500 g^{-1} of protein. Low mammalian cell expression has a detrimental impact on the efficiency of the process.

The storage of inclusion bodies for a longer period can be validated in most large-scale activities (such as two years).

In industrial production, holding periods can be long and costly; these are frequently disregarded when developing processes; it is advised that suitable timeframes be confirmed early on. This is a practical issue rather than a scientific one. It takes far longer to empty a 4000 l tank than it does to empty a 2 l flask. When designing a process that takes more than 8 h, realistic shift-change considerations are sometimes required. The validity of holding times is required.

Because there is always a maximum capacity for a process to be scaled economically, the scale-up factor utilized is dictated by the process economy. The manufacturing of insulin, for example, is measured in tons rather than grams. Due to CAPEX depreciation, the cost of scaling up a process from a 100 l fermenter to a 30 000 l fermenter is not always similar, making scaling less practical. This assertion goes against the belief that bigger bioreactors are more cost-effective. Because not all CMOs have all sizes of bioreactors, it is also a good idea to adjust the technique to the available bioreactor size when outsourcing. When only tiny fermenters are employed to process bacterial cultures, pooling inclusion bodies can be beneficial. Batch size and analytical procedures should, however, be carefully matched because the cost of analytical tests rises as batch size decreases.

All other volume parameters such as sample pH, conductivity, temperature, concentration, redox potential, holding time, reagent concentration, and precipitation time are held constant within interval limits, so the sum of sample and volume is the sole alteration in the phase scale-up. The precipitation procedure has not altered.

1.5.3 Downstream

The recombinant technology is linked to the expression method utilized, cell banking, fermentation and cell cultures, raw materials used, downstream processing, and the inadvertent introduction of adventitious agents (bacteria, viruses, mycoplasma, prions). Any hazardous contaminants or adventitious agents should be eliminated from the process design. In a downstream process at least three different chromatographic stages should be used, according to a rule of thumb. If insect cells, mammalian cells, or transgenic animals were used, a virus inactivation phase and an active virus filtering step should be considered. Except for microbial systems, endotoxins, nucleic acids, bioburden, viruses, and prions are all difficulties in all forms of expression. Viruses and prions are not a problem; prions are rarely a problem in other systems, except for transgenic animals. It is important to look at

the basic material used. Only biopharmaceutical-grade raw materials with extensive documentation on safety and quality should be utilized. Extending column life saves money; downstream processing expenses account for almost 70% of total production costs, principally in the cost of chromatographic media. Longer use, recharging, and other methods for extending column life must all be well validated and documented. Despite the high cost of chromatographic media, it is wise to select media based on criteria other than price per liter. Service, troubleshooting, procuring connecting media, columns, and equipment from the same vendor, as well as regulatory assistance, may be even more important. Such initiatives will aid in reducing the number of batches that fail. The key elements affecting the economy of chromatographic unit operations are media cost, binding power, recovery, column lifetime, linear flow, and shelflife.

Chromatography scaling generates more problems than any other phase in the process. Extra-column zone broadening can occur due to the various lengths and diameters of outlet pipes, valves, display cells, and other larger equipment. An increase in column diameter can limit flow rate due to diminished, supporting wall force (at constant pressure drop). The flow diminishes as the diameter of a column filled with Sepharose 6 FF is increased from 2.6 to 10 cm. Longer sample retention durations on the column can generate precipitation, which can clog pipes, valves, and chromatographic columns. The sample volume, sample load, column diameter, column area and volume, and flow rate are all proportionally changed (linear scale-up). However, the residence time is not changed (an alternative approach would keep residence time constant and allow for changes in both column areas and height). Within the interval limitations, sample pH, conductivity, temperature, concentration, redox potential, holding time, bed height, residence duration, linear flow rate, binding power, back pressure, buffers, equilibration, wash, elution, and clean-in-place (CIP) procedures all remain constant. Gradients should be changed in steps rather than in a linear method.

The filtering phase scale-up has no effect on the interval limits, pH, redox potential, temperature, concentration, conductivity, holding time, membrane type, transmembrane pressure, retentate pressure, feed pressure, crossflow velocity, filtrate velocity, wall flux, or CIP methods. Two factors that are linearly increased are sample volume and membrane area.

1.5.4 Facility

Facility expenses can be very high due to specialized area needs, specialized people requirements, environmental controls, and waste management requirements, among other things. If numerous drugs require distinct processing suites, a prospective biopharmaceutical marketer should consider outsourcing manufacture. Monitoring the environment is one of the control areas that is sometimes disregarded in budgeting; simply complying with monitoring regulations for a 5000 square foot plant will cost upwards of $2 million per year. Because the regulatory environment is still evolving, area standards are likely to change, resulting in large redesigning expenses, which is another reason to outsource production before the sector matures.

The price per gram of drug product is significantly decreased and the production period is cut by integrating process design, scale-up factor, and batch logistics to facility design. Many stages of set-up are required, including one for transferring technology to pilot scale, one for converting from pilot scale to first-stage production, and one for migrating from first-stage manufacturing to full-scale manufacture.

As part of the manufacturing process, all columns, tools, and utensils are cleaned, sterilized, and stored. Despite the fact that these issues are prevalent in small-scale production, they can add significant costs to a poorly constructed process and facility, as well as offer contamination hazards those regulatory authorities may not be comfortable with.

Labor-intensive procedures are expensive, and they can be reduced by automating and reducing unit operations. Each unit procedure raises the price while lowering the yield. If, on the other hand, insect cells, mammalian cells, or transgenic animals are employed for protein expression, the number of process steps will be limited due to the necessity to remove impurities and inactivate viruses. There is a tremendous drive to automate operations as more generic corporations enter the market to install more cost-effective technologies. Robots, continuous processing systems, and wave bioreactors are all being used to cut human resource expenses. A prospective manufacturer must evaluate all present and soon-to-be-available options when creating the technique. On the other hand, the FDA's validation guidelines for automated processes and computer systems in the United States should be implemented as soon as practicable.

When enormous amounts of trash are disposed of, environmental issues occur; for example, ammonium sulfate in large-scale projects can have a substantial detrimental influence on the environment.

The microbiological quality of the environment during various processing phases is crucial, in particular as the process moves downstream; stricter management and monitoring are recommended. In the atmosphere and locations where biopharmaceuticals are isolated, microbiological and other international contaminants should be maintained to a minimum. Biopharmaceuticals should be segregated and disposed of in the same way as biomaterials are. The National Institutes of Health (NIH) Guidelines (https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf) describe how to make and handle recombinant deoxyribonucleic acid (DNA) molecules, as well as organisms and viruses that include them. In the context of the NIH Guidelines recombinant DNA molecules are defined as: (i) molecules produced outside of living cells by joining natural or synthetic DNA segments to DNA molecules capable of replication in a living cell; or (ii) molecules produced by the replication of those mentioned in (i); or (iii) synthetic DNA segments that are likely to produce a potentially harmful polynucleotide or polypeptide. If the synthetic DNA segment is not produced *in vivo* as a physiologically active polynucleotide or polypeptide product, it is exempt from the NIH Guidelines.

Genomic DNA of plants and micro-organisms that have acquired a transposable part, even if the latter was provided via a no longer existing recombinant vector, is not subject to the NIH Guidelines unless the transposon itself contains recombinant DNA.

When processing biopharmaceuticals, the NIH guidance 'Experiments Involving More Than 10 l of Culture' should be followed: https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf. Sanitization of the utilized media is not required because most recombinant cell lines cannot survive outside of specialized media; nonetheless, some businesses do a decontamination technique, which adds to the cost of the process.

1.5.5 Equipment

Equipment interaction determines the chemicals employed; sodium chloride corrodes stainless steel, but sodium acetate does not. These issues should be addressed as early as possible in the development process.

Utensils relate to bags, filters, and any other equipment that needs to be updated on a regular basis. Sacks, filters, tubes, and other similar things have become ubiquitous in cleaning procedures to save money and time. The number of steps in a process has a direct impact on cost and yield; however, reducing the number of steps in a process may have an impact on robustness and safety, as well as additional regulatory testing expenses.

1.5.6 Validation

Validation of biological processes is a costly exercise that takes place throughout the manufacturing process. After all separation and purification procedures have been outlined in detail and flow charts have been provided, validation steps normally begin. There should be full explanations and specifications for all facilities, columns, reagents, buffers, and expected yields. According to the FDA, validation is defined as 'creating documented proof that provides a high degree of confidence that a given method can reliably generate a product that meets its predetermined requirements and quality features'. As a result, thorough documentary verification is required to validate the technique and demonstrate that it is repeatable. Validation reports for the various main processes will differ depending on the process in question; for example, if an ion-exchange column is used to extract endotoxins, evidence demonstrating that the process is reliably accurate to be achieved by measuring endotoxin levels before and after processing should be provided. It is vital to track the process before, during, and after it is done to evaluate the efficiency of each key purification stage. One common way for showing validation is to 'spike' the preparation with a known amount of contamination and then show that the contaminant is absent.

The target protein's nature, the expression method employed, and the need for protection, robustness, and cGMP compliance all have an impact on the process design. To create a safe product, approved raw materials must be employed, as well as any host, process, and product-related contaminants. In a method based on insect cells, mammalian cells, or transgenic animals, there are some general rules to follow, such as employing at least three chromatographic stages, virus inactivation, and filtration. The target protein's stability must be tracked during processing and storage. Process design is a small-scale job, and no process can be scaled up unless it

has undergone extensive testing on a small scale. The three most significant process maturity requirements are specifications, robustness, and cGMP compliance. Even if drug substance and drug product standards and acceptance criteria are not adequately described at this early stage, data from at least three small-scale batches must be provided and validated against available specifications and acceptance criteria. The technique must be dependable and achieve the desired result to some extent. It is critical to understand key parameters and how they interact before scaling up. It should be checked in cGMP to guarantee that the method follows its design and production protocols. Difficulties can be foreseen if the expression yield is low, the protein is unstable, the purification yield is low, or the procedure involves too many steps.

The move from laboratory to production scale is the most crucial component of scaling up. Not all pieces of equipment can be scaled. This should be the most significant consideration in laboratory-scale growth. Many providers deliver a varied selection of items in terms of capacity. Even if the initial cost of adopting this equipment is higher, single-source providers are frequently preferred. On a big scale, pump design might change from high precision piston or displacement pumps to rotary, diaphragm, or peristaltic pumps.

Low-volume multi-port valves are also replaced with simple one-way valves, which, when paired with large-scale tubing, can greatly enhance the volume of equipment accessories and result in increased dispersion of the target protein molecules. Stainless steel is frequently used in large-scale equipment, however, it cannot sustain high chloride salt concentrations. Scale-up difficulties to address with large-scale equipment include differences in chromatographic column physics of movement, choice, tubing placement, valve, reservoir, chemical resistance of building material, CIP choice, and SIP.

Process optimization, which is influenced by aspects such as labor, automation, lean management, column lifespan, utensil reuse, and batch preparation, should be distinguished from process design. The latter difficulties are handled at a later point in the manufacturing process, usually after the process design has been established.

1.5.7 Testing

Batch and fed-batch systems have a cheaper cost of in-process control than continuous cultures. The total cost, however, may not be that different, and the FDA's PAT project is worth examining. Milking techniques might result in small-volume bags, increasing the cost of analytical control systems. The cost estimates should include the end-of-production test for insect and mammalian cells, which includes sterility, fungus, mycoplasma, and virus testing.

When producers may employ analytical assays for quality testing as in-process testing when the process is properly developed, they can become less expensive. Because large-scale production demands more comprehensive testing processes, this is particularly crucial. The FDA's Process Analytical Technology (PAT) advice, which can be accessed at https://www.fda.gov/media/71012/download, is quite useful. During the development phase, this expanded method includes traditional

empirical testing as well as real-time monitoring of process parameters and responses. Better monitoring of parameters and responses can result in a reduction in analytical testing in the process control program.

Safety considerations come at a great price. Insect cells, mammalian cells, and transgenic animals can all be infected by viruses. Virus testing, virus reduction unit activities, and validation programs are required to provide product protection. The possibility of prion infection in sheep, goats, and cows, with a focus on managed herds, is being considered.

1.5.8 Quality

In-process control monitoring should be reduced to a bare minimum, and this can only be done with a well-defined system, which comes at a cost. The current tendency is to focus on tracking parameters and responses rather than in-process analytical approaches, allowing for real-time process management. The number of samples to examine has an inverse relationship with batch size; large batches reduce the cost of in-process control.

The usage of online data gathering systems is required for controlling and changing parameters that are critical to procedures. Because small-scale equipment lacks the same monitoring capabilities as large-scale instruments, this is a concern (which can be ordered with custom features).

Quality control testing is necessary for medications and drug products; nevertheless, when scaling up, reduced test programs should be reconsidered. Some test analyses introduced during the early development phase can be skipped from the batch release program because combined data has confirmed process robustness. It is important to explain why a certain analytical assay was dropped. Product testing may be reduced because of the FDA's PAT initiative.

Despite other validation criteria common to all testing forms, process and quality control testing are comprehensive (see USP, EP, or BP) and costly due to the nature of the tests required. In-process control assays may be justified if they give key process information during production or track a specific outcome that is important for in-process control in manufacturing processes. As a result, cutting costs means reducing the number of in-process analyses while preserving process control. It is typical practice to change the program as additional data becomes available and the process matures. The FDA's PAT strategy (http://www.fda.gov/cder/guidance/5815dft.htm) is another way to save money. To assure final product quality, PAT is a framework for planning, analyzing, and monitoring manufacturing through timely measurements of important quality and performance attributes of raw and in-process materials and processes. PAT's goal is to understand and supervise the manufacturing process because consistency cannot be checked into goods. This technique can be used to expand the in-process control software during the development phase to include traditional empirical testing and real-time monitoring of process parameters and reactions. The goal of the PAT system is to create and implement processes that can consistently provide a predetermined quality at the end of the manufacturing phase. These methods must follow the quality by design

concept, which reduces quality and regulatory risks while enhancing performance. A third option to save money is to reduce the price per sample by assuring a consistent supply of samples and shortening set-up and calibration times.

Quality control is performed on both the drug substance (DS) and the drug product (DP) (see BP/EP/USP). Testing typically involves 10–15 distinct analytical procedures and costs between $500 and $3000 for each sample. If outside facilities are invited in to conduct additional research, costs might increase. Outsourcing animal and viral testing can be exceedingly expensive. Outsourcing these assays, on the other hand, remains the favored strategy for avoiding the high expenses of maintaining animal housing or viral containment systems, as well as validating the procedures.

Process scaling must not compromise process integrity, robustness, or regulatory compliance, according to quality assurance systems. If the procedure is remodeled for scale-up, the veracity of preclinical data should be addressed. In resilient systems, unit operation parameters are tightly controlled, and parameters are defined in intervals rather than fixed points. The parameter intervals are validated and justified on a small scale (proved reasonable range), providing room to adjust the intervals according to large-scale needs. When employing the linear scale approach, these parameter intervals are kept constant. Reactor volumes, sample weights, and column diameters are all increased linearly.

1.5.9 Fill

Cost savings in the API formulation, fill, and pack activities may be achieved depending on the formulation. Because a lyophilized formulation would be more expensive, it should be preferred if a ready-to-inject formula can be developed. Syringes, pen systems, and other components included at this time greatly raise the cost of the product.

1.5.10 Water

The intended use of the finished product should determine the water quality. Process water can be of water for injection (WFI) consistency with CBER, for example. Purified water, on the other hand, might be used for *in vitro* testing. The approach determines the appropriate water quality for pharmaceuticals. Because processing is usually done in chilly rooms or at room temperature, the WFI system's self-sanitization to 80 °C is also lost.

Because of the difficult-to-sanitize design of processing equipment, some companies generate WFI by reverse osmosis rather than distillation to save money, resulting in polluted systems. Some threads or drips in a cold system provide a spot for microbes to dwell and proliferate. In some of the systems, a terminal sterilizing filter is used. The main worry is endotoxins, and the terminal filter can only mask the true quality of the WFI used. It is also vital to think about the disadvantages of depending on a 0.1 ml WFI sample to detect endotoxins from a device. Point-of-use heat exchangers, like those used in other WFI systems, can be utilized if cold WFI water is required.

Buffers can be prepared in sterile containers as non-pyrogenic sterile solutions. In smaller facilities, commercial sterile, non-pyrogenic buffer solutions are utilized. Non-sterile water that may be used as a reagent or as a buffer should be evaluated for stability and microbiological characteristics.

1.6 Facility design

Figure 1.2 depicts a typical biopharmaceutical production unit design. The manufacturing architecture of biological products is determined by the size of the production and the manner of production; in most situations the containment conditions noted above, as well as the necessity to process products under cleanroom conditions, are comparable to the processing of sterile products. The atmosphere should, in general, be like that of a sterile product preparation room. The following are the criteria for each of the major categories of biological product production work:

- *Working cell bank (WCB) and master cell bank (MCB)*: A separate room with a cold storage system (often a liquid nitrogen system) and a cold (−70 °C) cabinet must be made available for each GMC. The WCB is made from the cells from the MCB and both are kept in a safe location. A vaulted area with a class 100 000 atmosphere is recommended; normally, a 100–200 square foot area will suffice. Some manufacturers divide the room into two sections, each with its own entrance, one for MCB and the other for WCB. A negative-pressure transfer window allows a direct transfer from the WCB to the inoculum/culture room (see below) in some configurations. The MCB/WCB is recognized as a supply center at the time of usage and is processed by materials dispensing due to the necessity to maintain a defined area designation. In all circumstances, a duplicate MCB must be kept off-site from the immediate manufacturing area. An electronic recording system should be established to convey the temperature at which the GMCs are processed. These rooms should have a back-up power supply to ensure no power outages and alarms to record temperature fluctuations in the GMC storage cabinets. In addition, the chamber should have an automatic pressure differential and should be kept negative in relation to the hallway. To prevent cross-contamination of cultures, each GMC has its own room.

  Because it is the first room where culture tubes are opened to create WB or inoculum for fermentation, the inoculum room should be a class 10 000 room. In this chamber, a 4–8 l fermenter will be used to make the starter culture. The culture will then be transported directly to larger fermenters if the fermentation system is a closed inline system. When roller bottles are utilized, this room will be used as a staging location for preparing the culture for inoculation into the bottles, which will be done in a separate room due to the process's magnitude. The culture is handled in a biosafety laminar flow hood to keep the operator safe from contamination. The room should have a 10 000 rating with class 100 under the laminar flow hood. A negative-pressure passing carousel will usually connect this chamber to the fermentation,

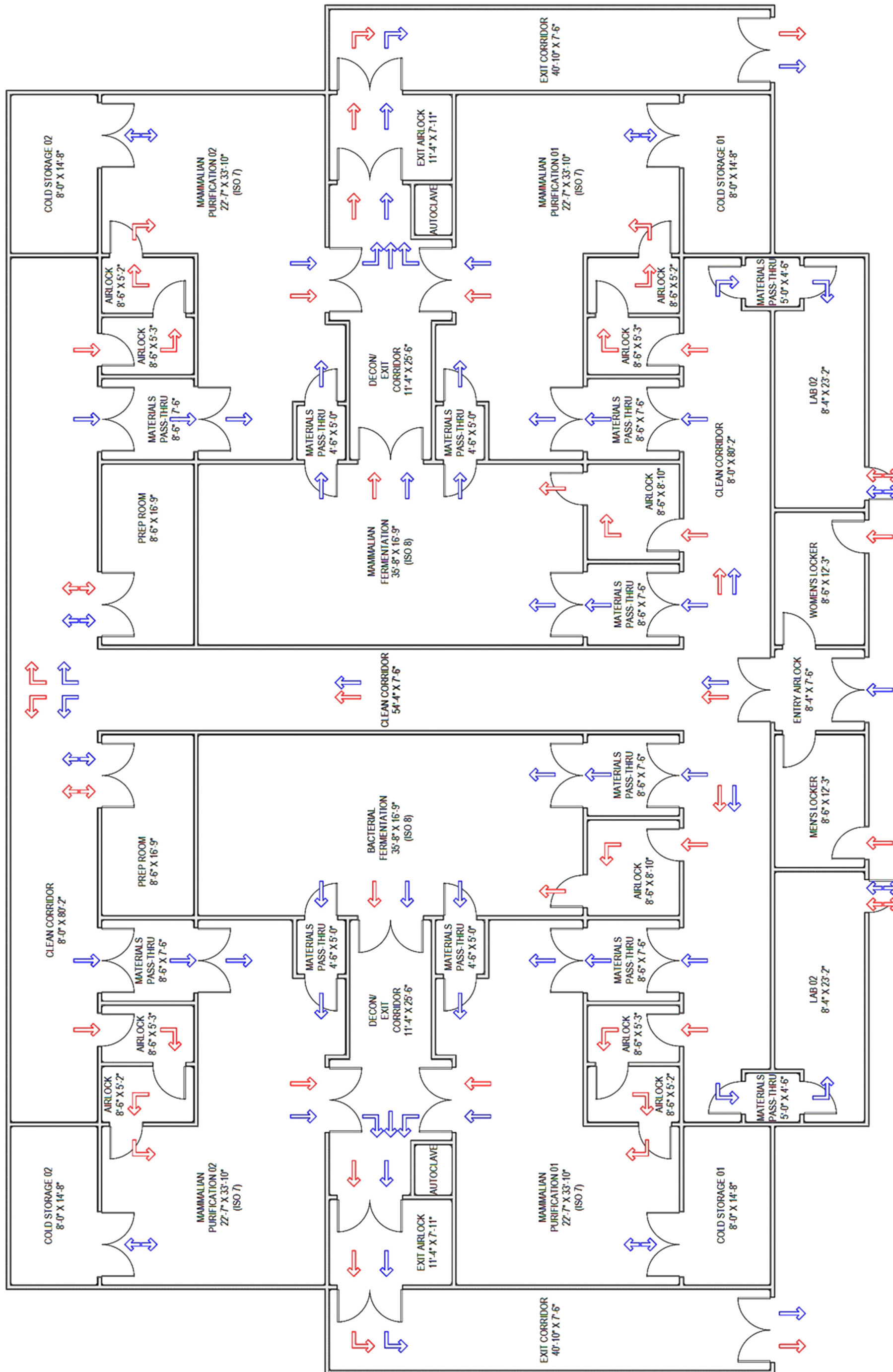

Figure 1.2. A typical biopharmaceutical manufacturing plant layout.

recovery, and roller bottle preparation areas. The amount of space available should be kept to a minimum (100–200 square feet).

- *Fermentation space*: Depending on the volume, this might be the facility's largest room or a group of rooms. Larger fermenters, such as those with capacity of 25 000 l or more, may require a three-stage fermentation process. This type of facility would most likely be 20 000 square feet or bigger, with multiple floors to accommodate massive fermenters. Fermenters with a capacity of 500–2000 l should be able to accommodate numerous low-dose biopharmaceuticals on a single level. The area categorization for this room is 100 000 unless a fully closed system is employed (which is suggested). Otherwise, unclassified pharmaceutical-grade classification can be utilized.

  This is where the fermentation is brought for the initial stage of processing (either from fermenters or roller bottles). If inclusion bodies are involved (as with the use of bacterial cultures), this room will feature a cold centrifuge and cell disrupters; for mammalian culture systems where the protein is secreted, this will be the initial stage of volume reduction by filtration. This chamber is also used to keep merchandise that need refolding in the 2–8 °C environment (provided as walk-in refrigerators). The area classification of this room remains at 100 000. This will require roughly 500 square feet of space.
- *Downstream processing space*: This is the second 10 000 square-foot classification room for the purifying process, with more space beneath laminar flow hoods. It is important to remember that each product requires its own set of contact equipment (columns, vessels, etc). A minimum of 1000 square feet is necessary; the size of the room will vary depending on the amount of output and the stages involved; large-scale filtering equipment may necessitate more than 5000 square feet and multiple floors in some circumstances. Some manufacturers have many locations for downstream processing; in these circumstances, suitable standard operating procedures (SOPs) for fermentation product packing and shipping should be in place.
- *Buffers and media preparation rooms*: Each phase requires its own buffer and media, which might be quite big depending on the procedure's scope. Switching to closed systems of media preparation and transfer to fermenters may be preferable when working with large fermenters (1000 l or more); however, most medium and small-scale operations require a large media preparation room (1500+ square feet) with 10 000 classification and work in a class 100 hood; the media prepared is then transferred to the storage area until dispensing.
- *Storage areas*: Many materials, such as media and buffer, require a lot of chilled space, therefore incoming material is kept in dedicated climate-controlled facilities. This is also a 100 000 classification area. During batch issues for production, a piece of the room is set aside for keeping materials from the WCB, media, and buffer rooms.
- *Finished product storage area*: The size of the room is dictated by the size of the dosing device, however, the concentrate is normally maintained refrigerated in a smaller room of around 100 square feet. The classification is the

same as the general pharmaceutical unclassified sector because the product is now sealed in its primary and secondary packaging.

1.6.1 Cleaning

Validation of cleaning procedures for handling equipment, including columns, should be done. In a multi-product operation, this is highly critical. The cleaning procedure's efficacy for final or intermediate items utilized in equipment provided by the equipment supplier. Validation evidence can demonstrate that the cleaning process decreases relevant contaminants to acceptable levels. Even with a significant number of washing cycles, all traces of the material will remain. The suitable residue amount, which is commonly represented in parts per million (ppm), must be justified by the maker. Endotoxins, germs, poisonous materials, and contaminated proteins should all be eliminated without compromising the effectiveness of the column. A written equipment cleaning protocol that outlines what should be done and what materials should be used should be in place. For fixed vessels, CIP is also employed. Diagrams should be produced in these circumstances to designate certain sections (such as valves) that are part of the cleaning procedure.

After the surface has been washed, it should be tested on a regular basis to confirm that it has been cleaned to the validated standard. A common procedure is to test the final rinse water or solvent for the presence of the cleaning agents last used in that piece of equipment. Always make a direct determination of the leftover substance.

The robustness of the analytical method utilized to classify the cleaning endpoints will be critical to the cleaning system's efficiency. Modern analytical instruments' sensitivity has allowed some detection thresholds to be decreased to parts per million (ppm) or even parts per billion (ppb). Residue limitations for each piece of equipment should be practical, feasible, and verifiable. There should be a scientific basis for establishing these thresholds, which must be documented. The likelihood of non-uniform residual distribution on a piece of equipment is another factor to consider. It is likely that the real average residue concentration is higher than what was found.

1.6.2 Filling and finishing

Biotechnology creates proteins and peptides, which are relatively unstable molecules in comparison to other medications; most biotechnology methods include switching proteins from one stabilizing or solubilizing buffer to another during the purification process. Finally, a solution is used to convert the protein to its final dose form, assuring long-term stability. These products frequently require lyophilization to achieve long-term stability due to the potential for degradation by different mechanisms such as deamidation, aggregation, oxidation, and possibly proteolysis by trace quantities of host cell proteases. The protein's final dosage type usually includes stabilizing agents that produce the ideal pH and solution conditions for long-term product stability and desirable attributes (tonicity). These substances include proteins, polyhydric alcohols, amino acids, sugars, bulking agents, inorganic

salts, and non-ionic surfactants. These excipients may also be required for the development of a lyophilized cake that is stable. Special requirements for lyophilized products, such as moisture level regulation, are usually mentioned in each USP monograph and may be necessary for product stability. Significantly, evaluating protein stability frequently needs a combination of analytical methods, each of which can be used to assess a particular route of protein degradation. The effects of temperature on protein structure make it difficult to forecast the shelflife of protein formulations using accelerated stability tests, resulting in non-Arrhenius behavior. As a result, determining the expiration dates of biotechnology-derived products frequently requires using real-time, prescribed storage environment stability tests.

Because biopharmaceuticals are not terminally sterilized, they require aseptic processing. Pollutants from a product's or system's manufacturing process are primarily a safety issue. The main sources of contamination are the cell-substrate (DNA, host cell proteins, and other biological materials, viruses), the medium (proteins, sera, and additives), and the purification procedure (process-related chemicals and product-related impurities).

Due to concerns about stability, many biopharmaceuticals are either refrigerated or lyophilized. Low temperatures and low moisture content often hinder microbial development. In aseptic processing of a non-preserved single dose biopharmaceutical (that is aseptically filled) maintained at room temperature as a solution, the limitations of a 0.1% media fill contamination rate should be tested.

Validation of the media fill and aseptic production processes should be properly documented. Because certain biopharmaceuticals are less stable than others, careful mixing and processing is required. Biopharmaceuticals often employ single filtration at low pressures, whereas aseptically packed parenteral typically use double filtration. As a result, comprehensive production requirements are required, including maximum filtration pressures.

It is important to keep an eye on the climate and accessibility for non-sterile biopharmaceutical batching. Due to the lack of preservatives in many of these goods, their inherent bacteriostatic and fungistatic activity, as well as their bioburden before sterilization, should be modest. The bioburden should be calculated until these bulk solutions are sterilized before loading. Batching or compounding of these bulk solutions should be monitored until the bulk solutions are purified to avoid any possible increase in microbiological levels (sterilized). At any microbiological stage, the possibility of an increase in endotoxins is a cause for concern. Compounding these materials should be done in a controlled atmosphere with enclosed tanks, in particular if the solution will be stored before sterilization. It is also a good idea to keep the time between formulation and sterilization in the production process as short as possible.

In-process testing ensures that an operation's true, real-time output is adequate and is a critical component of quality control. In-process controls include structure features, chromatographic profiles, protein species, protein concentrations, bioactivity, bioburden, and endotoxin levels, to name a few. The findings of the validation program must be coordinated with the establishment of acceptance criteria and the collection of in-process controls.

When loading biopharmaceuticals into ampoules or vials, many of the same challenges arise as when handling traditional medications. In established companies, these issues are usually resolved by adequate documentation. However, for a new biopharmaceuticals facility, developing and proving clinical efficacy and protection, as well as validating sterile operations, equipment, and systems, can be a lengthy process, particularly if specifications are unclear.

At least when first manufactured, the batch size will most likely be tiny. Due to the small batch size, filling lines cannot be as automated as other items filled in greater quantities. As a result, filling these products requires more employees, in particular for smaller, newer enterprises. This can lead to quality disparities. During the filling step, concerns such as inadequate clothing, inadequate environmental inspection systems, hand-stoppering of vials, particularly those that are lyophilized, and failure to validate some of the basic sterilizing processes arise. Due to people's active involvement, the number of persons involved in filling and aseptic manipulations should be maintained to a minimum. As part of an environmental program, microbiological samples acquired from employees working in aseptic manufacturing locations should be analyzed.

Another source of worry for product stability is the use of inert gas to substitute oxygen during processing and filling the solution. The concentrations of dissolved oxygen in the solution should be determined, just as they should be for other oxidizable compounds. Similarly, criteria such as line speed and the location of filling syringes in terms of closure should be included in the filling operation's validation to guarantee that oxygen-sensitive items are exposed to the least quantity of air possible (oxygen). In the absence of inert gas displacement, the manufacturer must show that the product is oxygen-free.

A machine would typically partly stopper vials to be lyophilized. When an operator manually sets the stopper, serious problems can arise. Another key difficulty with the filling process of a lyophilized product is the assurance of fill amounts. A low fill level implies that the vial is not fully functional. A low fill, unlike a powder or liquid fill, would be difficult to detect after lyophilization, particularly for a product with a milligram active component. Because of its clinical significance, sub-potency in a vial can be a highly significant clinical situation.

1.7 Testing

The tests mentioned below can be used for in-process, bulk, and product testing. The tests that are required will be determined by the method and the product's intended use.

a. *Quality*:
    - Color/appearance/clarity
    - Host cell DNA
    - Moisture content
    - Particulate analysis
    - pH determination

b. *Identity* (a single identity test might not be enough, it is necessary to confirm that the methods used have been checked):
   - Two-dimensional electrophoresis
   - Amino acid analysis
   - Amino acid sequencing
   - Capillary electrophoresis
   - Carbohydrate composition analysis (glycosylation)
   - ELISA
   - Gel electrophoresis
   - HPLC (chromatographic retention)
   - Immunoelectrophoresis
   - Immunoassays
   - Isoelectric focusing (IEF)
   - Mass spectroscopy
   - Molecular weight (SDS PAGE)
   - Peptide mapping (reduced/non-reduced)
   - Radioimmunoassay
   - SDS PAGE
   - Western blot

c. *Protein concentration/content*:
   - Amino acid analysis
   - Biuret method
   - HPLC
   - Lowry
   - Partial sequence analysis
   - Protein quantitation
   - UV spectrophotometry

d. *Purity* (The finished product's relative lack of extraneous substance, whether harmful to the user or destructive to the product, is referred to as 'purity'. Purity, among other things, refers to the lack of residual moisture, other volatile chemicals, and pyrogenic compounds. Protein impurities are the most common pollutants. These issues could be caused by the fermentation process, the media, or the host organism. Endogenous retroviruses may be found in hybridomas used to generate monoclonal antibodies. Specific testing for these compounds is required for *in vivo* products. For the product to be safe and secure, extraneous antigenic proteins must be eliminated.)
   - Tests for protein impurities:
     - Electrophoresis
       i. SDS PAGE
       ii. IEF
     - Dimensional electrophoresis
     - Peptide mapping
     - Multi-antigen ELISA
     - HPLC size-exclusion

- HPLC reverse phase
- Tests for non-protein impurities:
  i. Endogenous pyrogen assay
  ii. Endotoxin testing
  iii. Limulus amebocyte lysate (LAL)
- USP rabbit pyrogen test

e. *Contamination with pyrogens*: (Pyrogen contamination should be tested by injecting the final product into rabbits or using the LAL assay. The same conditions that were used to approve natural products should be applied to biotech products. Some *in vitro* diagnostic products can contain endotoxins, which could cause the device to malfunction. It is also crucial to test products *in vivo* for pyrogens. Despite passing the LAL and rabbit pyrogen tests, certain biological pharmaceuticals are pyrogenic in humans. This could be due to elements that are solely pyrogenic in humans. An endogenous pyrogen assay is used to predict if human subjects will have a pyrogenic reaction. The finished product is cultivated *in vitro* using human blood mononuclear cells before being injected into rabbits as cell culture fluid. Fever in rabbits indicates the presence of a pyrogenic substance in humans.):
   - Endogenous pyrogen assay
   - Limulus amebocyte lysate (LAL)
   - USP rabbit pyrogen test

f. *Viral contamination* (Viral contamination tests should be tailored to the cell-substrate and culture conditions used. It should be shown that the final product is free of detectable adventitious viruses.):
   - Hemadsorptions embryonated egg testing
   - Mouse antibody production (MAP)
   - Polymerase chain reaction (PCR)
   - The cytopathic effect in several cell types
   - Viral antigen and antibody immunoassay

g. *Contamination of nucleic acid* (The possibility of cellular transition events in the receiver raises worries about nucleic acid impurities. The extent to which additional host cell DNA is removed at each stage of the purification process can be demonstrated in pilot studies by looking at the amount of nucleic acid extracted at each phase. The theoretical degree of nucleic acid elimination during purification would be revealed in this type of analysis. Using appropriate probes, such as nick-translated host cell and vector DNA, and hybridization examination of immobilized contaminating nucleic acid, direct analyses of nucleic acid in various output batches of the final product should be carried out. The theoretical concerns about converting DNA generated from the cell-substrate rate would be alleviated by a general reduction of contaminating nucleic acid.):
   - DNA hybridization (dot blot)
   - Polymerase chain reaction (PCR)

h. *Protein contamination*:
    - SDS PAGE
    - PLC
    - IEF
i. *Foreign protein contamination*:
    - Immunoassays
    - Radio immunoassays
    - ELISA
    - Western blot
    - SDS page
    - Two-dimensional electrophoresis
j. *Microbial contamination* (Appropriate microbial contamination examinations should be carried out to ensure there are no identifiable bacteria (aerobes and anaerobes), fungus, yeast, or mycoplasma.):
    - Total yeasts and molds and heterotrophic plate count
    - LAL/pyrogen
    - Mycoplasma test
    - Total plate count
    - USP sterility test
k. *Chemical contaminants*: Other sources of contamination, such as allergies, petroleum oils, residual solvents, cleaning agents, column leachable contaminants, and so on, must be considered.
l. *(Activity) potency* ('Potency' is defined as a product's specific aptitude or capability, as determined by appropriate laboratory tests or properly monitored clinical outcomes gained by using the product in the manner intended to accomplish a certain result. To measure the potency of a chemical, potency testing should involve either *in vitro* or *in vivo* tests, or both, that are particularly devised for that substance. Cross-reference in-house biological potency standards with international (World Health Organization (WHO), National Institute of Biological Standards and Control (NIBSC)) or national (National Institutes of Health (NIH), National Cancer Institute) standards to assess the bioactivity of a product's activity.):
    - Biochemical/biophysical assays
    - Cell culture bioassays:
        - Antigen/antibody specificity and functional activity evaluation
        - HPLC-validated to correlate certain peaks the to biological activity
        - Identifying agents that could have a negative impact on potency
        - Immunoblotting/radio- or enzyme-linked immunoassays
    - Potency limits
    - Receptor based immunoassays:
        - Various immunodiffusion methods (single/double)
    - Whole animal bioassays
m. *Stability* (A product's capacity to keep its identity, power, consistency, purity, protection, and efficacy over time while staying within existing requirements

is referred to as 'stability'. Studies to support the specified date period should be undertaken on the finished product. Real-time stability data would be required to support the proposed dating period. Some of the things that can be examined include potency, pH, transparency, color, particles, physiochemical stability, moisture, and preservatives. As a back-up, data from accelerated stability testing might be employed. In accelerated testing or stress tests, exaggerated storage conditions are utilized to increase the ratio of chemical or physical deterioration of a substance or commodity. The goal is to identify kinetic parameters that can be used to estimate the approximate expiration date. A commodity's stress testing is extensively used to discover potential concerns that may develop during storage and shipment, as well as to determine the product's expiration date. This could involve studies on the effects of temperature changes in transportation and storage settings. These tests should produce a credible dating period under real-world settings, utilizing the containers and closures intended for the advertised product. Gentle mixing and processing, as well as a single low-pressure filtration, may be required for some biotechnologically produced proteins. The manufacturing instructions must be comprehensive, with maximum filtration pressures defined, in order to preserve product stability. The number of preservatives in products that contain preservatives to prevent microbial contamination should be monitored. This can be accomplished through microbiological challenge tests (such as the USP antimicrobial preservative effectiveness test) or chemical assays for the preservative.):

- It is critical to keep an eye on the stability test environment (light, temperature, humidity, and residual moisture).
- Container/closure system used for bulk storage (i.e. extractables, chemical modification of the protein, and changes in stopper formulations that may change the extractable profile).
- Identify materials that could lead to product instability and conduct tests for aggregation, denaturation, fragmentation, deamination, photolysis, and oxidation.
- Tests to determine aggregates or degradation products:
  - Two-dimensional electrophoresis
  - Gel filtration
  - HPLC
  - IEF
  - Ion-exchange chromatography
  - Peptide mapping
  - Performance testing
  - Potency assays
  - SDS PAGE
  - Spectrophotometric methods

n. *Batch-to-batch consistency* (Demonstrating lot-to-lot consistency with respect to established release conditions is the most important criterion

for determining whether a manufacturer is creating a standardized and reliable product.):

- Identity, purity, and practical operation are all examples of uniformity
- Stability

o. *Testing control*: Each study's acceptance criteria must be determined, as well as a quality control program for the drug substance and drug product. Setting acceptance criteria becomes a constant effort as more data becomes accessible during growth (and scale-up). A batch is released if all analytical findings are within the prescribed parameters. A high acceptance rate can be expected if the procedure is efficient and compliant, implying that regulatory agencies and manufacturers generally have similar opinions. The issues outlined above must be carefully considered by the process designer while designing the purification process (the design principle). The majority of the design can be finished before entering the laboratory due to the limits governing biopharmaceutical production. Process modeling is a step in the laboratory's experimental design phase for optimization and testing that occurs before the laboratory's experimental design phase. Quality control systems for biotechnology-derived goods are quite like quality control systems for conventional pharmaceutical products in areas such as raw material testing and release, manufacturing and process control documentation, and aseptic processing. Many of the strategies employed in the research of low molecular weight pharmaceutical goods are also applied to quality management systems for biotechnology-derived products. This entails employing chemical reference standards and proven methodologies to assess a wide variety of identified and suspected product contaminants as well as potential breakdown products. Quality control procedures for biotechnology-derived products are generally comparable to those designed for conventional biologicals in terms of evaluating product sterility, product protection in laboratory animals, and product potency. Quality management systems for biotechnology-derived goods and conventional pharmaceuticals employ quite different methodologies to assess product identity, accuracy, purity, and impurity profiling.

p. Furthermore, biotechnology quality control frequently necessitates a combination of end product and validated in-process testing and process validation to assure the elimination of undesirable actual or potential contaminants to regulatory agency standards. A thorough investigation of the manufacturing organism (cell), a thorough analysis of cell growth/propagation methods, and a thorough examination of the final product recovery process are often required for biotechnology-derived products. The sophistication of quality control systems for biotechnology-derived products is influenced by the size and structural properties of the product and manufacturing process. The laboratory controls are similar to those necessary in conventional cGMP/GLP enforcement for all pharmaceutical goods, with additional attention paid to specific ingredients and their handling:

q. Workers in laboratories should be properly trained for their jobs.

r. Laboratory equipment utilized in the measurement, evaluation, and storage of raw materials, product samples, and reference reagents should be maintained, calibrated, and controlled.
s. *Validation*: All laboratory procedures should be evaluated using the equipment and reagents used in the study techniques. If the manufacturer or requirements of important equipment/reagents changed, revalidation would be required. Raw data should be used to support validation criteria in submitted applications.
t. *Standards of reference*: Reference standards should be well-defined and registered, as well as preserved, safeguarded, and used properly during testing.
u. If not properly preserved, laboratory cultures and reagents such as enzymes, antibodies, test reagents, and other labile components will degrade.
v. *SOPs in the laboratory*: Written, appropriate, and followed procedures are essential. The quality control samples should be separated and processed accurately.

1.8 Documentation process

The development program includes project planning, cell banking, process improvement, analytical methods, scale-up, manufacture, stability tests, reference material preparation, and quality assurance. The effort would eventually lead to the approval of a manufacturing and control method for the product. You will have to submit a lot of papers to receive a license (new drug application, biological license application). However, many of the tasks performed during growth and scale-up are not included in the above applications. It is the project owner's responsibility to provide construction documentation to regulatory authorities for evaluation. It is important to remember that 'if it isn't publicized, it hasn't been done'.

A significant percentage of the required documentation can be produced ahead of time (e.g. cell banking reports, unit operation descriptions, development reports, analytical method descriptions, batch records). Other reports (such as summary reports) are published alongside the experimental work. While such reports do not explain the end result, they are extremely helpful in informing future users about the reasons behind their decisions. As a result, the tech transfer kit should include overview reports.

Hundreds, if not thousands, of documents are produced by the drug development program, each prepared by a separate person from a different government and, in some cases, a different corporation. Any of these documents may be needed in the future, thus a complete documentation system is essential to keep track of them. The tracking technique requires the authenticity of the paper, which includes information about the author, date, edition, business, and facility.

1.9 Conclusion

Understanding the critical elements of bioprocessing allows manufacturers to establish efficient manufacturing facilities that can produce cGMP compliant

biopharmaceuticals. Every step of the process requires a different set of environment controls, in-process testing, and output validation. A well-designed and validated facility also reduces the cost of the product significantly. Given the myriad of factors that can affect the quality of biopharmaceuticals, advanced planning and maintaining the change control is essential. This chapter presented most elements required for creating biopharmaceutical manufacturing by presenting the conditions required for each step of the process required for biopharmaceuticals.

Full list of references

Suggested reading

Preface

Arora R 2010 *Medicinal Plant Biotechnology* (Cambridge, MA: CAB International)

Atun R A and Sheridan D J 2007 *Innovation in the Biopharmaceutical Industry* (Hackensack, NJ: World Scientific)

Bawa R, Szebeni J, Webster T J and Audette G F 2018 *Immune Aspects of Biopharmaceuticals and Nanomedicines* (Singapore: Pan Stanford)

Beck A 2013 *Glycosylation Engineering of Biopharmaceuticals: Methods and Protocols* (New York: Humana)

Behera B K 2021 *Biopharmaceuticals: Challenges and Opportunities* 1st edn (Boca Raton, FL: CRC Press)

Brooks G 1998 *Biotechnology in Healthcare: An Introduction to Biopharmaceuticals* (London: Pharmaceutical Press)

Burke M and Walmsley S 2001 *Biopharmaceuticals: A New Era of Discovery in the Biotechnology Revolution?* (Richmond: PJB)

Castilho L R 2008 *Animal Cell Technology: From Biopharmaceuticals to Gene Therapy* (New York: Taylor and Francis)

Cavagnaro J A 2008 *Preclinical Safety Evaluation of Biopharmaceuticals: A Science-Based Approach to Facilitating Clinical Trials* (Hoboken, NJ: Wiley)

Crick F and Knablein J 2005 *Modern Biopharmaceuticals: Design, Development and Optimization* (Weinheim: Wiley)

Das T K 2014 *Biophysical Methods for Biotherapeutics: Discovery and Development Applications* (Hoboken, NJ: Wiley)

Dutton R L and Scharer J M 2007 *Advanced Technologies in Biopharmaceutical Processing* 1st edn (Ames, IA: Blackwell)

Geigert J 2019 *The Challenge of CMC Regulatory Compliance for Biopharmaceuticals* 3rd edn (Cham: Springer)

Grindley J N and Ogden J E 2000 *Understanding Biopharmaceuticals: Manufacturing and Regulatory Issues* (Denver, CO: Interpharm)

Hefferon K L 2010 *Biopharmaceuticals in Plants: Toward the Next Century of Medicine* (Boca Raton, FL: CRC Press/Taylor and Francis)

Hill R G and Rang H P 2013 *Drug Discovery and Development: Technology in Transition* 2nd edn (Edinburgh: Churchill Livingstone/Elsevier)

Ho R J Y and Gibaldi M 2013 *Biotechnology and Biopharmaceuticals: Transforming Proteins and Genes into Drugs* 2nd edn (Hoboken, NJ: Wiley-Blackwell)

Houde D J and Berkowitz S A 2015 *Biophysical Characterization of Proteins in Developing Biopharmaceuticals* (Amsterdam: Elsevier)

Huxsoll J F 1994 *Quality Assurance for Biopharmaceuticals* (New York: Wiley)

Jameel F and Hershenson S 2010 *Formulation and Process Development Strategies for Manufacturing Biopharmaceuticals* (Oxford: Wiley)

Jørgensen L and Nielsen H M 2009 *Delivery Technologies for Biopharmaceuticals: Peptides, Proteins, Nucleic Acids and Vaccines* (Hoboken, NJ: Wiley)

Knäblein J 2013 *Modern Biopharmaceuticals: Recent Success Stories* (Weinheim: Wiley-Blackwell)

Komives C and Zhou W 2019 *Bioprocessing Technology for Production of Biopharmaceuticals and Bioproducts* 1st edn (Hoboken, NJ: Wiley)

Krause S O 2007 *Validation of Analytical Methods for Biopharmaceuticals: A Guide to Risk-Based Validation and Implementation Strategies* (Bethesda, MD: PDA)

Lee C-J 2006 *Clinical Trials of Drugs and Biopharmaceuticals* (Boca Raton, FL: Taylor and Francis)

Niazi S 2018 *Biosimilarity: The FDA Perspective* (Boca Raton, FL: CRC Press)

Niazi S 2015 *Biosimilars and Interchangeable Biologicals: Strategic Elements* (Boca Raton, FL: CRC Press)

Niazi S 2015 *Biosimilars and Interchangeable Biologics: Tactical Elements* (Boca Raton, FL: CRC Press)

Niazi S 2012 *Disposable Bioprocessing Systems* (Boca Raton, FL: CRC Press)

Niazi S 2003 *Filing Patents Online* (Boca Raton, FL: CRC Press)

Niazi S and Brown J L 2015 *Fundamentals of Modern Bioprocessing* (Boca Raton, FL: CRC Press)

Niazi S 2007 and 2014 *Handbook of Bioequivalence Testing* (New York: Informa Healthcare)

Niazi S 2005 *Handbook of Biogeneric Therapeutic Proteins: Manufacturing, Regulatory, Testing and Patent Issues* (Boca Raton, FL: CRC Press)

Niazi S 2002, 2009 and 2020 *Handbook of Pharmaceutical Manufacturing Formulations: Sterile Products* vol 6 2nd edn (New York: Informa Healthcare)

Niazi S 2009 *Handbook of Pharmaceutical Manufacturing Formulations: Compressed Solids* vol 1 2nd edn (New York: Informa Healthcare)

Niazi S 2004, 2009 and 2020 *Handbook of Pharmaceutical Manufacturing Formulations: Uncompressed Solids* vol 2 2nd edn (New York: Informa Healthcare)

Niazi S 2004, 2009 and 2020 *Handbook of Pharmaceutical Manufacturing Formulations: Liquid Products* vol 3 2nd edn (New York: Informa Healthcare)

Niazi S 2004, 2009 and 2020 *Handbook of Pharmaceutical Manufacturing Formulations: Semisolid Products* vol 4 2nd edn (New York: Informa Healthcare)

Niazi S 2004, 2009 and 2020 *Handbook of Pharmaceutical Manufacturing Formulations: Over the Counter Products* vol 5 2nd edn (New York: Informa Healthcare)

Niazi S 2006 and 2019 *Handbook of Preformulation: Chemical, Biological and Botanical Drugs* (New York: Informa Healthcare)

Niazi S 2004 and 2020 Pharmacokinetic and pharmacodynamic modeling in early drug development *The Process of New Drug Discovery and Development* ed C G Smith and J T O'Donnell (2nd edn) (New York: CRC Press)

Niazi S 1979 *Textbook of Biopharmaceutics and Clinical Pharmacokinetics* (New York: Wiley)

Prazeres D M F 2011 *Plasmid Biopharmaceuticals: Basics, Applications, and Manufacturing* (Oxford: Wiley-Blackwell)

Rathore A S and Mhatre R 2009 *Quality by Design for Biopharmaceuticals: Principles and Case Studies* (Oxford: Wiley)

Rathore A S and Sofer G K 2005 *Process Validation in Manufacturing of Biopharmaceuticals: Guidelines, Current Practices, and Industrial Case Studies* (Boca Raton, FL: Taylor and Francis)

Rathore A S and Sofer G K 2012 *Process Validation in Manufacturing of Biopharmaceuticals* 3rd edn (Boca Raton, FL: Taylor and Francis/CRC Press)

Rathore A S 2012 *Process Validation in Manufacturing of Biopharmaceuticals* 3rd edn (Hoboken, NJ: CRC Press)
Razinkov V I and Kleemann G R 2017 *High-throughput Formulation Development of Biopharmaceuticals: Practical Guide to Methods and Applications* (Amsterdam: Elsevier/ Woodhead)
Rehbinder E 2009 *Pharming: Promises and Risks of Biopharmaceuticals Derived from Genetically Modified Plants and Animals* (Berlin: Springer)
Reid E C T Q 2007 A tool-kit for in-process determination and control of structural and conformational authenticity of complex biopharmaceuticals *PhD Thesis* University of London
Rickwood S and Southworth A 1995 *New Technologies in Biopharmaceuticals* (London: Financial Times Pharmaceuticals and Healthcare)
Rojanasakul Y and Wu-Pong S 2008 *Biopharmaceutical Drug Design and Development* 2nd edn (Totowa, NJ: Humana)
Rosales Mendoza S 2016 *Algae-Based Biopharmaceuticals.* (Switzerland: Springer)
Sandset T 2020 *'Ending AIDS' in the Age of Biopharmaceuticals: The Individual, the State and the Politics of Prevention* 1st edn (New York: Taylor & Francis)
Schmidt S R 2013 *Fusion Protein Technologies for Biopharmaceuticals: Applications and Challenges* (Hoboken, NJ: Wiley)
Segarra C 2013 Development of an integrated platform for the production of recombinant biopharmaceuticals *PhD Thesis* University of Sheffield
Takahashi M 2015 *Proc. 2015 Int. Conf. on Medicine and Biopharmaceuticals (China, 15–16 August)*
Tavakoli-Keshe R 2014 Quantifying the impact of the physical environment during processing and storage of biopharmaceuticals *PhD Thesis* University College London
Thomaz-Soccol V, Pandey A and Resende R R 2017 Current developments in biotechnology and bioengineering *Human and Animal Health Applications* (Amsterdam: Elsevier)
Tovey M G 2011 *Detection and Quantification of Antibodies to Biopharmaceuticals: Practical and Applied Considerations* (Hoboken, NJ: Wiley)
Walsh G and Murphy B 1999 *Biopharmaceuticals, an Industrial Perspective* (Dordrecht: Kluwer Academic)
Walsh G 2007 *Pharmaceutical Biotechnology: Concepts and Applications* (Chichester: Wiley)
Walsh G 2009 *Post-translational Modification of Protein Biopharmaceuticals* (Weinheim: Wiley)
Weert M and Møller E H 2008 *Immunogenicity of Biopharmaceuticals* (New York: Springer)
Wittrup K D and Verdine G L 2012 *Protein Engineering for Therapeutics. Part B* 1st edn (San Diego, CA: Academic)
Yang H 2017 *Emerging Nonclinical Biostatistics in Biopharmaceutical Development and Manufacturing* (Boca Raton, FL: CRC Press/Taylor and Francis)

Chapter 1

Baeshen M N *et al* 2015 Production of biopharmaceuticals in *E. coli*: current scenario and future perspectives *J. Microbiol. Biotechnol.* **25** 953–62
Baghban R *et al* 2019 Yeast expression systems: overview and recent advances *Mol. Biotechnol.* **61** 365–84
Berlec A and Strukelj B 2013 Current state and recent advances in biopharmaceutical production in *Escherichia coli*, yeasts and mammalian cells *J. Ind. Microbiol. Biotechnol.* **40** 257–74

Contreras-Gómez A, Sánchez-Mirón A, García-Camacho F, Molina-Grima E and Chisti Y 2014 Protein production using the baculovirus-insect cell expression system *Biotechnol. Prog.* **30** 1–18

Dhara V G, Naik H M, Majewska N I and Betenbaugh M J 2018 Recombinant antibody production in CHO and NS0 cells: differences and similarities *BioDrugs* **32** 571–84

Dumont J, Euwart D, Mei B, Estes S and Kshirsagar R 2016 Human cell lines for biopharmaceutical manufacturing: history, status, and future perspectives *Crit. Rev. Biotechnol.* **36** 1110–22

Dyson M R 2016 Fundamentals of expression in mammalian cells *Adv. Exp. Med. Biol.* **896** 217–24

Fernández F J and Vega M C 2016 Choose a suitable expression host: a survey of available protein production platforms *Adv. Exp. Med. Biol.* **896** 15–24

Grilo A L and Mantalaris A 2019 Apoptosis: a mammalian cell bioprocessing perspective *Biotechnol. Adv.* **37** 459–75

Gupta S K and Shukla P 2017 Microbial platform technology for recombinant antibody fragment production: a review *Crit. Rev. Microbiol.* **43** 31–42

Hacker D L and Balasubramanian S 2016 Recombinant protein production from stable mammalian cell lines and pools *Curr. Opin. Struct. Biol.* **38** 129–36

Jazayeri S H, Amiri-Yekta A, Bahrami S, Gourabi H, Sanati M H and Khorramizadeh M R 2018 Vector and cell line engineering technologies toward recombinant protein expression in mammalian cell lines *Appl. Biochem. Biotechnol.* **185** 986–1003

Krämer O, Klausing S and Noll T 2010 Methods in mammalian cell line engineering: from random mutagenesis to sequence-specific approaches *Appl. Microbiol. Biotechnol.* **88** 425–36

Kunert R and Reinhart D 2016 Advances in recombinant antibody manufacturing *Appl. Microbiol. Biotechnol.* **100** 3451–61

Lalonde M E and Durocher Y 2017 Therapeutic glycoprotein production in mammalian cells *J. Biotechnol.* **251** 128–40

Li F, Vijayasankaran N, Shen A Y, Kiss R and Amanullah A 2010 Cell culture processes for monoclonal antibody production *MAbs* **2** 466–79

Mahalik S, Sharma A K and Mukherjee K J 2014 Genome engineering for improved recombinant protein expression in *Escherichia coli Microb. Cell Fact.* **13** 177

Omasa T, Onitsuka M and Kim W D 2010 Cell engineering and cultivation of Chinese hamster ovary (CHO) cells *Curr. Pharm. Biotechnol.* **11** 233–40

Porowińska D, Wujak M, Roszek K and Komoszyński M 2013 Prokariotyczne systemy ekspresyjne [Prokaryotic expression systems] *Postepy Hig. Med. Dosw.* **67** 119–29

Ritacco F V, Wu Y and Khetan A 2018 Cell culture media for recombinant protein expression in Chinese hamster ovary (CHO) cells: history, key components, and optimization strategies *Biotechnol. Prog.* **34** 1407–26

Sanchez-Garcia L, Martín L, Mangues R, Ferrer-Miralles N, Vázquez E and Villaverde A 2016 Recombinant pharmaceuticals from microbial cells: a 2015 update *Microbial. Cell Fact.* **15** 1

Saeed A F H and Awan S A 2016 Advances in monoclonal antibodies production and cancer therapy *MOJ Immunol.* **3** 00099

Tripathi N K and Shrivastava A 2019 Recent developments in bioprocessing of recombinant proteins: expression hosts and process development *Front. Bioeng. Biotechnol.* **7** 420

Türkanoğlu Özçelik A, Yılmaz S and Inan M 2019 *Pichia pastoris* promoters *Methods Mol. Biol.* **1923** 97–112

Ullrich K K, Hiss M and Rensing S A 2015 Means to optimize protein expression in transgenic plants *Curr. Opin. Biotechnol.* **32** 61–7

Wells E and Robinson A S 2017 Cellular engineering for therapeutic protein production: product quality, host modification, and process improvement *Biotechnol. J.* **12** 1600105

Xiao-Jie L, Hui-Ying X, Zun-Ping K, Jin-Lian C and Li-Juan J 2015 CRISPR-Cas9: a new and promising player in gene therapy *J. Med. Genet.* **52** 289–96

Zhou Y, Lu Z, Wang X, Selvaraj J N and Zhang G 2018 Genetic engineering modification and fermentation optimization for extracellular production of recombinant proteins using *Escherichia coli Appl. Microbiol. Biotechnol.* **102** 1545–56

Zhu J and Hatton D 2018 New mammalian expression systems *Adv. Biochem. Eng. Biotechnol.* **165** 9–50

Chapter 2

Ahmad M, Hirz M, Pichler H and Schwab H 2014 Protein expression in *Pichia pastoris*: recent achievements and perspectives for heterologous protein production *Appl. Microbiol. Biotechnol.* **98** 5301–17

Bandaranayake A D and Almo S C 2014 Recent advances in mammalian protein production *FEBS Lett.* **588** 253–60

Barnes L M, Moy N and Dickson A J 2006 Phenotypic variation during cloning procedures: analysis of the growth behavior of clonal cell lines *Biotechnol. Bioeng.* **94** 530–7

Bleckmann M, Schmelz S, Schinkowski C, Scrima A and van den Heuvel J 2016 Fast plasmid based protein expression analysis in insect cells using an automated SplitGFP screen *Biotechnol. Bioeng.* **113** 1975–83

Butler M and Spearman M 2014 The choice of mammalian cell host and possibilities for glycosylation engineering *Curr. Opin. Biotechnol.* **2014** 107–12

Chapple S D and Dyson M R 2014 Expression screening in mammalian suspension cells *Methods Mol. Biol.* **1091** 143–9

Choi B K *et al* 2003 Use combinatorial genetic libraries to humanize N-linked glycosylation in the yeast *Pichia pastoris Proc. Natl Acad. Sci.* **100** 5022–7

Chusainow J *et al* 2009 A study of monoclonal antibody-producing CHO cell lines: what makes a stable high producer? *Biotechnol. Bioeng.* **102** 1182–96

Costa A R, Rodrigues M E, Henriques M, Azeredo J and Oliveira R 2010 Guidelines to cell engineering for monoclonal antibody production *Eur. J. Pharm. Biopharm.* **74** 127–38

Costa A R, Rodrigues M E, Henriques M, Oliveira R and Azeredo J 2013 Glycosylation: impact, control and improvement during therapeutic protein production *Crit. Rev. Biotechnol.* **34** 281–99

Dahodwala H, Nowey M, Mitina T and Sharfstein S T 2012 Effects of clonal variation on growth, metabolism, and productivity in response to trophic factor stimulation: a study of Chinese hamster ovary cells producing a recombinant monoclonal antibody *Cytotechnology* **64** 27–41

Davies S L, Lovelady C S, Grainger R K, Racher A J, Young R J and James D C 2013 Functional heterogeneity and heritability in CHO cell populations *Biotechnol. Bioeng.* **110** 260–74

Fahad S *et al* 2015 Recent developments in therapeutic protein expression technologies in plants *Biotechnol. Lett.* **37** 265–79

Fann C H, Guirgis F, Chen G, Lao M S and Piret J M 2000 Limitations to the amplification and stability of human tissue-type plasminogen activator expression by Chinese hamster ovary cells *Biotechnol. Bioeng.* **69** 204–12

Farrokhi N, Hrmova M, Burton R A and Fincher G B 2009 Heterologous and cell free protein expression systems *Methods Mol. Biol.* **513** 175–98

Fischer S, Handrick R and Otte K 2015 The art of CHO cell engineering: a comprehensive retrospect and future perspectives *Biotechnol. Adv.* **33** 1878–96

Ghaderi D, Zhang M, Hurtado-Ziola N and Varki A 2012 Production platforms for biotherapeutic glycoproteins, occurrence, impact, and challenges of non-human sialylation *Biotechnol. Genetic Eng. Rev.* **28** 147–75

Gong Z, Nielsen J and Zhou Y J 2017 Engineering robustness of microbial cell factories *Biotechnol. J.* **12** 1700014

Guerfal M, Claes K, Knittelfelder O, De Rycke R, Kohlwein S D and Callewaert N 2013 Enhanced membrane protein expression by engineering increased intracellular membrane production *Microb. Cell Fact.* **12** 122

Gupta S K and Shukla P 2017 Gene editing for cell engineering: trends and applications *Crit. Rev. Biotechnol.* **37** 672–84

Gupta S K and Shukla P 2018 Glycosylation control technologies for recombinant therapeutic proteins *Appl. Microbiol. Biotechnol.* **102** 10457–68

Hefferon K 2017 Reconceptualizing cancer immunotherapy based on plant production systems *Future Sci. OA* **3** Fso217

Hochkoeppler A 2013 Expanding the landscape of recombinant protein production in *Escherichia coli Biotechnol. Lett.* **35** 1971–81

James D C *et al* 1995 N-glycosylation of recombinant human interferon-gamma produced in different animal expression systems *Biotechnology* **13** 592–6

Jayapal K P, Wlaschin K F, Hu W and Yap M G 2007 Recombinant protein therapeutics from CHO cells-20 years and counting *Chem. Eng. Prog.* **103** 40

Jun S C, Kim M S, Hong H J and Lee G M 2006 Limitations to the development of humanized antibody producing Chinese hamster ovary cells using glutamine synthetase-mediated gene amplification *Biotechnol. Prog.* **22** 770–80

Karste K, Bleckmann M and van den Heuvel J 2017 Not limited to *E. coli*: versatile expression vectors for mammalian protein expression *Methods Mol. Biol.* **1586** 313–24

Kelly B J, King L A and Possee R D 2016 Introduction to baculovirus molecular biology *Methods Mol. Biol.* **1350** 25–50

Kim J Y, Kim Y G and Lee G M 2012 CHO cells in biotechnology for production of recombinant proteins: current state and further potential *Appl. Microbiol. Biotechnol.* **93** 917–30

Kim S J and Lee G M 1999 Cytogenetic analysis of chimeric antibody-producing CHO cells in the course of dihydrofolate reductase-mediated gene amplification and their stability in the absence of selective pressure *Biotechnol. Bioeng.* **64** 741–9

Ladisch M R and Kohlmann K L 1992 Recombinant human insulin *Biotechnol. Prog.* **8** 469–78

Le L T M, Nyengaard J R, Golas M M and Sander B 2018 Vectors for expression of signal peptide-dependent proteins in baculovirus/insect cell systems and their application to expression and purification of the high-affinity immunoglobulin gamma Fc receptor I in complex with its gamma chain *Mol. Biotechnol.* **60** 31–40

Lee J S, Grav L M, Lewis N E and Faustrup Kildegaard H 2015 CRISPR/Cas9-mediated genome engineering of CHO cell factories: application and perspectives *Biotechnol. J.* **10** 979–94

Lindgren K, Salmén A, Lundgren M, Bylund L, Ebler A, Fäldt E, Sö'rvik L, Fenge C and Skoging-Nyberg U 2009 Automation of cell line development *Cytotechnology* **59** 1–10

Łojewska E, Kowalczyk T, Olejniczak S and Sakowicz T 2016 Extraction and purification methods in downstream processing of plant-based recombinant proteins *Protein Expr. Purif.* **120** 110–17

Lomonossoff G P and D'Aoust M A 2016 Plant-produced biopharmaceuticals: a case of technical developments driving clinical deployment *Science* **353** 1237–40

Mattanovich D, Branduardi P, Dato L, Gasser B, Sauer M and Porro D 2012 Recombinant protein production in yeasts *Methods Mol. Biol.* **824** 329–58

O'Callaghan P M, Berthelot M E, Young R J, Graham J W A, Racher A J and Aldana D 2015 Diversity in host clone performance within a Chinese hamster ovary cell line *Biotechnol. Prog.* **2015** 1187–200

Parola C, Mason D M, Zingg A, Neumeier D and Reddy S T 2018 Genome engineering of hybridomas to generate stable cell lines for antibody expression *Methods Mol. Biol.* **1850** 79–111

Porter A J, Racher A J, Preziosi R and Dickson A J 2010 Strategies for selecting recombinant CHO cell lines for cGMP manufacturing: improving the efficiency of cell line generation *Biotechnol. Prog.* **26** 1455–64

Poulain A, Mullick A, Massie B and Durocher Y 2019 Reducing recombinant protein expression during CHO pool selection enhances frequency of high-producing cells *J. Biotechnol.* **296** 32–41

Puck T T, Sanders P and Petersen D 1964 Life cycle analysis of mammalian cells. II. Cells from the Chinese hamster ovary grown in suspension culture *Biophys. J.* **1964** 441–50

Rayder R A 2008 Expression systems for process and product improvement *BioProc. Int.* **6** 4–9

Rue S M, Anderson P W, Gaylord M R, Miller J J, Glaser S M and Lesley S A 2019 A high-throughput system for transient and stable protein production in mammalian cells *Methods Mol. Biol.* **2025** 93–142

Somogyi M *et al* 2019 A versatile modular vector set for optimizing protein expression among bacterial, yeast, insect and mammalian hosts *PLoS One* **14** e0227110

Tripathi N K and Shrivastava A 2019 Recent developments in bioprocessing of recombinant proteins: expression hosts and process development *Front. Bioeng. Biotechnol.* **7** 420

Urlaub G and Chasin L A 1980 Isolation of Chinese hamster cell mutants deficient in dihydrofolate reductase activity *Proc. Natl Acad. Sci. USA* **77** 4216–20

Vijayachandran L S *et al* 2011 Robots, pipelines, polyproteins: enabling multiprotein expression in prokaryotic and eukaryotic cells *J. Struct. Biol.* **175** 198–208

Walsh G 2018 Biopharmaceutical benchmarks 2018 *Nat. Biotechnol.* **36** 1136–45

Xu X *et al* 2011 The genomic sequence of the Chinese hamster ovary (CHO)-K1 cell line *Nat. Biotechnol.* **29** 735–41

Yao J, Weng Y, Dickey A and Wang K Y 2015 Plants as factories for human pharmaceuticals: applications and challenges *Int. J. Mol. Sci.* **16** 28549–65

Zhang H *et al* 2013 Rational development of a serum-free medium and fed-batch process for a GS-CHO cell line expressing recombinant antibody *Cytotechnology* **65** 363–78

Chapter 3

Bailey C G, Tait A S and Sunstrom N A 2002 High-throughput clonal selection of recombinant CHO cells using a dominant selectable, and amplifiable metallothionein-GFP fusion protein *Biotechnol. Bioeng.* **80** 670–6

Barron N *et al* 2011 Engineering CHO cell growth, and recombinant protein productivity by overexpression of miR-7 *J. Biotechnol.* **151** 204–11

Bebbington C R, Renner G, Thomson S, King D, Abrams D and Yarranton G T 1992 High-level expression of a recombinant antibody from myeloma cells using a glutamine synthetase gene as an amplifiable selectable marker *Biotechnology* **10** 169–75

Benhar I 2001 Biotechnological applications of phage, and cell display *Biotechnol. Adv.* **19** 1–33

Browne S M and Al-Rubeai M 2007 Selection methods for high-producing mammalian cell lines *Trends Biotechnol.* **25** 425–32

Campbell M, Corisdeo S, McGee C and Kraichely D 2010 Utilization of site-specific recombination for generating therapeutic protein producing cell lines *Mol. Biotechnol.* **2010** 199–202

Chu L and Robinson D K 2001 Industrial choices for protein production by large-scale cell culture *Curr. Opin. Biotechnol.* **12** 180–7

Chung M E *et al* 2017 Enhanced integration of large DNA into *E. coli* chromosome by CRISPR or Cas9 *Biotechnol. Bioeng.* **114** 172–83

Cicchini C *et al* 2002 Searching for DNA–protein interactions by lambda phage display *J. Mol. Biol.* **322** 697–706

Cranenburgh R M, Lewis K S and Hanak J A 2004 Effect of plasmid copy number, and lac operator sequence on antibiotic-free plasmid selection by operator-repressor titration in *Escherichia coli J. Mol. Microbiol. Biotechnol.* **7** 197–203

Derouazi M *et al* 2006 Genetic characterization of CHO production host DG44, and derivative recombinant cell lines *Biochem. Biophys. Res. Commun.* **340** 1069–77

Diepenbruck C, Klinger M, Urbig T, Baeuerle P and Neef R 2013 Productivity, and quality of recombinant proteins produced by stable CHO cell clones can be predicted by transient expression in HEK cells *Mol. Biotechnol.* **54** 497–503

Evans K *et al* 2015 Assurance of monoclonality in one round of cloning through cell sorting for single cell deposition coupled with high resolution cell imaging *Biotechnol. Prog.* **31** 1172–8

Fan L, Kadura I, Krebs L E, Hatfield C C, Shaw M M and Frye C C 2012 Improving the efficiency of CHO cell line generation using glutamine synthetase gene knockout cells *Biotechnol. Bioeng.* **109** 1007–15

FDA 1996 Guidance for Industry: for the submission of chemistry, manufacturing, and controls information for a therapeutic recombinant DNA-derived product or a monoclonal antibody product for *in vivo* use 96D-0344 https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-submission-chemistry-manufacturing-and-controls-information-therapeutic

Galanis M, Irving R A and Hudson P J 2001 Bacteriophage library construction, and selection of recombinant antibodies *Curr. Protoc. Immunol.* **ch 17** unit 17.1

Gupta S K and Shukla P 2016 Advanced technologies for improved expression of recombinant proteins in bacteria: perspectives, and applications *Crit. Rev. Biotechnol.* **36** 1089–98

Gurtu V, Yan G and Zhang G 1996 IRES bicistronic expression vectors for efficient creation of stable mammalian cell lines *Biochem. Biophys. Res. Commun.* **229** 295–8

Hexham J M 1998 Production of human Fab antibody fragments from phage display libraries *Methods Mol. Biol.* **80** 461–74

Ho S C *et al* 2012 IRES-mediated Tricistronic vectors for enhancing generation of high monoclonal antibody expressing CHO cell lines *J. Biotechnol.* **157** 130–9

Huang W, McKevitt M and Palzkill T 2000 Use of the arabinose p_{bad} promoter for tightly regulated display of proteins on bacteriophage *Gene* **251** 187–97

ICH Q5D Quality of biotechnological or biological products: derivation and characterization of cell substrates used for production of biotechnological or biological products; https://www.ema.europa.eu/en/ich-q5d-derivation-characterisation-cell-substrates-used-production-biotechnologicalbiological

Jazayeri S H, Amiri-Yekta A, Bahrami S, Gourabi H, Sanati M H and Khorramizadeh M R 2018 Vector and cell line engineering technologies toward recombinant protein expression in mammalian cell lines *Appl. Biochem. Biotechnol.* **185** 986–1003

Kaufman R J 1990 Selection, and coamplification of heterologous genes in mammalian cells *Methods Enymol.* **185** 537–66

Kim J M *et al* 2004 Improved recombinant gene expression in CHO cells using matrix attachment regions *J. Biotechnol.* **107** 95–105

Kim S H and Lee G M 2007 Differences in optimal pH and temperature for cell growth and antibody production between two Chinese hamster ovary clones derived from the same parental clone *J. Microbiol. Biotechnol.* **17** 8

Kim S J, Kim N S, Ryu C J, Hong H J and Lee G M 1998 Characterization of chimeric antibody producing CHO cells in the course of dihydrofolate reductase-mediated gene amplification, and their stability in the absence of selective pressure *Biotechnol. Bioeng.* **58** 73–84

Kim T K and Eberwine J H 2010 Mammalian cell transfection: the present, and the future *Anal. Bioanal. Chem.* **397** 3173–8

Kingston R E, Kaufman R J, Bebbington C R and Rolfe M R 2002 Amplification using CHO cell expression vectors *Curr. Protoc. Mol. Biol.* **ch 16** unit 16.23

Kito M, Itami S, Fukano Y, Yamana K and Shibui T 2002 Construction of engineered CHO strains for high-level production of recombinant proteins *Appl. Microbiol. Biotechnol.* **60** 442–8

Klickstein L B *et al* 1991 Ligation of linkers or adapters to double-stranded cDNA *Current Protocols in Molecular Biology* ed S K Smith (New York: Wiley)

Klottrup K J, Miro-Quesada G, Flack L, Pereda I and Hawley-Nelson P 2018 Measuring the aggregation of CHO cells before single cell cloning allows a more accurate determination of the probability of clonality *Biotechnol. Prog.* **34** 593–601

Knox R *et al* 2013 A streamlined implementation of the glutamine synthetase-based protein expression system *BMC Biotechnol.* **13** 74

Krämer O, Klausing S and Noll T 2010 Methods in mammalian cell line engineering: from random mutagenesis to sequence-specific approaches *Appl. Microbiol. Biotechnol.* **88** 425–36

Lai T, Yang Y and Ng S K 2013 Advances in Mammalian cell line development technologies for recombinant protein production *Pharmaceuticals* **6** 579–603

Laitinen O H *et al* 2005 A multipurpose vector system for the screening of libraries in bacteria, insect, and mammalian cells, and expression in vivo *Nucleic Acids Res.* **33** e42

Lindgren K, Salmén A, Lundgren M, Bylund L, Ebler A, Fäldt E, Sö'rvik L, Fenge C and Skoging-Nyberg U 2009 Automation of cell line development *Cytotechnology* **59** 1–10

Liu M *et al* 2019 Soluble expression of single-chain variable fragment (scFv) in *Escherichia coli* using superfolder green fluorescent protein as fusion partner *Appl. Microbiol. Biotechnol.* **103** 6071–9

Mahalik S, Sharma A K and Mukherjee K J 2014 Genome engineering for improved recombinant protein expression in *Escherichia coli Microb. Cell Fact.* **13** 177

Matsumura T *et al* 2014 Single-cell cloning, and expansion of human induced pluripotent stem cells by a microfluidic culture device *Biochem. Biophys. Res. Commun.* **453** 131–7

Meng Y G, Liang J, Wong W L and Chisholm V 2000 Green fluorescent protein as a second selectable marker for selection of high producing clones from transfected CHO cells *Gene* **242** 201–7

Metta M K, Kunaparaju R K and Tantravahi S 2016 Rapid amplification system for recombinant protein production in Chinese hamster ovary (CHO) cells *Cell Mol. Biol.* **62** 101–6

Mohammed Y, El-Baky N A, Redwan N A and Redwan E M 2012 Expression of human interferon-α8 synthetic gene under P_{BAD} promoter *Biochemistry* **77** 1210–19

Mostafa H E, Heller K J and Geis A 2002 Cloning of *Escherichia coli* lacZ, and lacY genes, and their expression in *Gluconobacter oxydans*, and *Acetobacter liquefaciens Appl. Environ. Microbiol.* **68** 2619–23

Murphy K C, Campellone K G and Poteete A R 2000 PCR-mediated gene replacement in *Escherichia coli Gene* **246** 321–30

Naddafi F, Davami F, Tabarzad M, Barkhordari F and Shirazi F H 2019 Construction of a mammalian IRES-based expression vector to amplify a bispecific antibody; blinatumomab *Iran J. Pharm. Res.* **18** 2117–23

Nakamura T and Omasa T 2015 Optimization of cell line development in the GS-CHO expression system using a high-throughput, single cell-based clone selection system *J. Biosci. Bioeng.* **120** 323–9

Ng S K 2012 Generation of high-expressing cells by methotrexate amplification of destabilized dihydrofolate reductase selection marker *Methods Mol. Biol.* **801** 161–72

Öztürk S, Ergün B G and Çalık P 2017 Double promoter expression systems for recombinant protein production by industrial microorganisms *Appl. Microbiol. Biotechnol.* **101** 7459–75

Paraskevopoulou V and Falcone F H 2018 Polyionic tags as enhancers of protein solubility in recombinant protein expression *Microorganisms* **6** 47

Peakman T C *et al* 1994 Comparison of expression of a humanized monoclonal antibody in mouse NSO myeloma cells, and Chinese hamster ovary cells *Hum. Antibodies Hybridomas* **5** 65–74

Romanov V I 2003 Phage display selection, and evaluation of cancer drug targets *Curr. Cancer Drug Targets* **3** 119–29

Sabido A, Martínez L M, de Anda R, Martínez A, Bolívar F and Gosset G 2013 A novel plasmid vector designed for chromosomal gene integration, and expression: use for developing a genetically stable *Escherichia coli* melanin production strain *Plasmid* **69** 16–23

Singh R P and al-Rubeai M 1998 Apoptosis, and bioprocess technology *Adv. Biochem. Eng. Biotechnol.* **62** 167–84

Srirangan K, Loignon M and Durocher Y 2020 The use of site-specific recombination, and cassette exchange technologies for monoclonal antibody production in Chinese hamster ovary cells: retrospective analysis, and future directions *Crit. Rev. Biotechnol.* **40** 833–51

Stefan A, Schwarz F, Bressanin D and Hochkoeppler A 2010 Shine–Dalgarno sequence enhances the efficiency of lacZ repression by artificial anti-lac antisense RNAs in *Escherichia coli J. Biosci. Bioeng.* **110** 523–8

Sørensen H P and Mortensen K K 2005 Soluble expression of recombinant proteins in the cytoplasm of *Escherichia coli Microb. Cell Fact.* **4** 1

Tripathi N K and Shrivastava A 2019 Recent developments in bioprocessing of recombinant proteins: expression hosts and process development *Front. Bioeng. Biotechnol.* **7** 420

Velez-Suberbie M L, Betts J P J, Walker K L, Robinson C, Zoro B and Keshavarz-Moore E 2018 High throughput automated microbial bioreactor system used for clone selection, and rapid scale-down process optimization *Biotechnol. Prog.* **34** 58–68

Wang Z *et al* 2002 Dual gene amplification, and selection system with dihydrofolate reductase, and glutamine synthetase genes effectively increase the foreign gene expression *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi* **16** 59–61 (in Chinese)

Xiao-Jie L, Hui-Ying X, Zun-Ping K, Jin-Lian C and Li-Juan J 2015 CRISPR-Cas9: a new and promising player in gene therapy *J. Med. Genet.* **52** 289–96

Yeo J H M *et al* 2017 Optimized selection marker, and CHO host cell combinations for generating high monoclonal antibody producing cell lines *Biotechnol. J.* **12** 1700175

Yoshimoto N *et al* 2013 An automated system for high-throughput single cell-based breeding *Sci. Rep.* **3** 1191

Zaworski P G and Gill G S 1993 Serum-free transfection, and selection in Chinese hamster ovary (CHO) cells *Biotechniques* **15** 863–6

Chapter 4

Akiba T and Fukimbara T 1973 Fermentation of volatile substrate in a tower-type fermenter with a gas entrainment process *J. Ferment. Technol.* **51** 134–41

Amanullah A *et al* 2010 Novel micro-bioreactor high throughput technology for cell culture process development: reproducibility and scalability assessment of fed-batch CHO cultures *Biotechnol. Bioeng.* **106** 57–67

Amer M, Feng Y and Ramsey J D 2019 Using CFD simulations and statistical analysis to correlate oxygen mass transfer coefficient to both geometrical parameters and operating conditions in a stirred-tank bioreactor *Biotechnol. Prog.* **35** e2785

Bailey J E and Ollis D F 1986 *Biochemical Engineering Fundamentals* 2nd edn (New York: McGraw-Hill)

Biomedical N NOVA BioProfile FLEX Instruction Manual

Bonham-Carter J and Jerry S 2011 A brief history of perfusion processing *Bioprocess Int.* **9** 24–32

Butt J B 1999 *Reaction Kinetics and Reactor Design* 2nd edn (New York: Marcel Dekker)

Campani G, Ribeiro M P, Horta A C, Giordano R C, Badino A C and Zangirolami T C 2015 Oxygen transfer in a pressurized airlift bioreactor *Bioprocess. Biosyst. Eng.* **38** 1559–67

Chisti Y 1992 Assure bioreactor sterility *Chem. Eng. Prog.* **88** 80–95

Chopra J and Sen R 2018 Process optimization involving critical evaluation of oxygen transfer, oxygen uptake and nitrogen limitation for enhanced biomass and lipid production by oleaginous yeast for biofuel application *Bioprocess. Biosyst. Eng.* **41** 1103–13

Clincke M F *et al* 2013 Very high density of Chinese hamster ovary cells in perfusion by alternating tangential flow or tangential flow filtration wave bioreactorTM—part II: applications for antibody production and cryopreservation *Biotechnol. Prog.* **29** 768–77

Clincke M F, Mölleryd C, Zhang Y, Lindskog E, Walsh K and Chotteau V 2013 Very high density of CHO cells in perfusion by ATF or TFF in WAVE bioreactortm. Part I effect of the cell density on the process *Biotechnol. Prog.* **29** 754–67

Doran P M 2013 *Mass Transfer* 2nd edn (New York: Academic), ch 10 pp 379–444

Doran P M 2012 *Bioprocess Engineering Principles.* 2nd edn (New York: Academic)

Freyer S A, König M and Künkel A 2004 Validating shaking flasks as representative screening systems *Biochem. Eng. J.* **17** 169–73

GE Healthcare 2005 *Microcarrier Cell Culture: Principles and Methods* (Amersham: GE Heathcare)

Hsu W T and Aulakh R P S 2012 Advanced microscale bioreactor system: a representative scale-down model for benchtop bioreactors *Cytotechnology* **64** 667–78

Kirk T V and Szita N 2013 Oxygen transfer characteristics of miniaturized bioreactor systems *Biotechnol. Bioeng.* **110** 1005–19

Kostov Y, Harms P, Randers-Eichhorn L and Rao G 2001 Low-cost microbioreactor for high-throughput bioprocessing *Biotechnol. Bioeng.* **72** 346–52

Legmann R, Schreyer H B, Combs R G, McCormick E L, Russo A P and Rodgers S T 2009 A predictive high-throughput scale-down model of monoclonal antibody production in CHO cells *Biotechnol. Bioeng.* **104** 1107–20

Lourenço N D, Lopes J A, Almeida C F, Sarraguça M C and Pinheiro H M 2012 Bioreactor monitoring with spectroscopy and chemometrics: a review *Anal. Bioanal. Chem.* **404** 1211–37

Mandenius C F 2016 *Challenges for Bioreactor Design and Operation* (Weinheim: Wiley), pp 1–34

Morandi M and Valeri A 1988 Industrial scale production of *β*-interferon *Adv. Biochem. Eng. Biotechnol.* **37** 57–72

Glacken M W 1991 Bioreactor control and optimization *Biotechnology* **17** 373–404

Pollock J, Ho S V and Farid S S 2013 Fed-batch and perfusion culture processes: economic, environmental, and operational feasibility under uncertainty *Biotechnol. Bioeng.* **110** 206–19

Prave P 1987 *Fundamentals of Biotechnology* (Weinheim: Wiley)

Shuler M L and Kargi F 2006 *Bioprocess Engineering Basic Concepts* 2nd edn (Englewood Cliffs, NJ: Prentice-Hall)

Singh V 1999 Disposable bioreactor for cell culture using wave-induced agitation *Cytotechnology* **30** 149–58

Sola C and Godia F 1995 *Bioreactor System Design* (New York: Marcel Dekker), pp 511–52

Spier R E, Griffiths J B and Berthold W 2013 *Animal Cell Technology: Products of Today, Prospects for Tomorrow* (Amsterdam: Elsevier)

Stephanopoulos G, Rehm H J and Reed G (ed) 1993 *Biotechnology* 2nd edn (New York: Wiley)

Williams J A 2002 Keys to bioreactor selections *Chem. Eng. Prog.* **98** 34–41

Wilson G 2005 Evaluation of a novel micro-bioreactor system for cell culture optimisation *Cell Technology for Cell Products, Proc. of the 19th ESACT Meeting* (Berlin: Springer), p 611

YSI. YSI 2900 Series Biochemistry Analyzers Operations and Maintenance Manual

Zhou X and Xu Y 2017 Improvement of fermentation performance of *Gluconobacter oxydans* by combination of enhanced oxygen mass transfer in compressed-oxygen-supplied sealed system and cell-recycle technique *Bioresour. Technol.* **244** 1137–41

Zhu M M, Goyal A, Rank D L, Gupta S K, Boom T V and Lee S S 2005 Effects of elevated pCO_2 and osmolality on growth of CHO cells and production of antibody-fusion protein B1: a case study *Biotechnol. Prog.* **21** 70–7

Chapter 5

Ali S, Perez-Pardo M A, Aucamp J P, Craig A, Bracewell D G and Baganz F 2012 Characterization and feasibility of a miniaturized stirred tank bioreactor to perform *E. coli* high cell density fed-batch fermentations *Biotechnol. Prog.* **28** 66–75

Andersen D C and Reilly D E 2004 Production technologies for monoclonal antibodies and their fragments *Curr. Opin. Biotechnol.* **15** 456–62

Arora M 2015 Cell culture media: a review *Mater. Methods* **2015** 174

Bauer S and White M D 1976 Pilot scale exponential growth of *Escherichia coli* to high cell concentration with temperature variation *Biotech. Bioeng.* **18** 839–46

Berrill A, Ho S V and Bracewell D G 2008 Ultra scale-down to define and improve the relationship between flocculation and disc-stack centrifugation *Biotechnol. Prog.* **24** 426–31

Bielser J M, Wolf M, Souquet J, Broly H and Morbidelli M 2018 Perfusion mammalian cell culture for recombinant protein manufacturing—a critical review *Biotechnol. Adv.* **36** 1328–40

Buchsteiner M, Quek L E, Gray P and Nielsen L K 2018 Improving culture performance and antibody production in CHO cell culture processes by reducing the Warburg effect *Biotechnol. Bioeng.* **115** 2315–27

Caillava A J, Ortiz G E, Melli L J, Ugalde J E, Ciocchini A E and Comerci D J 2019 Improving bioreactor production of a recombinant glycoprotein in *Escherichia coli*: effect of specific growth rate on protein glycosylation and specific productivity *Biotechnol. Bioeng.* **116** 1427–38

Chen K, Liu Q, Xie L Z, Sharp P A and Wang D I 2001 Engineering of a mammalian cell line for reduction of lactate formation and high monoclonal antibody production *Biotechnol. Bioeng.* **72** 55–61

Cruz H J, Moreira J L and Carrondo M J 1999 Metabolic shifts by nutrient manipulation in continuous cultures of BHK cells *Biotechnol. Bioeng.* **66** 104–13

Donini M and Marusic C 2019 Current state-of-the-art in plant-based antibody production systems *Biotechnol. Lett.* **41** 335–46

Dusseljee P J B and J F 1990 Instrumentation and control ed B McNeil and L M Harvey *Fermentation, A Practical Approach* (Oxford: IRL Press), pp 149–71

Ehret J, Zimmermann M, Eichhorn T and Zimmer A 2019 Impact of cell culture media additives on IgG glycosylation produced in Chinese hamster ovary cells *Biotechnol. Bioeng.* **116** 816–30

Eibl R and Eibl D 2007 Disposable bioreactors for cell culture based-bioprocessing *ACHEMA Worldwide News* 8–10

Fan Y, Ley D and Andersen M R 2018 Fed-batch CHO cell culture for lab-scale antibody production *Methods Mol. Biol.* **1674** 147–61

FDA 2004 *Guidance for Industry PAT-A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance*

Fleischaker R J and Sinskey A J 1981 Oxygen demand and supply in cell culture *Appl. Microbiol. Biotechnol.* **12** 193–7

Fletcher T 2005 Designing culture media for recombinant protein production: a rational approach *Bioprocess Int.* **3** 30–6

Freshney R I 2010 Defined culture media and supplements and serum-free culture media *Culture of Animal Cells: A Manual of Basic Technique* 6th edn (New York: Wiley), pp 99–132

Gaglione R *et al* 2019 Cost-effective production of recombinant peptides in *Escherichia coli N. Biotechnol.* **51** 39–48

Gawlitzek M, Estacio M, Furch T and Kiss R 2009 Identification of cell culture conditions to control N-glycosylation site-cccupancy of recombinant glycoproteins expressed in CHO cells *Biotechnol. Bioeng.* **103** 1164–75

Glick B R and Patten C L *Molecular Biotechnology: Principles and Applications of Recombinant DNA* 5th edn (New York: Wiley)

Gomez N, Barkhordarian H, Lull J, Huh J, GhattyVenkataKrishna P and Zhang X 2019 Perfusion CHO cell culture applied to lower aggregation and increase volumetric productivity for a bispecific recombinant protein *J. Biotechnol.* **304** 70–7

Gomis-Fons J, Löfgren A, Andersson N, Nilsson B, Berghard L and Wood S 2019 Integration of a complete downstream process for the automated lab-scale production of a recombinant protein *J. Biotechnol.* **301** 45–51

Gupta S K and Shukla P 2017 Microbial platform technology for recombinant antibody fragment production: a review *Crit. Rev. Microbiol.* **43** 31–42

Halling P J 1990 pH, dissolved oxygen and related sensors ed B McNeil and L M Harvey *Fermentation: A Practical Approach* (Oxford: IRL Press), pp 131–47

Hammett K, Kuchibhatla J, Hunt C, Holdread S and Brooks J 2007 Developing chemically defined culture media through DOE: complete optimization with increased protein production in less than 8 Months ed R Smith *Cell Technology for Cell Products.* (Dordrecht: Springer), pp 683–91

Harcum S 2005 *Protein Glycosylation* (Boca Raton, FL: CRC Press)

He W *et al* 2017 Expression, purification, and renaturation of a recombinant peptide-based HIV vaccine in *Escherichia coli Can. J. Microbiol.* **63** 493–501

Heidemann R, Zhang C, Qi H, Larrick Rule J, Rozales C and Park S 2000 The use of peptones as medium additives for the production of a recombinant therapeutic protein in high density perfusion cultures of mammalian cells *Cytotechnology* **32** 157–67

Hennicke J, Reinhart D, Altmann F and Kunert R 2019 Impact of temperature and pH on recombinant human IgM quality attributes and productivity *N. Biotechnol.* **50** 20–6

Hill W J and Hunter W G 1966 A review of response surface methodology: a literature survey *Technometrics* **8** 571–90

Huang C J, Lin H and Yang X 2012 Industrial production of recombinant therapeutics in *Escherichia coli* and its recent advancements *J. Ind. Microbiol. Biotechnol.* **39** 383–99

Hwang H G *et al* 2016 Recombinant glargine insulin production process using *Escherichia coli J. Microbiol. Biotechnol.* **26** 1781–9

Johnson K 2016 Strategies for optimized cell culture medium design *BPI Int. Conf. (Boston, MA)*

Kandula S, Babu S, Jin M and Shukla A A 2009 Design of a filter train for precipitate removal in monoclonal antibody downstream processing *Biotechnol. Appl. Biochem.* **54** 149–55

Karengera E, Robotham A, Kelly J, Durocher Y, De Crescenzo G and Henry O 2018 Concomitant reduction of lactate and ammonia accumulation in fed-batch cultures: impact on glycoprotein production and quality *Biotechnol. Prog.* **34** 494–504

Kelley B 2009 Industrialization of mAb production technology: the bioprocessing industry at a crossroads *MAbs* **1** 443–52

Knorre W A, Deckwer W D, Korz D, Pohl H D, Riesenberg D and Ross A 1991 High cell density fermentation of recombinant *Escherichia coli* with computer controlled optimal growth rate *Ann. NY Acad. Sci.* **646** 300–6

Lech K *et al* 1994 Growth in liquid culture media *Current Protocols in Molecular Biology* ed K Struhl (New York: Wiley)

Lee G M and Kildegaard H F 2020 *Cell Culture Engineering: Recombinant Protein Production* (New York: Wiley)

Leone S, Sannino F, Tutino M L, Parrilli E and Picone D 2015 Acetate: friend or foe? Efficient production of a sweet protein in *Escherichia coli* BL21 using acetate as a carbon source *Microb. Cell Fact.* **14** 106

Li B and Sha M 2016 Scale-up of *Escherichia coli* fermentation from small scale to pilot scale using Eppendorf fermentation systems *Eppendorf Application Note* 2016;306

Li F, Hashimura Y, Pendleton R, Harms J, Collins E and Lee B 2006 A systematic approach for scale-down model development and characterization of commercial cell culture processes *Biotechnol. Prog.* **22** 696–703

Li F, Vijayasankaran N, Shen A Y, Kiss R and Amanullah A 2010 Cell culture processes for monoclonal antibody production *MAbs* **2** 466–79

Lourenço N D, Lopes J A, Almeida C F, Sarraguça M C and Pinheiro H M 2012 Bioreactor monitoring with spectroscopy and chemometrics: a review *Anal. Bioanal. Chem.* **404** 1211–37

Lutz H *et al* 2015 Robust depth filter sizing for centrate clarification *Biotechnol. Prog.* **31** 1542–50

Merten O W 1999 Safety issues of animal products used in serum-free medium *Dev. Biol. Stand.* **99** 167–80

Mirro R 2011 Introduction to *Escherichia coli* cultivation in a stirred-tank bioreactor *Eppendorf Application Note* 2011 p 251

Monteil D T *et al* 2016 A comparison of orbitally-shaken and stirred-tank bioreactors: pH modulation and bioreactor type affect CHO cell growth and protein glycosylation *Biotechnol. Prog.* **32** 1174–80

Monteil D T and Kuan J 2018 Bench-scale stirred-tank bioreactor for recombinant protein production in Chinese hamster ovary (CHO) cells in suspension *Methods Mol. Biol.* **1850** 133–45

Mori H, Yano T, Kobayashi T and Shimizu S 1979 High density cultivation of biomass in fed-batch system with DO-stat *J. Chem. Eng.* **12** 313–19

Perez-Pardo M A, Ali S, Balasundaram B, Mannall G J, Baganz F and Bracewell D G 2011 Assessment of the manufacturability of *Escherichia coli* high cell density fermentations *Biotechnol. Prog.* **27** 1488–96

Perez-Zabaleta M, Guevara-Martínez M, Gustavsson M, Quillaguamán J, Larsson G and van Maris A J A 2019 Comparison of engineered *Escherichia coli* AF1000 and BL21 strains for *(R)*-3-hydroxybutyrate production in fed-batch cultivation *Appl. Microbiol. Biotechnol.* **103** 5627–39

Picanco-Castro V and Swiech K *Recombinant Glycoprotein Production: Methods and Protocols* (New York: Springer)

Pollock S V H and Farid S S 2013 Fed-batch and perfusion culture processes: economic, environmental, and operational feasibility under uncertainty *Biotechnol. Bioeng.* **110** 206–19

Priyanka K J, Gomes J and Rathore A S 2019 Implementing process analytical technology for the production of recombinant proteins in *Escherichia coli* using an advanced controller scheme *Biotechnol. J.* **14** e1800556

Saldova R *et al* 2017 Advances in analytical methodologies to guide bioprocess engineering for bio-therapeutics *Methods* **116** 63–83

Schmideder A and Weuster-Botz D 2017 High-performance recombinant protein production with *Escherichia coli* in continuously operated cascades of stirred-tank reactors *J. Ind. Microbiol. Biotechnol.* **44** 1021–9

Schwarz H *et al* 2020 Small-scale bioreactor supports high density HEK293 cell perfusion culture for the production of recombinant Erythropoietin *J. Biotechnol.* **309** 44–52

Seth G *et al* 2013 Development of a new bioprocess scheme using frozen seed train interculture mediates to initiate CHO cell culture manufacturing campaigns *Biotechnol. Bioeng.* **110** 1376–85

Toussaint C, Henry O and Durocher Y 2016 Metabolic engineering of CHO cells to alter lactate metabolism during fed-batch cultures *J. Biotechnol.* **217** 122–31

Tripathi N K and Shrivastava A 2019 Recent developments in bioprocessing of recombinant proteins: expression hosts and process development *Front. Bioeng. Biotechnol.* **7** 420

Singh V 2016 Strategies for fermentation medium optimization: an in-depth review *Front. Microbiol.* **7** 2087

Velur Selvamani R S, Friehs K and Flaschel E 2014 Extracellular recombinant protein production under continuous culture conditions with *Escherichia coli* using an alternative plasmid selection mechanism *Bioprocess. Biosyst. Eng.* **37** 401–13

Vijayasankaran N, Li J, Shawley R, Chen A, Shiratori M and Gawlitzek M 2010 Animal cell culture culture media ed M C Flickinger *Encyclopedia of Industrial Biotechnology, Bioprocess, Bioseparation, and Cell Technology* (New York: Wiley)

Vogg S, Wolf M K F and Morbidelli M 2018 Continuous and integrated expression and purification of recombinant antibodies *Methods Mol. Biol.* **1850** 147–78

Voulgaris I, Chatel A, Hoare M, Finka G and Uden M 2016 Evaluation of options for harvest of a recombinant *E. coli* fermentation producing a domain antibody using ultra scale-down techniques and pilot-scale verification *Biotechnol. Prog.* **32** 382–92

Warikoo V *et al* 2012 Integrated continuous production of recombinant therapeutic proteins *Biotechnol. Bioeng.* **109** 3018–29

Wurm F M 2004 Production of recombinant protein therapeutics in cultivated mammalian cells *Nat. Biotechnol.* **22** 1393–8

Xie L Z and Wang D I C 1996 High cell density and high monoclonal antibody production through medium design and rational control in a bioreactor *Biotechnol. Bioeng.* **51** 725–9

Yang J D, Lu C, Stasny B, Henley J, Guinto W and Gonzalez C 2007 Fed-batch bioreactor process scale-up from 3 L to 2,500 L scale for monoclonal antibody production from cell culture *Biotechnol. Bioeng.* **98** 141–54

Yee L and Blanch H W 1992 Recombinant protein expression in high cell density fed-batch cultures of *Escherichia coli Biotechnology* **10** 1550–6

Zare H, Mir Mohammad Sadeghi H and Akbari V 2019 Optimization of fermentation conditions for reteplase expression by *Escherichia coli* using response surface methodology *Avicenna J. Med. Biotechnol.* **11** 162–8

Zhou Y, Lu Z, Wang X, Selvaraj J N and Zhang G 2018 Genetic engineering modification and fermentation optimization for extracellular production of recombinant proteins using *Escherichia coli Appl. Microbiol. Biotechnol.* **102** 1545–6

Zhu M M, Goyal A, Rank D L, Gupta S K, Boom T V and Lee S S 2005 Effects of elevated pCO_2 and osmolality on growth of CHO cells and production of antibody-fusion protein B1: a case study *Biotechnol. Prog.* **21** 70–7

Zhang H, Wang H, Liu M, Zhang T, Zhang J, Wang X and Xiang W 2013 Rational development of a serum-free medium and fed-batch process for a GS-CHO cell line expressing recombinant antibody *Cytotechnology* **65** 363–78

Zieliński M *et al* 2019 Expression and purification of recombinant human insulin from *E. coli* 20 strain *Protein Expr. Purif.* **157** 63–9

Chapter 6

Ambler C 1961 Theory of centrifugation *Ind. Eng. Chem.* **53** 430–3

Asenjo J A and Patrick I 1990 Large-scale protein purification *Protein Purification Applications: A Practical Approach* ed E L V Harris and S Angal (Oxford: IRL Press), pp 1–27

Bakeev K A 2010 *Process Analytical Technology: Spectroscopic Tools and Implementation Strategies for the Chemical and Pharmaceutical Industries* 2nd edn (New York: Wiley)

Baneyx F 1999 Recombinant protein expression in *Escherichia coli Curr. Opin. Biotechnol.* **10** 411–21

Berthold W and Kempken R 1994 Interaction of cell culture with downstream purification: a case study *Cytotechnology* **15** 229–42

Besnard L *et al* 2016 Clarification of vaccines: an overview of filter based technology trends and best practices *Biotechnol. Adv.* **34** 1–3

Bessette P H, Aslund F, Beckwick J and Georgiou G 1999 Efficient folding of proteins with multiple disulfide bonds in the *Escherichia coli* cytoplasm *Proc. Natl Acad. Sci. USA* **96** 13703–8

Boychyn M, Doyle W, Bulmer M, More J and Hoare M 2000 Laboratory scaledown of protein purification processes involving fractional precipitation and centrifugal recovery *Biotechnol. Bioeng.* **69** 1–10

Brinkmann A, Elouafiq S, Pieracci J and Westoby M 2018 Leveraging single-pass tangential flow filtration to enable decoupling of upstream and downstream monoclonal antibody processing *Biotechnol. Prog.* **34** 405–11

Brorson K, Krejci S, Lee K, Hamilton E, Stein K and Yuan X 2003 Bracketed generic inactivation of rodent retroviruses by low pH treatment for monoclonal antibodies and recombinant proteins *Biotechnol. Bioeng.* **82** 321–9

Burgess R R 2009 Refolding solubilized inclusion body proteins *Methods Enzymol.* **463** 259–82

Burgstaller D, Jungbauer A and Satzer P 2019 Continuous integrated antibody precipitation with two-stage tangential flow microfiltration enables constant mass flow *Biotechnol. Bioeng.* **116** 1053–65

Cai K, Anderson J, Orchard J D, Afdahl C D, Dickson M and Li Y 2019 Virus removal robustness of ion exchange chromatography *Biologicals* **58** 28–34

Carta G and Jungbauer A 2010 *Introduction to Protein Chromatography* (Weinheim: Wiley), pp 57–84

Carta G and Jungbauer A 2010 *Protein Chromatography* (Weinheim: Wiley), p 346

Clark E D 2001 Protein refolding for industrial processes *Curr. Opin. Biotechnol.* **12** 202–7

Challener C 2018 Evolving UF/DF capabilities *BioPharm Int.* **31** 24–7

Connell-Crowley L *et al* 2012 Cation exchange chromatography provides effective retrovirus clearance for antibody purification processes *Biotechnol. Bioeng.* **109** 157–65

Dizon-Maspat J, Bourret J, D'Agostini A and Li F 2012 Single pass tangential flow filtration to debottleneck downstream processing for therapeutic antibody production *Biotechnol. Bioeng.* **109** 962–70

Ellgaard T W, Bindslev L and Kamstrup S 2017 Evaluation of the virus clearance capacity and robustness of the manufacturing process for the recombinant factor VIII protein, turoctocog alfa *Protein Expr. Purif.* **129** 94–100

FDA 1984 *Biologics. Points to Consider the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology* (FDA Department of Health and Human Services)

FDA 1999 International Committee on Harmonisation. Q5a: viral safety evaluation of biotechnology products derived from cell lines of human or animal origin

Fang M and Huang H L 2001 Advances in *in vitro* refolding of inclusion body proteins *Sheng Wu Gong Cheng Xue Bao* **17** 608–12

Freydell E J, van der Wielen L A, Eppink M H and Ottens M 2011 Techno-economic evaluation of an inclusion body solubilization and recombinant protein refolding process *Biotechnol. Prog.* **27** 1315–28

GE Healthcare 1998 *Protein Purification Handbook* (Uppsala: GE HEalthcare Bio-Sciences AB), p 97

GE Healthcare 2006 *Break Through the Bottleneck* (Amersham: GE Healthcare)

GE Healthcare 2006 Sanitization of ÄKTA process with sodium hydroxide *Process Chromatography* (Amersham: GE Healthcare Life Sciences)

GE Healthcare 2016 GE: your reliable downstream bioprocessing *Our Proven AxiChrom™ Columns*

GE Healthcare 2016 Optimizing productivity on high capacity Protein-A affinity medium *GE Healthcare Application Notes* (Amersham: GE Healthcare Life Sciences)

Hagel L, Jagschies G and Sofe G 2008 Separation technologies *Handbook of Process Chromatography* 2nd edn (New York: Academic), ch 4 pp 81–125

Hagel L, Jagschies G and Sofer G 2007 Production scenarios *Handbook of Process Chromatography: Development, Manufacturing, Validation and Economics* ed L Hagel, G Jagschies and G Sofer 2nd edn (New York: Academic), pp 22–39

Hashemzadeh M S, Mohammadi M, Ghaleh H E G, Sharti M, Choopani A and Panda A K 2020 Expression, solubilization, refolding and final purification of recombinant proteins as expressed in the form of 'classical inclusion bodies' in *E. coli Protein Pept. Lett.* **28** 122–30

Iammarino M, Chandler M, Roush D and Goklen K 2007 Impact of cell density and viability on primary clarification of mammalian cell broth *Bioprocess Int.* **20**

Janson J-C 2011 Introduction to chromatography *Protein Purification: Principles, High Resolution Methods, and Applications* 3rd edn (New York: Wiley), pp 23–50

Janson J C and Hedman P 1987 On the optimization of process chromatography of proteins *Biotechnol. Prog.* **3** 9–13

Janson J C and Ryden L 1998 *Protein Purification: Principles, High Resolution Methods, and Applications* 2nd edn (New York: Wiley-LISS)

Joseph A *et al* 2016 A scale-down mimic for mapping the process performance of centrifugation, depth and sterile filtration *Biotechnol. Bioeng.* **113** 1934–41

Kelley B D 2007 Very large-scale monoclonal antibody purification: the case for conventional unit operations *Biotechnol. Prog.* **23** 995–1008

Kunert R and Reinhart D 2016 Advances in recombinant antibody manufacturing *Appl. Microbiol. Biotechnol.* **100** 3451–61

Laska M E, Brooks R P, Gayton M and Pujar N S 2005 Robust scale-up of dead end filtration: impact of filter fouling mechanisms and flow distribution *Biotechnol. Bioeng.* **92** 308–20

Liu H F, Ma J, Winter C and Bayer R 2010 Recovery and purification process development for monoclonal antibody production *MAbs* **2** 480–99

Looby M *et al* 2011 Application of quality by design principles to the development and technology transfer of a major process improvement for the manufacture of a recombinant protein *Biotechnol. Prog.* **27** 1718–29

Mark Buswell A, Ebtinger M, Vertés A A and Middelberg A P 2002 Effect of operating variables on the yield of recombinant trypsinogen for a pulse-fed dilution-refolding reactor *Biotechnol. Bioeng.* **77** 435–44

Marshak D R *et al* 1996 Purification of a recombinant protein overproduced in *Escherichia coli Strategies for Protein Purification and Characterization: A Laboratory Course Manual* (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp 245–62

Maurizi M R 1992 Proteases and protein degradation in *Escherichia coli Experientia* **48** 178–201

McNerney T *et al* 2015 PDADMAC flocculation of Chinese hamster ovary cells: enabling a centrifuge-less harvest process for monoclonal antibodies *MAbs* **7** 413–28

Metzger K F J *et al* 2020 IGF1 inclusion bodies: a QbD based process approach for efficient USP as well as early DSP unit operations *J. Biotechnol.* **312** 23–34

Nelson C A, Lee C A and Fremont D H 2014 Oxidative refolding from inclusion bodies *Methods Mol. Biol.* **1140** 145–57

Panda A K 2003 Bioprocessing of therapeutic proteins from the inclusion bodies of *Escherichia coli Adv. Biochem. Eng. Biotechnol.* **85** 43–93

Patra A K, Mukhopadhyay R, Mukhija R, Krishnan A, Garg L C and Panda A K 2000 Optimization of inclusion body solubilization and renaturation of recombinant human growth hormone from *Escherichia coli Protein Expr. Purif.* **18** 182–92

Pérez M *et al* 2011 Validation of model virus removal and inactivation capacity of an erythropoietin purification process *Biologicals* **39** 430–7

WHO 2013 Protein products prepared by recombinant DNA technology *WHO Technical Report Series*

Rathore A S, Yu M, Yeboah S and Sharma A 2008 Case study and application of process analytical technology (PAT) towards bioprocessing: use of on-line high-performance liquid 13 chromatography (HPLC) for making real-time pooling decisions for process chromatography *Biotechnol. Bioeng.* **100** 306–16

Roque A C A, Silva C S O and Taipa M A 2007 Affinity-based methodologies and ligands for antibody purification: advances and perspectives *J. Chromatogr.* A **1160** 44–55

Roush D J and Lu Y 2008 Advances in primary recovery: centrifugation and membrane technology *Biotechnol. Prog.* **24** 488–95

Saraswat M, Musante L, Ravidá A, Shortt B, Byrne B and Holthofer H 2013 Preparative purification of recombinant proteins: current status and future trends *BioMed Res. Int.* **2013** 312709

Saxena A, Tripathi B P, Kumar M and Shahi V K 2009 Membrane-based techniques for the separation and purification of proteins: an overview *Adv. Colloid Interface Sci.* **145** 1–22

Sharma K, Babu P V, Sasidhar P, Srinivas V K, Mohan V K and Krishna E 2008 Recombinant human epidermal growth factor inclusion body solubilization and refolding at large scale using expanded-bed adsorption chromatography from *Escherichia coli Protein Expr. Purif.* **60** 7–14

Shukla A A and Gottschalk U 2013 Single-use disposable technologies for biopharmaceutical manufacturing *Trends Biotechnol.* **31** 147–54

Shukla A K and Kandula J R 2008 Harvest and recovery of monoclonal antibodies from large-scale mammalian cell culture *BioPharm Int.* **21**

Singh A, Upadhyay V and Panda A K 2015 Solubilization and refolding of inclusion body proteins *Methods Mol. Biol.* **1258** 283–91

Singh A, Upadhyay V, Upadhyay A K, Singh S M and Panda A K 2015 Protein recovery from inclusion bodies of *Escherichia coli* using mild solubilization process *Microb. Cell Fact.* **14** 41

Singh N, Arunkumar A, Chollangi S, Tan Z G, Borys M and Li Z J 2016 Clarification technologies for monoclonal antibody manufacturing processes: current state and future perspectives *Biotechnol. Bioeng.* **113** 698–716

Swartz J P 2001 Advances in *Escherichia coli* production of therapeutic proteins *Curr. Opin. Biotechnol.* **12** 195–201

Thatcher D R, Wilks P and Chaudhuri J 1996 *Inclusion Bodies and Refolding* (Oxford: BIOS Scientific), pp 119–30

Tipton B, Larsen W, Beck J and O'Brien T 2002 Retrovirus and parvovirus clearance from an affinity column product using adsorptive depth filtration *BioPharm Int.* **15** 43–50

Tomic S *et al* 2015 Complete clarification solution for processing high density cell culture harvests *Sep. Purif. Technol.* **141** 269–75

Tran R 2014 Changing manufacturing paradigms in downstream processing and the role of alternative bioseparation technologies *J. Chem. Technol. Biotechnol.* **89** 1534–44

Vallejo L F and Rinas U 2004 Strategies for the recovery of active proteins through refolding of bacterial inclusion body proteins *Microb. Cell Fact.* **3** 11

WHO Guidelines on the quality , safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology, Annex 4, TRS No 987

Yamamoto S, Nakanishi K and Matsuno R 1988 *Ion-Exchange Chromatography of Proteins* (New York: Marcel Dekker)

Yavorsky D, Blanck R, Lambalot C and Brunkow R 2003 The clarification of bioreactor cell cultures for biopharmaceuticals *Pharm. Technol.* **27** 62–74

Yigzaw Y, Piper R, Tran M and Shukla A A 2006 Exploitation of the adsorptive properties of depth filters for host cell protein removal during monoclonal antibody purification *Biotechnol. Prog.* **22** 288–96

Zhou T, Wang L, Zhang Z and Jin M 2019 An enhanced regeneration strategy to improve microbial control and prolong resin lifetime for protein-A resin in large-scale monoclonal antibody (mAb) purification *J. Biotechnol.* **289** 118–25

Chapter 7

Bailey J E and Ollis D F 1986 *Biochemical Engineering Fundamentals* 2nd edn (New York: McGraw-Hill)

Beck A *et al* 2013 Analytical characterization of biosimilar antibodies and Fc-fusion proteins *Trends Anal. Chem.* **48** 81–95

Beck A 2013 Characterization of therapeutic antibodies and related products *Anal. Chem.* **85** 715–36

Behme S 2015 *Manufacturing of Pharmaceutical Proteins* (New York: Wiley)

Blanch H W and Clark D S 1996 *Biochemical Engineering* (New York: Marcel Dekker)

Butt J B 1999 *Reaction Kinetics and Reactor Design* 2nd edn (New York: Marcel Dekker)

Cai X, Wake A and Gouty D 2013 Analytical and bioanalytical assay challenges to support comparability studies for biosimilar drug development *Bioanalysis* **5** 517–20

CMC Biotech Working Group 2009 *A-Mab: A Case Study in Bioprocess Development* (Tampa, FL: International Society for Pharmaceutical Engineering) 2009

CHMP Guideline on Similar Biological Medicinal Products (European Medicines Agency) 2005 p 437

Doran P M 1995 *Bioprocess Engineering Principles* (New York: Academic)

FDA 2011 *Guidance for Industry: Process Validation-General Principles and Practices*

FDA 2004 *Guidance for Industry: PAT—A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance, Food and Drug Administration* (Center for Drug Evaluation and Research (CDER))

FDA 2007 *Guidance for Industry and review Staff: Target Product Profile—A Strategic Development Process Tool*

Hahn R 2006 Comparison of protein A affinity sorbents III. Life time study *J. Chromatogr.* A **1102** 224

ICH 2009 *ICH Harmonized Tripartite Guideline: Pharmaceutical Development Q8* (R2)

ICH 2005 Q9 *Quality Risk Management* (ICH Harmonized Tripartite)

Ishihara T, Kadoya T and Yamamoto S 2007 Application of a chromatography model with linear gradient elution experimental data to the rapid scale-up in ion-exchange process chromatography of proteins *J. Chromatogr.* A **1162** 34–40

Jagschies G, Sofer G K and Hagel L 2007 *Handbook of Process Chromatography: Development, Manufacturing, Validation and Economics* (New York: Academic)
Kumar V and Rathore A S 2014 Two-stage chromatographic separation of aggregates for monoclonal antibody therapeutics *J. Chromatogr.* A **14** 155–62
Legmann R *et al* 2009 A predictive high-throughput scale-down model of monoclonal antibody production in CHO cells *Biotechnol. Bioeng.* **104** 1107–20
Levine H L and Jagschies G (ed) 2010 CMC activities for monoclonal antibody development *The Development of Therapeutic Monoclonal Antibody Products: A Comprehensive Guide to CMC Activities from Clone to Clinic* ch 3 pp 36–59
Lute S *et al* 2008 Robustness of virus removal by protein A chromatography is independent of media lifetime *J. Chromatogr.* A **1205** 17–25
Ma N K 2002 Fabrication and use of a transient contraction flow device to quantify the sensitivity of mammalian and insect cells to hydrodynamic forces *Biotechnol. Bioeng.* **80** 428–36
Miró-Quesada G 2008 A Bayesian approach for multiple response surface optimization in the presence of noise variables *J. Appl. Stat.* **31** 251–70
Morandi M and Valeri A 1988 Industrial scale production of β-interferon *Adv. Biochem. Eng. Biotechnol.* **37** 57–72
Peterson J J 2004 A posterior predictive approach to multiple response surface optimization *J. Qual. Technol.* **36** 139–53
Peterson J J 2008 A Bayesian approach to the ICH Q8 definition of design space *J. Biopharm. Stat.* **18** 959–75
Peterson J J Q 2009 A Bayesian reliability approach to multiple response optimization with seemingly unrelated regression models *Qual. Technol. Quant. Manag.* **6** 353–69
Prave P 1987 *Fundamentals of Biotechnology* (Weinheim: Wiley)
Rathore A S 2009 Follow-on protein products: scientific issues, developments and challenges *Trends Biotechnol.* **27** 698–705
Rathore A S 2009 Roadmap for implementation of quality by design (QbD) for biotechnology products *Trends Biotechnol.* **27** 546–53
Rathore A S and Mhatre R 2008 *Quality by Design for Biopharmaceuticals: Principles and Case Studies* (New York: Wiley)
Rathore A S and Winkle H 2009 Quality by design for biopharmaceuticals *Nat. Biotechnol.* **27** 26–34
Rathore A S *et al* 2015 Continuous processing for production of biopharmaceuticals *Prep. Biochem. Biotechnol.* **45** 836–49
Rathore A S *et al* 2015 Process analytical technologies in biopharmaceutical process development *J. Chem. Technol. Biotechnol.* **90** 213–14
Rosati S *et al* 2013 Tackling the increasing complexity of therapeutic monoclonal antibodies with mass spectrometry *Trends Anal. Chem.* **48** 72–80
Schellekens H 2004 How similar do 'biosimilars' need to be? *Nat. Biotechnol.* **22** 1357–9
Shukla A A 2017 Evolving trends in mAb production processes *Bioeng. Transl. Med.* **2** 58–69
Shuler M L and Kargi F 2006 *Bioprocess Engineering Basic Concepts* 2nd edn (Englewood Cliffs, NJ: Prentice-Hall)
Singleton C A 2014 MS in the analysis of biosimilars *Bioanalysis* **6** 1627–37
Stephanopoulose G, Rehm H-J and Reed G (ed) 1993 *Bioprocessing Biotechnology* vol 3 2nd edn (New York: Wiley)
Stockdale G C 2009 Finding design space and a reliable operating region using a multivariate Bayesian approach with experimental design *Qual. Technol. Quant. Manag.* **6** 391–408

Tsuruta L R, Santos M and Moro A M 2015 Biosimilars advancements: moving on to the future *Biotechnol. Prog.* **31** 1139–49

Walsh G 2014 Biopharmaceutical benchmarks 2014 *Nat. Biotechnol.* **32** 992–1000

Weinacker D *et al* 2013 Applications of recombinant *Pichia pastoris* in the healthcare industry *Braz. J. Microbiol.* **44** 1043–8

Williams J A 2002 Keys to bioreactor selections *Chem. Eng. Prog.* **98** 34–41

Xie H *et al* 2010 Rapid comparison of a candidate biosimilar to an innovator monoclonal antibody with advanced liquid chromatography and mass spectrometry technologies *MAbs* **2** 379–94

Chapter 8

ASTM E2281-03 Standard Practice for Process and Measurement Capability Indices

ASTM E2474-06 Standard Practice for Pharmaceutical Process Design Utilizing Process Analytical Technology

ASTM E2476-09 Standard Guide for Risk Assessment and Risk Control as it Impacts the Design, Development, and Operation of PAT Processes for Pharmaceutical Manufacture

ASTM E2500-07 Standard Guide for Specification, Design, and Verification of Pharmaceutical and Biopharmaceutical Manufacturing Systems and Equipment

ASTM E2709-10 Standard Practice for Demonstrating Capability to Comply with a Lot Acceptance Procedure

Childress L, Gay A, Zargar A and Ito M K 2013 Review of red yeast rice content and current Food and Drug Administration oversight *J. Clin. Lipidol.* **7** 117–22

EMA 2002 Compilation of Community Procedures on Inspections and Exchange of Information *FDA (CBER) Validation of Procedures for Processing of Human Tissues Intended for Transplantation, Guidance for Industry* https://ema.europa.eu/en/documents/regulatory-procedural-guideline/compilation-community-procedures-inspections-exchange-information_en.pdf

European Commission Good Manufacturing Practice (GMP) guidelines *EudraLex* vol 4 https://ec.europa.eu/health/documents/eudralex/vol-4_en

FDA (CDER, CBER, CVM, and ORA) 2006 *Quality Systems Approach to Pharmaceutical Current Good Manufacturing Practice Regulations, Guidance for Industry*

FDA (CDER, CVM, and ORA) 2004 *PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance, Guidance for Industry*

FDA (CDER) 2006 *Investigating Out-of-Specification (OOS) Test Results for Pharmaceutical Production, Guidance for Industry*

FDA.HHS 2006 Current good manufacturing practice regulation and investigational new drugs. Direct final rule *Fed. Regist.* **71** 2458–62

FDA.HHS 2008 Current good manufacturing practice and investigational new drugs intended for use in clinical trials. Final rule *Fed. Regist.* **73** 40453–63

FDA/Global Harmonization Task Force (GHTF; Medical Devices) 2004 *Quality Management Systems–Process Validation* 2nd edn

FDA/ICH (CDER and CBER) 2009 *Q10 Pharmaceutical Quality System Guidance for Industry*

FDA/ICH, (CDER and CBER) 2001 *Q7 Good Manufacturing Practice for Active Pharmaceutical Ingredients, Guidance for Industry*

FDA/ICH, (CDER and CBER) 2009 *Q8(R2) Pharmaceutical Development, Guidance for Industry*

FDA/ICH, (CDER and CBER) 2006 *Q9 Quality Risk Management, and Industry Guidance*

Geigert J 2004 *The Challenge of CMC Regulatory Compliance for Biopharmaceuticals* (New York: Kluwer Academic/Plenum)

Geigert J 2019 *The Challenge of CMC Regulatory Compliance for Biopharmaceuticals* 3rd edn (Cham: Springer)

Huxsoll J F 1994 *Quality Assurance for Biopharmaceuticals* (New York: Wiley)

Lai H and Chen Q 2012 Bioprocessing of plant-derived virus-like particles of Norwalk virus capsid protein under current good manufacture practice regulations *Plant Cell Rep.* **31** 573–84

Rathore A S and Mhatre R 2009 *Quality by Design for Biopharmaceuticals: Principles and Case Studies* (Hoboken, NJ: Wiley)

Rathore A S and Sofer G K 2005 *Process Validation in Manufacturing of Biopharmaceuticals: Guidelines, Current Practices, and Industrial Case Studies* (Boca Raton, FL: Taylor and Francis)

Rathore A S and Sofer G K 2012 *Process Validation in Manufacturing of Biopharmaceuticals* 3rd edn (Boca Raton, FL: Taylor and Francis/CRC Press)

Sheets R L, Rangavajhula V, Pullen J K, Butler C, Mehra V, Shapiro S and Pensiero M 2015 Now that you want to take your HIV/AIDS vaccine/biological product research concept into the clinic: what are the 'cGMP'? *Vaccine* **33** 1757–66

Tolbert W R and Rupp R G 1989 Manufacture of pharmaceutical proteins from hybridomas and other cell substrates *Dev. Biol. Stand.* **70** 49–56

Wang L, Zheng H, Ren X and Sun H 2016 Trend analysis of FDA warning letters issued to medical products about violations to current good manufacturing practices (CGMP) between 2007 and 2014 *Ther. Innov Regul. Sci.* **50** 312–18

Werz W, Hoffmann H, Haberer K and Walter J K 1997 Strategies to avoid virus transmissions by biopharmaceutic products *Arch. Virol. Suppl.* **13** 245–56

Chapter 9

Brown J *et al* 2014 Scale-up of microbial fermentation using recombinant *E. coli* in HyPerforma 30 L and 300 L single-use fermentors *Application Note* CO29180 Thermo Fisher Scientific, San Jose, CA https://thermofisher.com/content/dam/LifeTech/Documents/PDFs/CO29180-SUF-Launch-AppNotes-Scale-Up%20of%20Microbial-Global-FLR_V2.pdf

Belongia B, Blanck R and Tingley S 2003 Single-use disposable filling for sterile pharmaceuticals *Pharm. Eng.* **23** 26–134

Bisschops M, Frick L, Fluton S and Ransohoff T 2009 Single-use, continuous countercurrent, multicolumn chromatography *Bioprocess Int.* **7** S18–23

Boedeker B, Goldstein A and Mahajan E 2018 Fully single-use manufacturing concepts for clinical and commercial manufacturing and ballroom concepts *Adv. Biochem. Eng. Biotechnol.* **165** 179–210

Brower M, Hou Y and Pollard D 2015 Monoclonal antibody continuous processing enabled by single-use *Continuous Processing in Pharmaceutical Manufacturing* ed G Subramanian (New York: Wiley), pp 255–96

Dorival-García N and Bones J 2017 Monitoring leachables from single-use bioreactor bags for mammalian cell culture by dispersive liquid–liquid microextraction followed by ultra high performance liquid chromatography quadrupole time of flight mass spectrometry *J. Chromatogr.* A **1512** 51–60

Forums/extractables-and-leachables. 2016/04/11/ 2016;311844

Fuller M and Pora H 2008 Introducing disposable systems into biomanufacturing: a CMO case study *Bioprocess Int.* **6** 30–6

Galliher P M *et al* 2011 Single use bioreactor platform for microbial fermentation *Single-Use Technology in Biopharmaceutical Manufacture* ed R Eibl and D Eibl (New York: Wiley), pp 247–58

Ge X *et al* 2006 Validation of an optical sensor-based high-throughput bioreactor system for mammalian cell culture *J. Biotechnol.* **122** 293–306

Huether-Franken C M *et al* 2013 Scalability of parallel E. coli fermentations in BioBLU® f single-use bioreactors *Application Note* 293-I Eppendorf AG, Juelich, Germany https://eppendorf.com/product-media/doc/en/70274/DASGIP_Fermentors-Bioreactors_Application-Note_293_BioBLU-f_Scalability-Parallel-E-coli-Fermentations-BioBLU-f-Single-Bioreactors.pdf

Jacquemart R, Vandersluis M, Zhao M, Sukhija K, Sidhu N and Stout J 2016 A single-use strategy to enable manufacturing of affordable biologics *Comput. Struct. Biotechnol. J.* **14** 309–18

Jordi M A *et al* 2018 Qualitative assessment of extractables from single-use components and the impact of reference standard selection *J. Pharm. Biomed. Anal.* **150** 368–76

Kelly P S *et al* 2019 Improvements in single-use bioreactor film material composition leads to robust and reliable Chinese hamster ovary cell performance *Biotechnol. Prog.* **35** e2824

Krishnan R and Chen H 2012 Overview of single-use technologies for biologics production *Am. Pharm. Rev.* **15** 15–19

Kuczewski M, Schirmer E, Lain B and Zarbis-Papastoitsis G 2011 A single-use purification process for the production of a monoclonal antibody produced in a PER.C6 human cell line *Biotechnol. J.* **6** 56–65

Laukel M *et al* 2011 Single-use downstream processing for clinical manufacturing. Current capabilities and limitations *Bioprocess Int.* **S2** 14–21

Levine H L 2013 Going beyond flexible single use facilities for achieving efficient commercial manufacturing *Bus. Rev. Webinar*

Luitjens A, Lewis J and Pralong A 2012 Single-use biotechnologies and modular manufacturing environments invite paradigm shifts in bioprocess development and biopharmaceutical manufacturing *Biopharmaceutical Production Technology.* ed G Subramanian (New York: Wiley), pp 817–57

Mahajan E, Ray-Chaudhuri T and Vogel J D 2012 Standardization of single-use components extractable studies for industry *Pharm. Eng.* **32** 1–3

Minow B, Rogge P and Thompson K 2012 Implementing a fully disposable MAb manufacturing facility *Bioprocess Int.* **10** 48–57

Mire-Sluis A *et al* 2011 Extractables and leachables *Bioprocess Int.* **9** 14–23

Munro T P, Mahler S M, Huang E P, Chin D Y and Gray P P 2011 Bridging the gap: facilities and technologies for development of early stage therapeutic mAb candidates *MAbs* **3** 440–52

Nicholson P and Storm E 2011 Single-use tangential flow filtration in bioprocessing—an approach to design and development *Bioprocess Int.* **9** 38–47

Oosterhuis N M G and van den Berg H J 2011 How multipurpose is a single-use bioreactor? *BioPharm Int.* **24** 51–6

Pegel A, Reiser S, Steurenthaler M and Klein S 2011 Evaluating disposable depth filtration platforms for MAb harvest clarification *Bioprocess Int.* **9** 52–6

Pidgeon T 2010 Single-use technologies for fill-finish of clinical trials materials *Pharm. Technol.* s22–25

Pollard D J, Richardson D and Brower M 2017 Progress towards automated single-use continuous monoclonal antibody manufacturing via the protein *Continuous Biomanufacturing - Innovative Technologies and Methods: Innovative Technologies and Methods* ed G Subramanian (New York: Wiley)

Pollock J, Ho S V and Farid S S 2013 Fed-batch and perfusion culture processes: economic, environmental, and operational feasibility under uncertainty *Biotechnol. Bioeng.* **110** 206–19

Rawlings B and Pora H 2009 Environmental impact of single-use and reusable bioprocess systems *Bioprocess Int.* **7** 18–26

Riesen N and Eibl R 2011 Single-use bag systems for storage, transportation, freezing and thawing *Single-Use Technology in Biopharmaceutical Manufacture* ed R Eibl and D Eibl (Hoboken, NJ: Wiley), pp 14–20

Spichiger S and Spichiger-Keller U E 2011 New single-use sensors for online measurement of glucose and lactate: the answer to the PAT initiative *Single-Use Technology in Biopharmaceutical Manufacture* ed R Eibl and D Eibl (New York: Wiley), pp 295–9

Shukla A A, Hubbrard B, Tressel T, Guhan S and Low D 2007 Downstream processing of monoclonal antibodies *J. Chromatogr.* B **848** 28–39

Shukla A A and Gottschalk U 2013 Single-use disposable technologies for biopharmaceutical manufacturing *Trends Biotechnol.* **31** 147–54

Sinclair A, Leveen L, Monge M, Lim J A C and Cox S 2008 The environmental impact of disposable technologies—can disposables reduce your facility's environmental footprint? *BioPharm Int.* **21** 4–15

Sugimoto M A A *et al* 2017 Quality of bevacizumab (Avastin®) repacked in single-use glass vials for intravitreal administration *Arq. Bras. Oftalmol.* **80** 108–13

Thorne B A, Waugh S, Wilkie T, Dunn J and LaBreck M 2012 Implementing a single-use TFF system in a cGMP biomanufacturing facility *BioPharm Int.* **25** s20–6

US Department of Health and Human Services 2004 Pharmaceutical CGMPs: guidance for industry PAT—a framework for innovative pharmaceutical development, manufacturing and quality assurance *Food and Drug Administration*

Vachette E, Fenge C, Cappia J M, Delaunay L, Greller G and Magali B 2014 Robust and convenient single-use processing: superior strength and flexibility of flexsafe bag *Bioprocess Int.* **12** 23–5

Walter J K 1998 Strategies and considerations for advanced economy in downstream processing of biopharmaceutical proteins *Bioseparations, Processing, Quality and Characterisation, Economics, Safety and Hygiene* ed G Subramanian (New York: Wiley VCH), pp 447–60

Walther J, Hwang C, Konstantinov K, Godawat R, Abe Y and Sinclair A 2015 The business impact of an integrated continuous biomanufacturing platform for recombinant protein production *J. Biotechnol.* **213** 3–12

Warikoo V *et al* 2012 Integrated continuous production of recombinant therapeutic proteins *Biotechnol. Bioeng.* **109** 3018–29

Westbrook A *et al* 2014 Application of a two-dimensional single-use rocking bioreactor to bacterial cultivation for recombinant protein production *Biochem. Eng. J.* **88** 154–61

White T and Ott K 2015 Management, notification and documentation of single-use systems change orders: challenges and opportunities *Bioprocess Int.*

Wolton D, Heaven L, McFeaters S and Kodilkar M 2015 Standardization of disposables design: the path forward for a potential game changer *Bioprocess Int.* **2**

Zhao M, Vandersluis M, Stout J, Haupts U, Sanders M and Jacquemart R 2019 Affinity chromatography for vaccines manufacturing: finally ready for prime time? *Vaccine* **37** 5491–503

Chapter 10

Arnold L, Lee K, Rucker-Pezzini J and Lee J H 2019 Implementation of fully integrated continuous antibody processing: effects on productivity and COGm *Biotechnol. J.* **14** e1800061

Bakeev K A 2010 *Process Analytical Technology: Spectroscopic Tools and Implementation Strategies for the Chemical and Pharmaceutical Industries* 2nd edn (New York: Wiley)

Baur D *et al* 2018 Model assisted comparison of Protein A resins and multi-column chromatography for capture processes *J. Biotechnol.* **285** 64–73

Beni V, Nilsson D, Arven P, Norberg P, Gustafsson G and Turner A P F 2015 Printed electrochemical instruments for biosensors *ECS J. Solid State Sci.* **4** 3001–5

Berrie D M, Waters R C, Montoya C, Chatel A and Vela E M 2020 Development of a high-yield live-virus vaccine production platform using a novel fixed-bed bioreactor *Vaccine* **38** 3639–45

Bohonak D, Mehta U, Weiss E R and Voyta G 2020 Adapting virus filtration to enable intensified and continuous mAb processing *Biotechnol. Prog.* **37** e3088

Bracewell D G, Brown R A and Hoare M 2004 Addressing a whole bioprocess in real-time using an optical biosensor-formation, recovery and purification of antibody fragments from a recombinant *E. coli* host *Bioprocess. Biosyst. Eng.* **26** 271–82

Brestrich N, Briskot T, Osberghaus A and Hubbuch J 2014 A tool for selective inline quantification of co-eluting proteins in chromatography using spectral analysis and partial least squares regression *Biotechnol. Bioeng.* **111** 1365–73

Brestrich N, Sanden A, Kraft A, McCann K, Bertolini J and Hubbuch J 2015 Advances in inline quantification of co-eluting proteins in chromatography: process-data-based model calibration and application towards real-life separation issues *Biotechnol. Bioeng.* **112** 1406–16

Capito F, Skudas R, Kolmar H and Stanislawski B 2013 Host cell protein quantification by Fourier transform mid infrared spectroscopy (FT-MIR) *Biotechnol. Bioeng.* **110** 252–9

Carrondo M J T *et al* 2012 How can measurement, monitoring, modeling and control advance cell culture in industrial biotechnology? *Biotechnol. J.* **7** 1522–9

Chemmalil L *et al* 2020 Online/at-line measurement, analysis and control of product titer and critical product quality attributes (CQAs) during process development *Biotechnol. Bioeng.* **117** 3757–65

Chen P H, Cheng Y T, Ni B S and Huang J H 2020 Continuous cell separation using microfluidic-based cell retention device with alternative boosted flow *Appl. Biochem. Biotechnol.* **191** 151–63

De Luca C *et al* 2020 Modern trends in downstream processing of biotherapeutics through continuous chromatography: the potential of multicolumn countercurrent solvent gradient purification *Trends Analyt. Chem.* **132** 116051

Elich T, Goodrich E, Lutz H and Mehta U 2019 Investigating the combination of single-pass tangential flow filtration and anion exchange chromatography for intensified mAb polishing *Biotechnol. Prog.* **35** e2862

Esbensen K, Kirsanov D, Legin A, Rudnitskaya A, Mortensen J, Pedersen J, Vognsen L, Makarychev-Mikhailov S and Vlasov Y 2004 Fermentation monitoring using multisensor systems: feasibility study of the electronic tongue *Anal. Bioanal. Chem.* **378** 391–5

Feidl F *et al* 2020 Process-wide control and automation of an integrated continuous manufacturing platform for antibodies *Biotechnol. Bioeng.* **117** 1367–80

Fisher A C, Kamga M H, Agarabi C, Brorson K, Lee S L and Yoon S 2019 The current scientific and regulatory landscape in advancing integrated continuous biopharmaceutical manufacturing *Trends Biotechnol.* **37** 253–67

Flickinger M C 2013 *Upstream Industrial Biotechnology. Equipment, Process Design, Sensing, Control, and cGMP Operations* (New York: Wiley)

Gagnon M, Nagre S, Wang W, Coffman J and Hiller G W 2019 Novel, linked bioreactor system for continuous production of biologics *Biotechnol. Bioeng.* **116** 1946–58

Gagnon M, Nagre S, Wang W and Hiller G W 2018 Shift to high-intensity, low-volume perfusion cell culture enabling a continuous, integrated bioprocess *Biotechnol. Prog.* **34** 1472–81

Goussen C *et al* 2020 Viral clearance capacity by continuous protein A chromatography step using sequential multicolumn chromatography *J. Chromatogr.* B **1145** 122056

Gupta S K and Shukla P 2017 Sophisticated cloning, fermentation, and purification technologies for an enhanced therapeutic protein production: a review *Front. Pharmacol.* **8** 419

Hansen S K, Jamali B and Hubbuch J 2013 Selective high throughput protein quantification based on UV absorption spectra *Biotechnol. Bioeng.* **110** 448–60

Helal N A *et al* 2019 Integrated continuous manufacturing in pharmaceutical industry: current evolutionary steps toward revolutionary future *Pharm. Pat. Anal.* **8** 139–61

Hilbold N J *et al* 2017 Evaluation of several protein a resins for application to multi-column chromatography for the rapid purification of fed-batch bioreactors *Biotechnol. Prog.* **33** 941–53

Hogwood C E, Bracewell D G and Smales C M 2014 Measurement and control of host cell proteins (HCPs) in CHO cell bioprocesses *Curr. Opin. Biotechnol.* **30** 153–60

Heidemann R, Lünse S, Tran D and Zhang C 2010 Characterization of cell-banking parameters for the cryopreservation of mammalian cell lines in 100-mL cryobags *Biotechnol. Prog.* **26** 1154–63

Hughson M D, Cruz T A, Carvalho R J and Castilho L R 2017 Jul Development of a 3-step straight-through purification strategy combining membrane adsorbers and resins *Biotechnol. Prog.* **33** 931–40

Ichihara T, Ito T and Gillespie C 2019 Polishing approach with fully connected flow-through purification for therapeutic monoclonal antibody *Eng. Life Sci.* **19** 31–6

Kadlec P, Gabrys B and Strandt S 2009 Data-driven soft sensors in the process industry *Comput. Chem. Eng.* **33** 795–814

Kamga M H, Cattaneo M and Yoon S 2018 Integrated continuous biomanufacturing platform with ATF perfusion and one column chromatography operation for optimum resin utilization and productivity *Prep. Biochem. Biotechnol.* **48** 383–90

Kateja N, Agarwal H, Hebbi V and Rathore A S 2017 Integrated continuous processing of proteins expressed as inclusion bodies: GCSF as a case study *Biotechnol. Prog.* **33** 998–1009

Kelley B, Kiss R and Laird M 2018 A different perspective: how much innovation is really needed for monoclonal antibody production using mammalian cell technology? *Adv. Biochem. Eng. Biotechnol.* **165** 443–62

Kreyenschulte D, Paciok E, Regestein L, Bluemich B and Buechs J 2015 Online monitoring of fermentation processes via non-invasive low-field NMR *Biotechnol. Bioeng.* **112** 1810–21

Łącki K M and Riske F J 2020 Affinity chromatography: an enabling technology for large-scale bioprocessing *Biotechnol. J.* **15** e1800397

Lacki K M 2014 High throughput process development in biomanufacturing *Curr. Opin. Chem. Eng.* **6** 25–32

Lin H, Leighty R W, Godfrey S and Wang S B 2017 Principles and approach to developing mammalian cell culture media for high cell density perfusion process leveraging established fed-batch media *Biotechnol. Prog.* **33** 891–901

Lute S, Kozaili J, Johnson S, Kobayashi K and Strauss D 2020 Development of small-scale models to understand the impact of continuous downstream bioprocessing on integrated virus filtration *Biotechnol. Prog.* **36** e2962

Luttmann R, Bracewell D G, Cornelissen G, Gernaey K V, Glassey J, Hass V C, Kaiser C, Preusse C, Striedner G and Mandenius C F 2012 Soft sensors in bioprocessing: a status report and recommendations *Biotechnol. J.* **7** 1040–8

Merhar M, Podgornik A, Barut M, Jaksa S, Zigon M and Strancar A 2001 High performance reversed-phase liquid chromatography using novel CIM RP-SDVB monolithic supports *J. Liq. Chrom. Rel. Technol.* **24** 2429–43

National Academies of Sciences, Engineering, and Medicine; Division on Earth and Life Studies; Board on Chemical Sciences and Technology 2019 *Continuous Manufacturing for the Modernization of Pharmaceutical Production: Proceedings of a Workshop* (Washington, DC: National Academies Press)

Noui L, Hill J, Keay P J, Wang R Y, Smith T, Yeung K, Habib G and Hoare M 2002 Development of a high resolution UV spectrophotometer for at-line monitoring of bioprocesses *Chem. Eng. Process.* **41** 107–14

Odman P, Johansen C L, Olsson L, Gernaey K V and Lantz A E 2009 Online estimation of biomass, glucose and ethanol in *Saccharomyces cerevisiae* cultivations using in-situ multi-wavelength fluorescence and software sensors *J. Biotechnol.* **144** 102–12

Oh S K, Yoo S J, Jeong D H and Lee J M 2013 Real-time estimation of glucose concentration in algae cultivation system using Raman spectroscopy *Bioresour. Technol.* **142** 131–7

Ötes O, Flato H, Vazquez Ramirez D, Badertscher B, Bisschops M and Capito F 2018 Scale-up of continuous multi-column chromatography for the protein A capture step: from bench to clinical manufacturing *J. Biotechnol.* **281** 168–74

Ötes O, Flato H, Winderl J, Hubbuch J and Capito F 2017 Feasibility of using continuous chromatography in downstream processing: comparison of costs and product quality for a hybrid process vs a conventional batch process *J. Biotechnol.* **259** 213–20

Pais D A, Carrondo M J, Alves P M and Teixeira A P 2014 Towards real-time monitoring of therapeutic protein quality in mammalian cell processes *Curr. Opin. Biotechnol.* **30** 161–7

Papathanasiou M M, Quiroga-Campano A L, Steinebach F, Elviro M, Mantalaris A and Pistikopoulos E N 2017 Advanced model-based control strategies for the intensification of upstream and downstream processing in mAb production *Biotechnol. Prog.* **33** 966–88

Rajendran A, Paredes G and Mazzotti M 2009 Simulated moving bed chromatography for the separation of enantiomers *J. Chromatogr.* A **1216** 709–38

Rathore A S, Kumar D and Kateja N 2018 Recent developments in chromatographic purification of biopharmaceuticals *Biotechnol. Lett.* **40** 895–905

Roque A C A *et al* 2020 Anything but conventional chromatography approaches in bioseparation *Biotechnol. J.* **15** e1900274

Schofield M 2018 Current state of the art in continuous bioprocessing *Biotechnol. Lett.* **40** 1303–9

Shaikh S and Jaiswal P 2018 Biopharmaceuticals Market By Type (Monoclonal Antibody, Interferon, Insulin, Growth & Coagulation Factor, Erythropoietin, Vaccine, Hormone, and,

Others) and Application (Oncology, Blood Disorder, Metabolic Disease, Infectious Disease, Cardiovascular Disease, Neurological Disease, Immunology, and Others) - Global Opportunity Analysis And Industry Forecast, 2018-2025 *Allied Market Res*. https://www.alliedmarketresearch.com/biopharmaceutical-market

Sonnleitner B 2013 Automated measurement and monitoring of bioprocesses: key elements of the M(3)C strategy *Adv. Biochem. Eng. Biotechnol* **132** 1–33

Stanke M and Hitzmann B 2013 Automatic control of bioprocesses *Measurement, Monitoring, Modelling and Control of Bioprocesses* ed C F Mandenius and N Titchener-Hooker (Berlin: Springer), pp 35–63

Stepper L, Filser F A, Fischer S, Schaub J, Gorr I and Voges R 2020 Pre-stage perfusion and ultra-high seeding cell density in CHO fed-batch culture: a case study for process intensification guided by systems biotechnology *Bioprocess. Biosyst. Eng.* **43** 1431–43

Tao Y, Shih J, Sinacore M, Ryll T and Yusuf-Makagiansar H 2011 Development and implementation of a perfusion-based high cell density cell banking process *Biotechnol. Prog.* **27** 824–9

Xu J *et al* 2020 Biomanufacturing evolution from conventional to intensified processes for productivity improvement: a case study *MAbs* **12** 1770669

Xu S, Gavin J, Jiang R and Chen H 2017 Bioreactor productivity and media cost comparison for different intensified cell culture processes *Biotechnol. Prog.* **33** 867–78

Yilmaz D, Mehdizadeh H, Navarro D, Shehzad A, O'Connor M and McCormick P 2020 Application of Raman spectroscopy in monoclonal antibody producing continuous systems for downstream process intensification *Biotechnol. Prog.* **36** e2947

Yongky A *et al* 2019 Process intensification in fed-batch production bioreactors using non-perfusion seed cultures *MAbs* **11** 1502–14

Zhao R, Natarajan A and Srienc F 1999 A flow injection flow cytometry system for online monitoring of bioreactors *Biotechnol. Bioeng.* **62** 609–17