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# Chapter 1

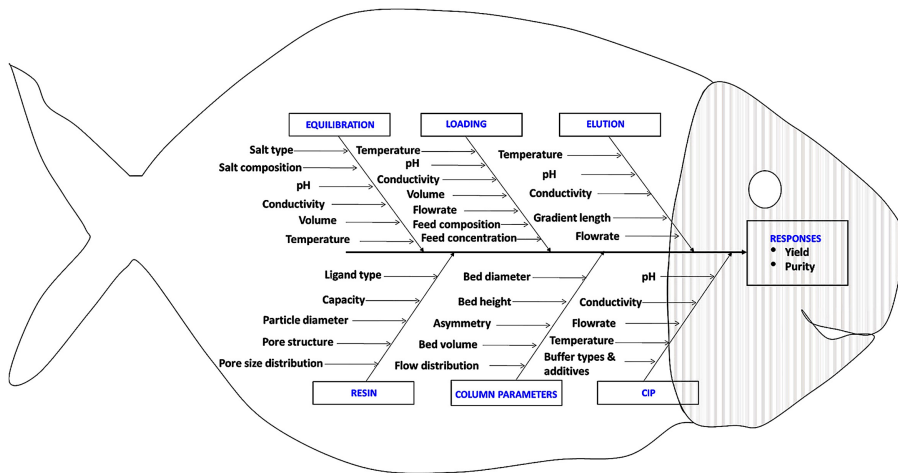
## Understanding bioprocessing

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 entire team to 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).



**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                           | <i>E. coli</i> | <i>Saccharomyces cerevisiae</i> ,<br><i>Pichia pastoris</i> | Lepidoptera     | CHO             | Cattle                 |
| Extracellular expression          | No             | Yes   | Yes             | Yes             | Yes                    |
| Host cell protein expression      | No             | No  | No              | No              | Yes                    |
| <i>In vitro</i> 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  $-70\text{ }^{\circ}\text{C}$ . 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 contaminants.
- 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 <i>E. coli</i> 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 <i>E. coli</i> 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. |  |
| <i>E. coli</i> (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 Formulation        | The purified bulk product.<br>Re-formulation of the drug substance preparing for administration to humans.   |
| Gram-positive bacteria | Drug product Fermentation         | The final product.<br>The expression of the target protein to the periplasmatic room or medium.  |
|                        | Harvest                           | Harvest of cells by centrifugation before cell disruption.<br>This step may be bypassed using expanded bed technology.                             |
|                        | Capture Intermediate purification | Purification of the target protein from the supernatant.<br>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 Formulation        | The purified bulk product.<br>Re-formulation of the drug substance preparing for administration to humans.   |
| Yeast                  | Drug substance Fermentation       | The final product.<br>The expression of the target protein to the medium.  |
|                        | Harvest                           | Harvest of cells by centrifugation before cell disruption.<br>This step may be bypassed using expanded bed technology.                             |
|                        | Capture Intermediate purification | Purification of the target protein from the supernatant or by expanded bed technology.<br>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 Formulation        | The purified bulk product.<br>Re-formulation of the drug substance preparing for administration to humans.   |
| Insect Cells           | Drug product Cell culture         | The final product.<br>The expression of the target protein to the medium.  |
|                        | Harvest                           | Harvest of cells by centrifugation before cell disruption.<br>This step may be bypassed using expanded bed technology.                             |
|                        | Capture                           | Purification of the target protein from the supernatant or by expanded bed technology.   |

|                            |  |  |
|----------------------------|--|--|
| Mammalian cells            | 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 Formulation   | The purified bulk product.<br>Re-formulation of the drug substance preparing for administration to humans.             |
|                            | Drug product   | The final product.   |
|                            | Cell culture   | The expression of the target protein to the medium.  |
|                            | Harvest  | Harvest of cells by centrifugation before cell disruption.<br>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.  |
| Transgenic animals         | 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 Formulation   | The purified bulk product.<br>Re-formulation of the drug substance preparing for administration to humans.             |
|                            | Drug product   | The final product.   |
|                            | 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 Formulation | The purified bulk product.<br>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  $\mu\text{m}$  filter. Representative samples of the DS are collected and analyzed following specified guidelines. The bulk DS is often kept frozen ( $-20\text{ }^{\circ}\text{C}$ ), 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.

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

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

$$\text{Fill and pack} = D1 \times D2 + D3$$

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

$$\text{Yield/batch} = (B3 \times A1 \times A3)/100 \ 000$$

**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   |
| 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   |

$\text{Cost/G} = \text{total cost/yield (per batch)}$

$\text{Cost per vial} = \text{cost per batch/D1}$

$\text{Cost per dose} = (\text{cost per batch/D1}) \times A6$

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<sup>-1</sup>, monoclonal antibodies cost \$150–200 g<sup>-1</sup>, 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<sup>-1</sup>. Assuming a daily milk volume of 20 l, a transgenic cow producing 20 g l<sup>-1</sup> 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,  $p\text{CO}_2$ , and  $p\text{O}_2$ , 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  $1 \text{ g l}^{-1}$ . *E. coli* produces a better yield of  $1\text{--}4 \text{ g l}^{-1}$ , while mammalian cells produce much higher amounts when used for antibodies. Transgenic animals produce the highest yield of  $5\text{--}40 \text{ g l}^{-1}$ . 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  $1000 \text{ g l}^{-1}$  with a 40% yield, the media cost contribution is roughly  $\$25 \text{ g}^{-1}$  of protein; when the expression level drops to  $10 \text{ g l}^{-1}$ , the media cost contribution rises to  $\$2500 \text{ g}^{-1}$  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](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](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 ( $-70\text{ }^{\circ}\text{C}$ ) 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,

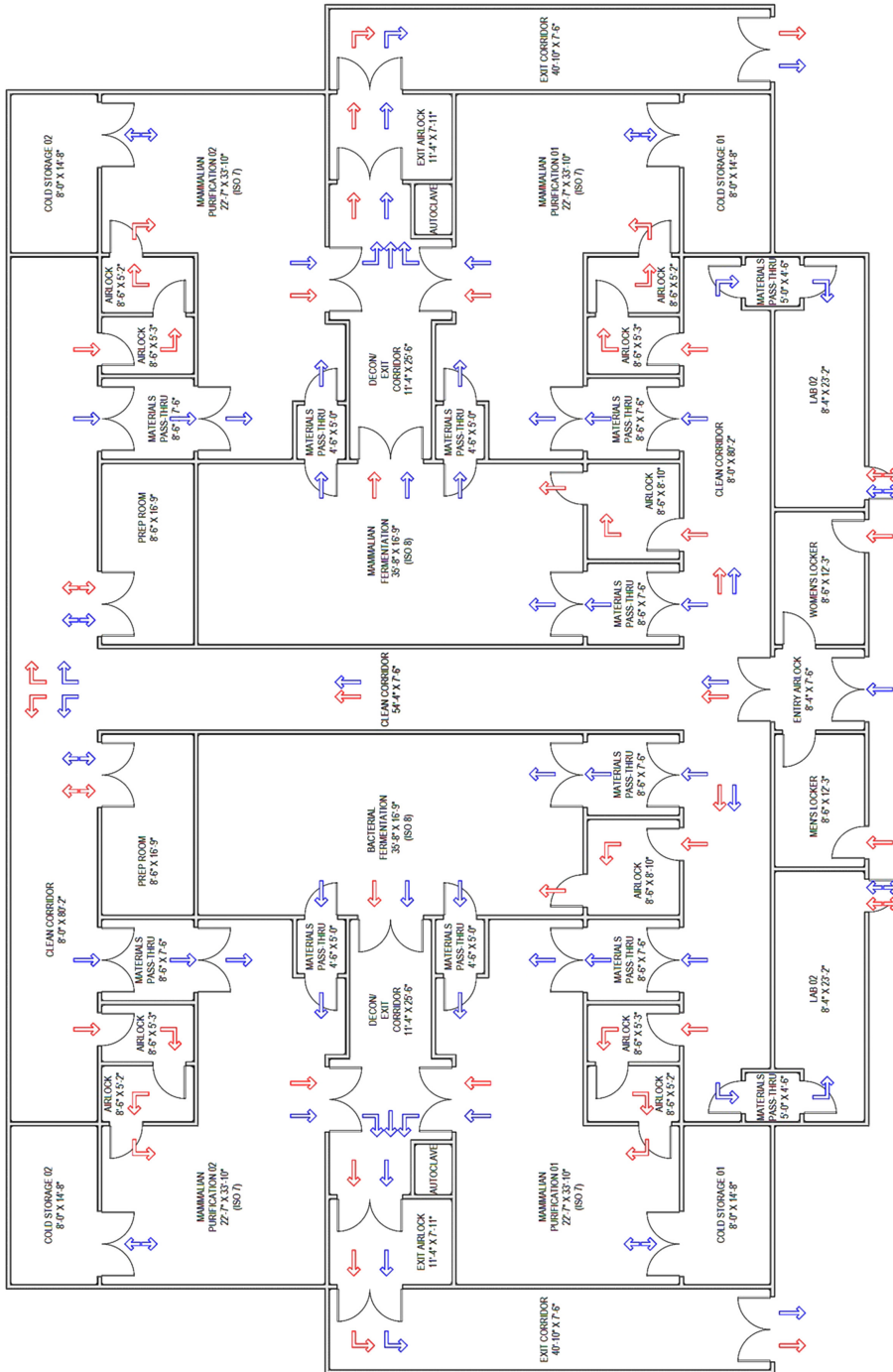


Figure 1.2. A typical biopharmaceutical manufacturing plant layout.



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 amoebocyte 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 amoebocyte 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 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.