Chapter 11

Downstream Processing

Objectives

This chapter provides an overview of downstream processing. After completing this chapter, students will be able to:

- describe the general elements of the downstream purification process for a typical monoclonal antibody-based therapeutic, starting with the bioreactor harvest pool and finishing with pre-formulation, pure drug product.
- explain the overall goals of the purification process from the standpoint of purity, yield, and efficiency as well as the general strategies employed to achieve these goals.
- define the specific methods and in-process tools and materials used in typical steps of the downstream process and the purposes and physical/chemical mechanisms occurring at each step, including:
  - clarification of the bioreactor harvest by depth filtration and/or centrifugation
  - capture chromatography for product concentration and initial purification
  - ultrafiltration and diafiltration of the product at various stages in the downstream process
  - intermediate purification chromatography steps
  - final polishing chromatography steps
- describe general economic factors contributing to the cost of goods produced and common considerations/strategies for optimizing and improving those process economics.
- classify specific departmental contributions to the ongoing operation and maintenance of the downstream unit operations within a facility.

Terms

Capture/recovery: the rapid separation of the product of interest from the cells of the bioreactor

Direct Flow Filtration (DFF): devices which allow the process fluid to cross the membrane in essentially a perpendicular flow direction; this provides little or no prevention of particulate build-up or the concentration of other elements that do not fit through the pore structure

Intermediate purification: the removal of bulk contaminants, including host cell proteins and adventitious viruses as well as any potential contaminating leachates from other in-process materials

Microfiltration: filtration that can be used at the start of the downstream process to clarify the feed beyond what was accomplished in the upstream harvest and centrifugation/clarification

Polishing: the elimination of trace contaminants and impurities, including inactive or unwanted isoforms of the desired therapeutic, or common impurities, including fragments or other chemical modifications thereof

Sterilizing grade filtration: direct flow filtration (oftentimes involving the use of *nanofiltration* cartridges) that eliminates microbial organisms and insoluble proteins; removes adventitious and endogenous viruses; and sterile filters the product in preparation for final formulation

Tangential Flow Filtration (TFF): devices that orient the membrane so that process flow sweeps across the active filtration surface, which minimizes pore plugging and surface fouling by concentrated reject elements of the feed

Ultrafiltration: filtration used between chromatography steps to concentrate the product and change the buffer conditions in order to prepare it for subsequent chromatography steps

Introduction to Downstream Processing

Downstream processing is the phase of biomanufacturing typically considered to begin with cell culture bioreactor harvest containing expressed active pharmaceutical ingredient API and finishing with a highly purified and appropriately concentrated product ready for final formulation and packaging.

This chapter uses the monoclonal antibody-based (mAb) drug category as the representative example target drug to consider, as this category currently comprises one of the most common biotherapeutic classes produced today. The annual production demands for mAb therapeutics are high, due to both their relatively lower potency when compared to other biopharmaceuticals and the related need for larger and more sustained dosing. The market requirement for a typical approved mAb is 10–100 kilograms/year, employing bioreactor batch sizes up to 20,000 liters; chromatography unit operations can range to two meters in diameter and packed resin bed volumes up to approximately 600 liters.

Various economic analyses estimate that process development and clinical manufacturing costs can constitute 40–60 percent of a drug's development cost. Along with commercial manufacturing, driven largely by downstream processing consumable material costs, this can reach up to 25 percent of the sales revenue for the drug.

The majority of biotherapeutic mAbs and their derivatives are all produced by a similar set of methods. They are recombinant proteins and are most often produced in batch-fed mammalian cell expression systems. They are then harvested and purified by a series of orthogonal chromatography steps with intermediate filtration and virus inactivation/clearance steps. Orthogonal chromatography involves using related complimentary chromatography processes that are complimentary and compatible forms of purification.

Mammalian cell lines are capable of mAb expression levels (titers) ranging widely from 0.5–5 g/liter harvest, with the appropriate degree of glycosylation. Glycosylation is the number and pattern of linked sugars at specific points in the drug polypeptide chains that enhance biological activity in the drug's therapeutic application. Mammalian cell expression systems result in relatively challenging feed streams. This is due in part to host cell-derived contaminants that are liberated by cell attrition and other expressed host cell proteins, along with media additives (serum supplements or protein/other additives such as growth promoters or stabilizers). Given the drugs' origins in mammalian systems, significant effort is required to ensure that no mammalian-infecting viruses reach the final drug. The final drugs must be free of adventitious agents such as bacterial pathogens or breakdown products (e.g., endotoxins, or cell wall components of Gram-negative bacteria).

Drug production of a required purity, efficacy, and reasonable cost per unit is the overall goal of the downstream purification process. There are, however, several additional factors to be considered in approaching this goal. For example, the operational goals evolve over the development lifecycle of the drug, from the development or pilot laboratory to ultimate commercial manufacture. In the early life of a drug candidate, developers may produce several variants of the primary molecule, which are rapidly screened for biological activity and stability. In parallel, an emphasis during this period is placed on the thorough characterization and

stability of the compound, with less emphasis on the methods for its purification. Once the definitive target compound and detailed structure are established, the focus is immediately shifted to regulatory viability, from safety (Clinical Phase I) to efficacy in test animal subjects (primarily in Phases I and II) to human testing of both (Phase III).

During these early, smaller scale stages of a drug's production, there is often little motivation to refine the downstream processing scheme. A potential failure in clinical trials would eliminate the need to carry the candidate forward in the development cycle. Often during this period there may also be commercial decisions related to the developing organization's economic condition, constraints, and commercial goals. It is quite common for the candidate drug to be developed initially in an academic entity or entrepreneurial biotechnology firm, which is solely interested in licensing it to a larger, established pharmaceutical firm. The larger firm, with its greater development resources, can then bring the drug candidate to maturity. The firm can make the candidate the subject of either 1) an Investigational New Drug (IND) filing for approval of sale in the United States or 2) another country's approval process/agency. Only then is focus given to the means by which the drug will be produced at larger scale.

Once a drug shows promise in clinical trials, there is often a need for rapid progression to small scale production; this reduces time invested in process optimization to ensure robust and high-yielding processes. Compressed marketing schedules can also limit operator/technician training opportunities; full validation of the impacts of critical operational variability; the thoroughness of raw material and equipment supply chain audits; the degree of automation planned for the processing equipment; etc. Compromises in each of these areas results in higher risks when transferring the process to manufacturing, with real potential for extremely low yields and contaminated/discarded batches.

Despite their intense attention to chromatography resins and other in-process, product-contacting materials (used to validate products as having low or no extractables or leachates), drug manufacturers must also prove that these contaminants are absent or below detectable levels (e.g., they are removed via the downstream process). The downstream processing purification efforts must also prove effective at removing impurities, or components distinguished from contaminants by having direct relation to the product of interest. For example, this can be incompletely expressed product, remnants of the proteolysis of target or other proteins, or aggregates of the target product.

Drug safety assurance is governed by federal regulatory agencies with many specific requirements. For example, the position of the FDA on virus safety can best be seen in this guidance:

> "All production processes should incorporate effective validated steps for the inactivation/removal of a wide range of viruses of diverse physicochemical characteristics...It is desirable to incorporate two distinct effective steps which complement each other in their mode of action."

Therefore, assurance of the absence of viruses typically dictates clearance using a combination of orthogonal methods within the downstream processes; these viruses can originate from the expression cell line by introduction from the environment or from growth medium, reagents, or other in-process materials. For each processing step, the effectiveness of removal or inactivation must be validated; this is accomplished through virus spiking studies, in which representative and/or model viruses are added at relatively high levels and then tracked through each step of the process via infectivity assays.

Goals of the Downstream Process

A typical process for a mAb is shown in Figure 11-1. The downstream process can be divided into three stages, each with the primary goals of the corresponding stage as follows:

Capture or **Recovery**: This involves the rapid separation of the product of interest from the cells of the bioreactor. The goal is:

- removal of all micro-particulates and colloidal materials (a mixture with one substance uniformly dispersed throughout another)
- removal of the majority of water, growth medium supplements, and small molecule solutes via product concentration
- isolation of product away from proteolytic enzymes or other degradative elements

Depending on the specificity of the capture tools (depth filters, affinity chromatography resins, etc.), the product may emerge from the recovery stage at purity levels already approaching 95 percent.

Intermediate purification: This involves the removal of bulk contaminants, including host cell proteins and adventitious viruses, as well as any potential contaminating leachates from other in-process materials. Often there are trace (but finite) specific levels of leached ligand from the capture resin (most commonly free protein A) that can be partially bound or otherwise co-eluted with the product of interest. Protein A is found in the bacteria *Staphylococcus aureus* and has the ability to bind with high affinity to proteins from various mammalian species.

Polishing: This involves the elimination of trace contaminants and impurities, including inactive or unwanted isoforms of the desired therapeutic or common impurities, including fragments or other chemical modifications thereof. An example would be a partially unfolded or incorrectly combined peptide chain or one with modified amino acid side chains (e.g., oxidized, cross-linked, deaminated, etc).

Both intermediate and polishing stages help to ensure the removal of adventitious (non-inherent) and endogenous (from within) viruses from the product, as well as remaining traces of DNA, endotoxins, and host cell proteins.

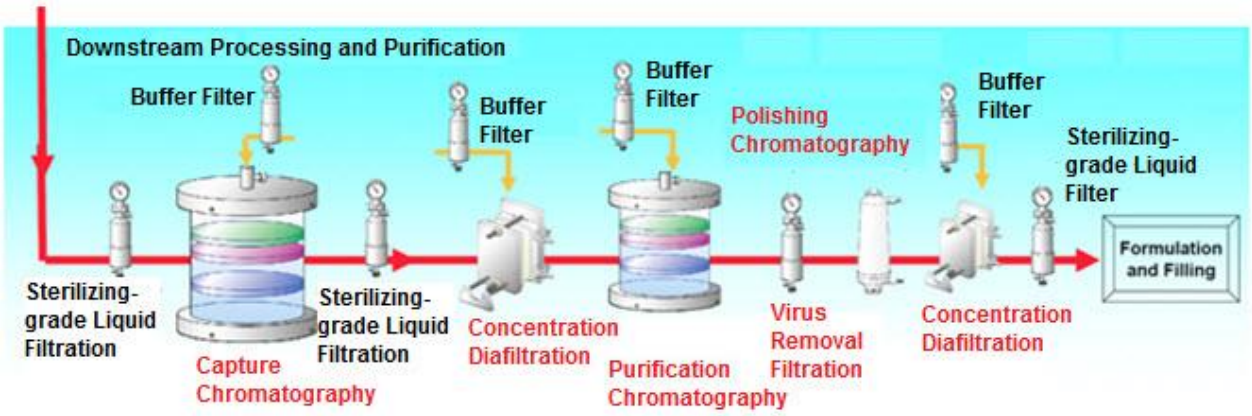


Figure 11-1. Typical downstream process for monoclonal antibody process (courtesy Pall Life Sciences)

Table 11-1: Commonly employed downstream processing methods (adapted from Kemp and O'Neil 2003)

Processing Method	Attributes	Benefits	Limitations
Clarification: (often considered part of the upstream processing train)			
Sedimentation-based Clarification	continuous centrifugation	capable of handling very large harvest volumes	open process-contamination and safety issues
Direct Flow Filtration	microporous		volume and throughput limited
	charged filter media		
	cellulose pads		
Tangential Flow Filtration	contained systems	capable of handling large harvest volumes	
Capture: (typically considered the first stage of downstream processing)			
Chromatography	Protein A affinity	high throughput, high purity	high initial cost
	other affinity ligands	high throughput	purity, regulatory acceptance
Simultaneous Clarification and Capture	cation exchange Expanded Bed Adsorption (EBA)	low cost media reduces unit operations	low throughput, feedstock preconditioning sensitive to feed variations and fouling, challenge for sanitization
Purification:			
Chromatography	ion exchange, HIC, IMAC, ceramic hydroxyapatite	variety of selectivities, high capacity, robust	often flow rate limited
Adsorptive Membrane	charged membranes	high throughput, contained, suited to trace contaminant removal	low capacities

Table 11-2: Typical contaminant clearance values from each chromatography stage

Contaminant	Capture load	Intermediate purification load	Polishing load
Host cell protein (ng/ml)	10^5	10^3	10
Endotoxin (EU/ml)	10^6	10	<1
DNA (pg/ml)	10^6	10^3	10^2

Downstream Process Unit Operations

Process filtration

Filtration is used at several stages in the downstream processing of the bioreactor harvest, as well as for the preparation of purified water and other processing fluids (buffers, sanitizing agents, etc.). Several filtration steps are integral to the Capture, Intermediate purification, and Polishing stages; these types of filtration fall into one of three general types:

- **Microfiltration** can be used at the start of the downstream process to clarify the feed beyond what was accomplished in the upstream harvest and centrifugation/clarification.
- **Ultrafiltration** is used between chromatography steps to concentrate the product and change the buffer conditions to prepare it for subsequent chromatography steps.
- **Sterilizing grade direct flow filtration**, oftentimes involving the use of *nanofiltration* cartridges, eliminates microbial organisms and insoluble proteins, removes adventitious and endogenous viruses, and sterile filters the product in preparation for final formulation.

Flow-through devices, assembled with the above membrane media, are formatted to affect two general flow types:

- **Direct Flow Filtration** devices allow the process fluid to cross the membrane in essentially a perpendicular flow direction; this provides little or no prevention of particulate build-up or the concentration of other elements that do not fit through the pore structure.
- **Tangential Flow Filtration** devices orient the membrane so that process flow sweeps across the active filtration surface, which minimizes pore plugging and surface fouling by concentrated reject elements of the feed.

Direct Flow Filtration (DFF)

A flat membrane disk is the simplest arrangement of the filter. However, as membrane surface area requirements increase, it is more common for that membrane to be folded into a pleated sheet either with or without a secondary supporting layer. The combined filtration layer is then

wrapped around a perforated collecting core. An advanced version of this type fits an even greater membrane area into the same cartridge volume by folding the pleats over (all in the same direction), allowing a greater length of membrane along each fold.

In the typical pleated membrane DFF cartridge, flow is directed from the inlet (through the media in a perpendicular or direct path across the membrane) to the outer surface of the media. The flow then collects in the core and exits the holder to the device's downstream port. Devices come in a range of membrane areas, cartridge/holder volumes, and materials of construction (Figure 11-2). At the process scale, most are sized as multiples of 10" cartridges, which are typically stacked end-to-end and combined into groups or rounds within a large steep housing (Figure 11-3).

Figure 11-2. Pall Life Sciences Ultipor® Direct Flow Filtration (DFF) cartridges of various scales (top) and typical automated process DFF system fitted with a multi-round assembly of cartridges in a single housing—partially lifted from its base to expose the cartridges (bottom)—courtesy Pall Life Sciences

The most common applications for membranes housed in the DFF format are the microfiltration applications of particulate removal, sterile filtration, and virus removal/clearance filtration. Virus clearance filtration is discussed in greater detail later in this chapter. Membrane pore size ratings are in the 0.1 µm to 10.0 µm range for particle and microbial removal and in the sub 0.1 µm pore size for virus filtration. The current standard pore size for sterilizing grade filters is 0.1 µm or 0.22 µm depending on the supplier. Specialized "nanofilters" are also marketed for filtration-based removal for certain virus classes.

Tangential Flow Filtration (TFF)

TFF is a rapid and efficient method for separating and purifying process flow. It can be used to recovery and purify solutions from small volumes (10 mL) up to thousands of liters. With TFF the feed flows tangentially over the surface of the membrane, where a portion flows through the membrane as permeate. Key operating variables include:

- transmembrane pressure (TMP)
- feed cross flow velocity (Δ P)
- increased turbulence (enhances mass transfer)
- process flux
- temperature
- volume concentration factor
- number of diafiltration wash volumes

Transmembrane pressure and cross flow velocity are important variables. The transmembrane pressure drives fluid through the membrane, taking the permeable molecules with it. Cross flow velocity is the rate that the solution flows through a feed channel and across the membrane. Its force sweeps away any molecules which might foul the membrane and cause filtrate flow to be restricted (Figure 11-3).

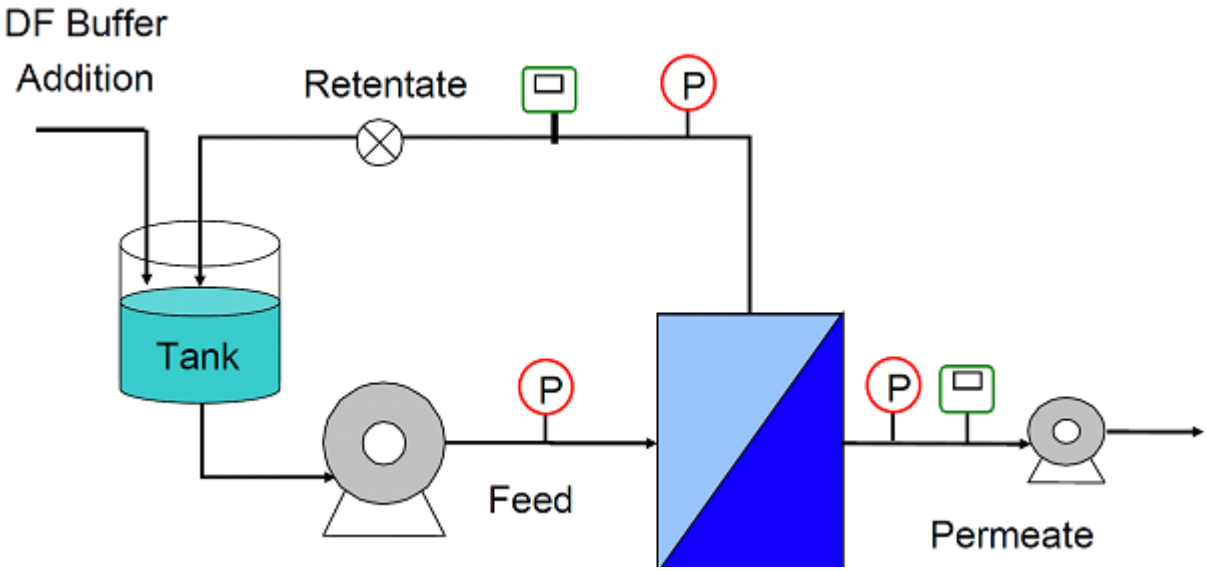


Figure 11-3. Basic schematic of a TFF unit operation

Chromatography (general)

Liquid chromatography is a purification method employing a packed resin bed, through which a solution containing a mixture of solutes is flowed; specific solutes are differentially bound or slowed as they contact the bed, while others pass through without interacting with the packed resin. The large majority of chromatography steps used in the purification of mAbs and other protein-based biotherapeutics are those in which some constituents bind or interact with a ligand of the stationary phase (packed resin) absolutely, while others pass through with no interaction. The bound components are then removed or eluted by the gradual or step-wise change of the composition of the mobile phase (such as through the use of buffers) run through the packed resin bed, such that bound feed constituents are ideally eluted separately from the product of interest. In bind-elute methods the product is what binds, while in flow-through methods the product passes through while other unwanted elements of the feed are temporarily bound. Figure 11-4 illustrates the separation of feed components in a bind-elute adsorptive chromatography method.

The functional or chromatographically-active groups of the stationary phase are:

- charged (as in ion exchange chromatography)
- of specific biochemical make-up with affinity for certain feed components (as in affinity chromatography)
- hydrophobic (hydrophobic interaction chromatography)
- some combination of the above (mixed mode)

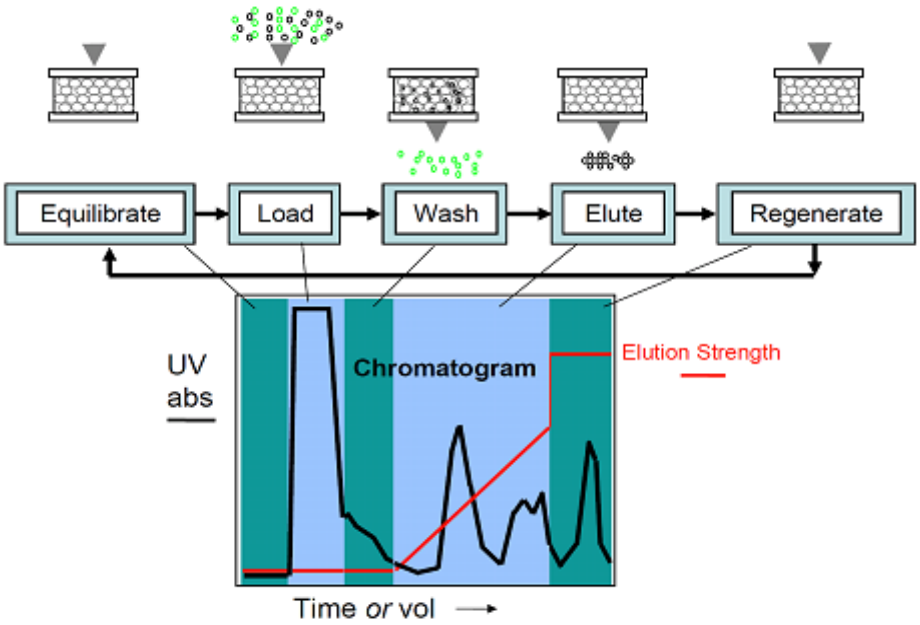


Figure 11-4. Chromatography cycle in bind-elute mode

The chromatographic media (resins) selected for various stages in the downstream process typically have the following properties in common:

- high particle porosity and internal pore surface area for high dynamic binding capacity of either the product of interest or contaminants and impurities
- relatively larger resin particle diameter as compared to analytical and other small scale resins so that resistance to flow is minimized
- ideally an average pore size to enable relatively less hindered diffusion of target molecules into the back, out of the pores and adsorptive surfaces; this is to maintain dynamic binding capacity at high levels without the need for slowing the flow rate.

All of these attributes are intended to maximize the volumetric and mass throughput of each chromatography step. Figure 11-5 lists the key characteristics of resin particles.

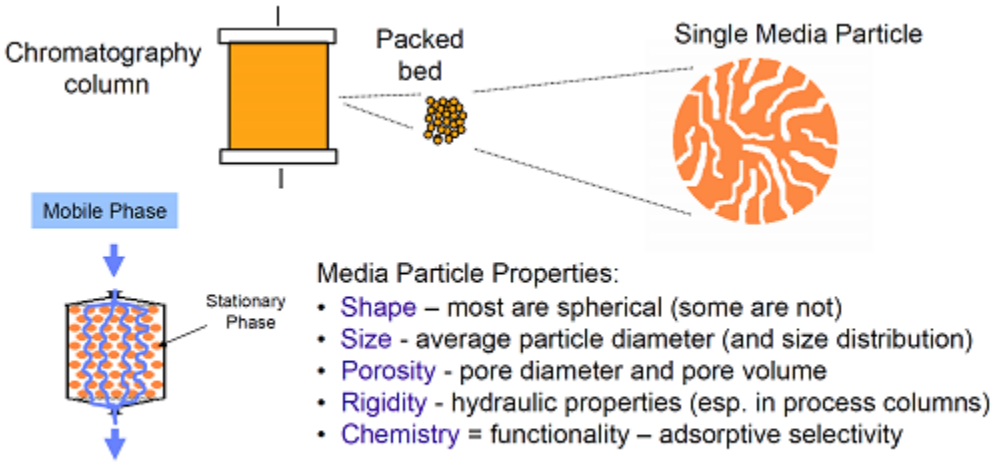


Figure 11-5. Key characteristics of the packed bed resin particles

Multiple chromatographic steps are typically combined in a single downstream process in order to exploit differing properties of the product of interest or contaminants; this allows for selectivity and purifying power for each step. Intermediate processing steps, performed between chromatographic steps, are integrated with the chromatographic steps to prepare the feed for subsequent steps, such as changes to the constituent concentrations. Furthermore, the solution ionic strength or pH can be altered to best utilize the selectivity of the chromatographic steps.

Typically, process development of the downstream processing/purification sequence has been completed at the laboratory/bench scale prior to the need for quantitative production. Oftentimes, however, product stability and purity improvements continue as the process is scaled through the pilot stage to the full production scale.

Chromatography scale-up principles

Oftentimes the scale of each of the upstream and downstream steps increases no more than 10–20 times that of the prior scale. For example, the pilot scale purification batches would be produced in bioreactors ranging in a volume of 200–1000 liters and columns in the 10–25cm diameter size (< 2–10 liter bed volumes). The full production scale-up resulting from those batches will often include 10 thousand or 20 thousand liter bioreactors, resulting in proportionately larger harvest volumes.

Chromatography unit operations are linearly scaled by keeping the bed height (L) constant and increasing the column diameter, resulting in a scaling factor (*n*) equal to the increase in column cross-sectional area (A). Linear velocity, defined as the volumetric flow rate divided by the column cross sectional area (Q/A) remains constant at all scales. The times for each step in the cycle of equilibration-load-wash-elution and regeneration also remain constant. All other key parameters, including load capacity, increase by *n*. The scaling factor is applied as summarized in Figure 11-6.

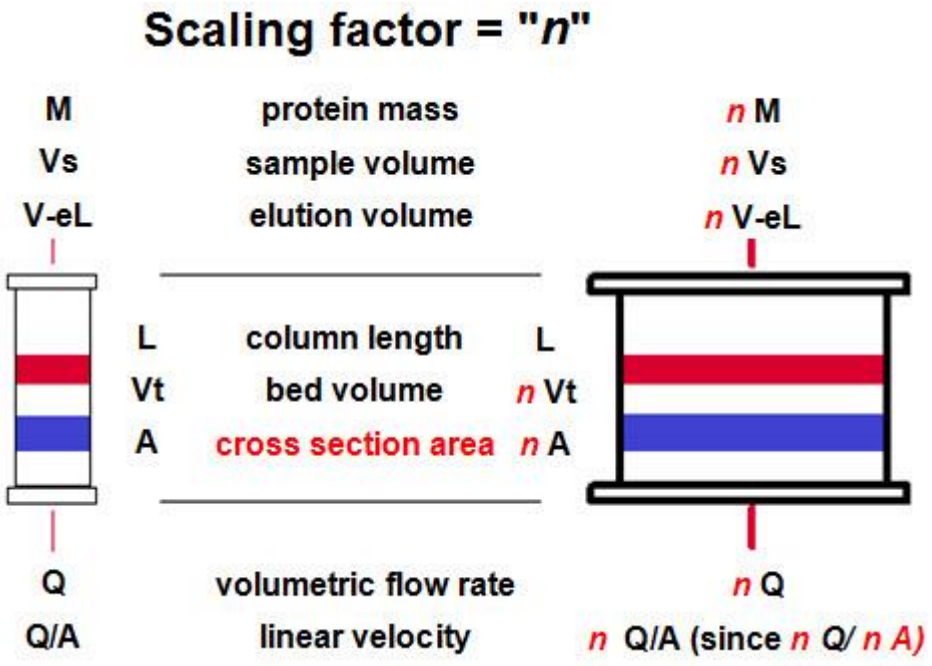


Figure 11-6: Linear scale-up of a chromatographic unit operation

It is important to note that the first column step (the protein A affinity step) would not necessarily need to increase by the same factor as the clarified bioreactor harvest or feed volume. It is often more economical to split the feed and run several cycles on smaller columns then pool the eluted/recovered product for the subsequent processing step, reducing the scale of the capture step. With protein A resins averaging $10,000+/liter versus ~$1000/liter for ion exchange or other functional classes, a smaller protein A capture column saves on resin costs, reduces the column and system sizes and costs, and cuts down on space requirements in the recovery suite.

The example scale-up in Table 11-3 shows how this can be achieved for a protein A capture step.

Table 11-3: Use of a smaller capture column by adding cycles enabled by more rapid process velocity (from Kemp and O'Neil [2003])

Parameter	Case 1	Case 2
sample volume (litres)	10,000	10,000
sample concentration (g/l)	1	1
loading flow velocity (cm/hr)	500	750
elution regeneration flow (cm/hr)	500	1000
column diameter (cm)	80	60
bed height (cm)	20	25
column volume (litres)	100	71
number of cycles	5	6
time (hours)	7.8	7.7

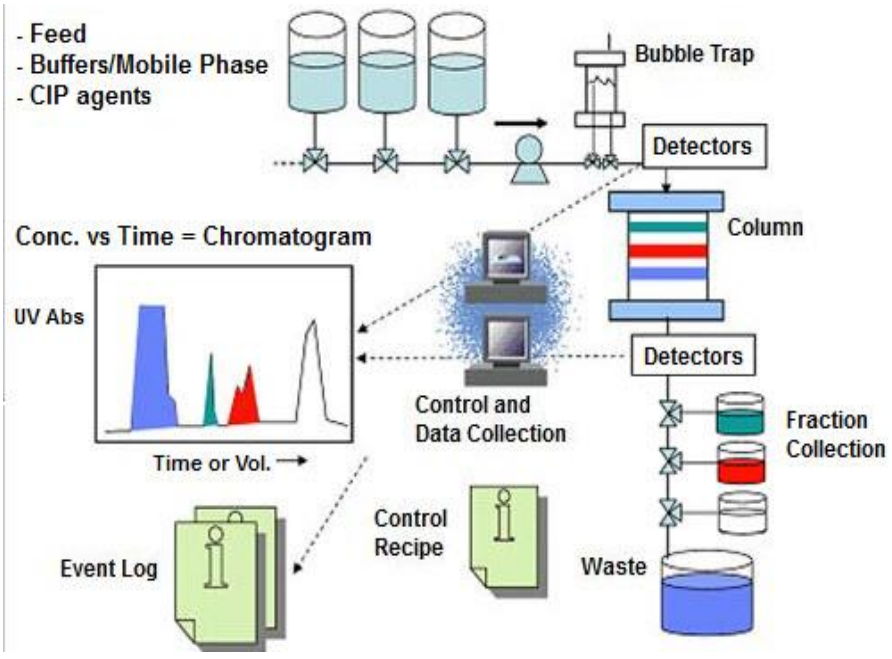


Figure 11-7. Components of a liquid chromatography unit operation

Pilot and production-scale chromatography systems are often constructed with stainless steel piping trains to maximize durability and cleanability. Two mobile phase pumps enable a wide selection of process fluids (equilibration, wash, sample feed, elution, regeneration, and sanitization solutions) and/or proportional binary mixing to adjust conditions of conductivity and/or pH in continuous gradients for differential elution of bound feed components.

A series of pre- and post-column sensors (flow, conductivity, pH, flow rate, pressure, etc.) are linked to a microprocessor-based controller. This provides monitoring and control of the unit operation, often using dynamic feedback of the flow, conductivity, and/or pH parameters. Pre-column sensors are often used to protect the column, while post-column sensors are focused on recording the outcome of the fractionation process (e.g., by optical density over time or eluted volume). A series of automated valves direct the active flow pathway, each often equipped with a proximity sensor to record their position; in combination with pump and mixing control, these can ensure properly-executed recipes for process cycling.

As with all biomanufacturing equipment, the flow path and product-contacting surfaces must be constructed of FDA-conforming materials, and all areas must be easily cleaned between cycles. The overall pathways must be free of dead legs (improper flow) or poorly flushed segments; this is handled using extremely low hold-up sanitary valve blocks, allowing multiple flow directions that eliminate or reduce trapped liquid at intersections. A representative process drawing is shown in Figure 11-8. A photo of a commercial unit is shown in Figures 11-9.

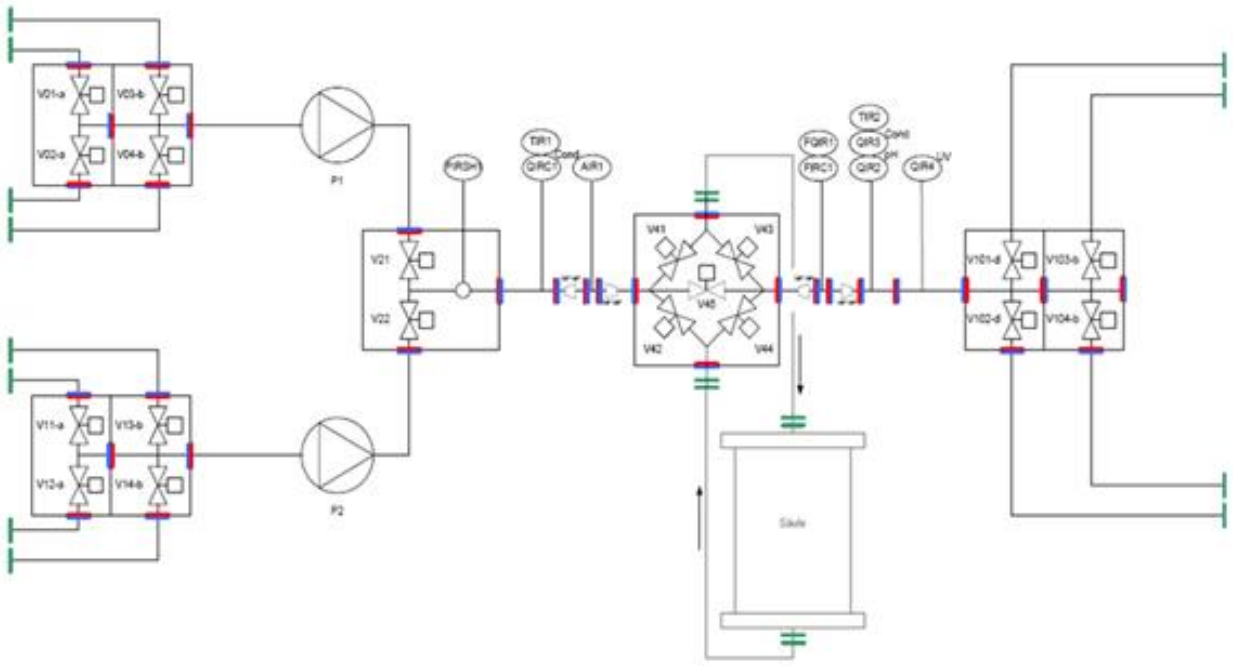


Figure 11-8. Piping & Instrumentation Diagram (P&ID)
of a process chromatography system (courtesy Pall Life Sciences)

Figure 11-9. Example #1 of modern automated chromatography system (courtesy Pall Life Sciences)

Viral clearance by filtration and inactivation

The FDA-provided guidance on the effective virus reduction and inactivation states that:

> "Confidence that the infectious virus is absent from the final product will in many instances not be derived solely from direct testing for their presence, but also from demonstration that the purification regimen is capable of removing and/or inactivating the viruses."
>
> (Source: FDA/ICH Harmonized Tripartite Guideline, "Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin," 1997.)

It is common for filter manufacturers to establish performance data for filtration membranes and devices by use of model agents as surrogates for other actual potential viral agents of similar characteristics (e.g., DNA- or RNA-containing, enveloped/non-enveloped). For example, bacteriophage PR772 (Enterobacteria phage), a 63–82 nm icosahedral (essentially spherical), non-enveloped, non-aggregating phage, is used as a model for HCV, HIV, HTLV. The bacteriophage PP7 (Pseudomonas phage), a 25 nm icosahedral, non-enveloped, non-aggregating virus, is used to model parvoviruses (B19, PPV) and other small viruses (e.g., HAV, poliovirus, and MMV [MVM]).

For each processing step, the effectiveness of removal or inactivation must be validated by the biomanufacturer using virus challenge or spiking studies, in which representative and/or model viruses are added at known high titers then tracked through each step of the process typically via infectivity assay. Along with specific and intentional virus removal or inactivation steps, the viral clearance or titer reduction values are determined for most all steps in the process. This uses the similar challenge of spiking tests whether or not those steps are present in the downstream processing train for purification purposes other than virus removal (e.g., capture chromatography, TFF, buffer exchange, intermediate and polishing chromatography steps, etc.).

In Table 11-4 some typical clearance levels are listed for several model viruses across the major steps in an example monoclonal antibody process. For this process, a worst case exposure was assumed of 8 logs of the virus, represented by Xenotropic Murine Leukemia Virus (XMuLV), so the assurance for its removal (if present at the start of the process) was nearly 11 logs titer reduction, or considerably more than required to completely remove it from the process stream.

Table 11-4. Typical downstream virus clearance results from spiking validation trials (* XMuLv = murine leukemia v.; PRV = pseudorabies v.; MMV = minute murine v.)

Process Step	Model Virus* / titer reduction (TR) at each step:		
	XMuLV	PRV	MMV
Affinity Chromatography	2.82 $\pm$ 0.14	-	1.33 $\pm$ 0.54
Low pH Elution/Hold	$\geq$ 5.02 $\pm$ 0.31	$\geq$ 4.73 $\pm$ 0.25	-
Chromatography 2	3.29 $\pm$ 0.28	-	2.14 $\pm$ 0.24
Membrane Filtration	$\geq$ 5.22 $\pm$ 0.37	$\geq$ 5.14 $\pm$ 0.36	1.41 $\pm$ 0.61
Chromatography 3	2.48 $\pm$ 0.55	$\geq$ 5.49 $\pm$ 0.43	-
Total Log Reduction	$\geq$18.83	$\geq$ 15.36	4.88

For mAb processes, a very effective viral inactivation step (for enveloped viruses in particular) is conveniently a low pH exposure hold at pH 2.5–4.0, the elution buffer of the protein A affinity capture column. This, in combination with a dedicated virus filtration step, provides the orthogonal approaches accepted by regulatory entities as assuring virus clearance.

In plasma fractionation, as well as in some non-antibody recombinant proteins/products, an accepted approach has been the disruption of enveloped viruses by addition of small amounts

of a solvent and detergent mixture, namely 1% tri-n-butyl-phosphate (TNBP) as the solvent, combined with a non-ionic detergent such as 1% Triton-X or Tween. This solvent/detergent (S/D) treatment, originated at the New York Blood Center in the 1980s, is the most widespread method of viral inactivation in plasma fractionation processes and is used in the production of some recombinant proteins.

Further validation/qualification of filtration media and chromatography sorbents

Typically, before process development is completed it is likely that the membrane and chromatography media have been evaluated and selected for use in the process. By this time a membrane or sorbent supplier will routinely be asked to comment on and often participate in studies (with the drug producer) demonstrating compatibility of the media with the drug. This is to show that there is negligible loss of product by irreversible binding to the membrane or retention by the cassette/other retaining device.

For a membrane filtration device, it is common to run sterilizing grade filter compatibility studies by spiking a sample of the drug product at various stages in the process with bacterial or bacterial spores, then showing microbial clearance as well as the maintenance of the drugs chemical stability or even its biological activity.

For some chromatography media there may be a known degree of leaching of the ligand into the process stream. The ligand in this case is covalently attached to the resin and has an affinity for the biotherapeutic. In these cases there must be validation of the chemical nature/form and a specific quantitative method for detecting the ligand in the process stream. Proof must be provided that it is removed from the drug substance downstream from that step in the process train. This is exemplified by studies that show that for a mAb, any free protein A ligand leaching from the capture sorbent upstream is cleared. This is because the cation exchange step is conducted in buffer conditions that disrupt the antibody-to-protein A association. The free protein A is then passed through as the antibody binds in the bind/elute cation exchange step.

Typically, validation of contaminant removal is accomplished by spiking a known and excessive quantity of the contaminant into the train and tracking it downstream to prove the ability of those steps to remove it. This assures that the much lower concentrations (typically logs 10 lower) that occur through leaching into the process stream are removed. Thus it will always be of no consequence when considering final drug purity and safety. Table 11-5 is an example summary of the overall reduction of levels of various contaminants and impurities (isoforms) from a therapeutic mAb product.

Table 11-5. Common process constituents and methods of removal or purification (UF = ultrafiltration MF = microfiltration) (adapted from Kemp and O'Neil [2003])

Component	Culture harvest Level	Final Product Level	Conventional Method
Therapeutic Antibody	0.1-1.5 g/l	1-10 g/l	UF/Chromatography
Isoforms	various	monomer	Chromatography
Serum and host proteins	0.1-3.0 g/l	< 0.1-10 mg/l	Chromatography
Cell debris and colloids	10^6/ml	none	MF
Bacterial pathogens	various	$<10^{-6}$/dose	MF
Virus pathogens	various	$<10^{-6}$/dose (12 LRV)	Virus filtration
DNA	1 mg/l	10 ng/dose	Chromatography
Endotoxins	various	<0.25 EU/ml	Chromatography
Lipids, surfactants	0-1 g/l	<0.1-10 mg/l	Chromatography
Buffer	growth media	stability media	UF
Extractables/leachables	various	<0.1-10 mg/l	UF/ Chromatography
Purification reagents	various	<0.1-10mg/l	UF

Unit Operation Preparations

Column packing and packed bed qualification

The chromatographic unit must operate consistently and invariably through scale-up and ongoing production. It is critical that the packed chromatography column possess reliable, repeatable chromatographic performance and long term bed stability to enable multiple re-uses. Therefore, methods for initially forming the packed bed must be repeatable. With advances in hardware design, column packing has become increasingly mechanized by use of pumped delivery of resin slurry (also referred to as Pack-in-Place). Furthermore, hydraulics or other drive mechanisms are increasingly being used for incorporating movement of the column adjuster (top) end cell to the top surface of the packed bed. These steps can be accomplished

with automated packing systems. This will eventually be accompanied by electronic recording of the packing parameters, including:

- resin delivery velocity
- the packing pressure pattern
- the rate and force with which the adjuster is moved to its final position

The key parameters to control reproducibly are:

- rate of resin consolidation in the column
- pattern of pressure as the bed forms
- final compression factor (the ratio of gravity-settled versus packed bed volume)

All of the above are chosen as a function of both the resin compressibility and a group of other physical behaviors that affect the pressure drop relative to flow velocity across the forming bed. In addition, for some resins having extremely high surface charge density or extremely hydrophobic functional groups, the conditions of ionic strength and pH of the packing buffer can help to consolidate resin particles (to overcome surface-to-surface repulsive forces).

The final packing velocity and pressure drop across the formed bed should approximate the critical or maximum pressure and velocity for that resin in the bed geometry being packed, figures 11-10 and 11-11. Once reaching these conditions, as with any column packing event, the challenge is to re-position the column adjustable end cell down on the formed bed, with the least amount of recoil (relaxation) and/or disruption of the bed top surface. This is most easily and reproducibly achieved with the use of hydraulically-assisted adjuster movement as part of the packing procedure.

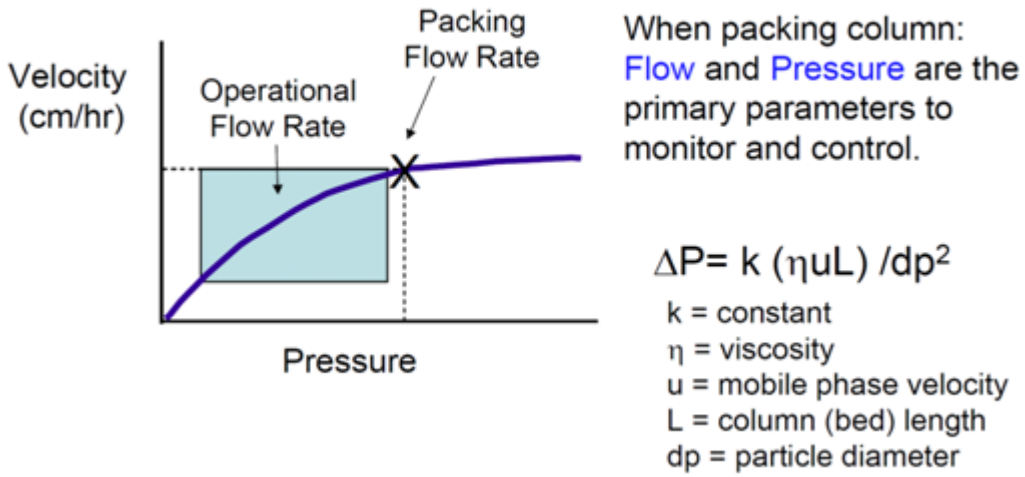


Figure 11-10. Packing and Operational flow rates and parameters that impact pressure versus flow

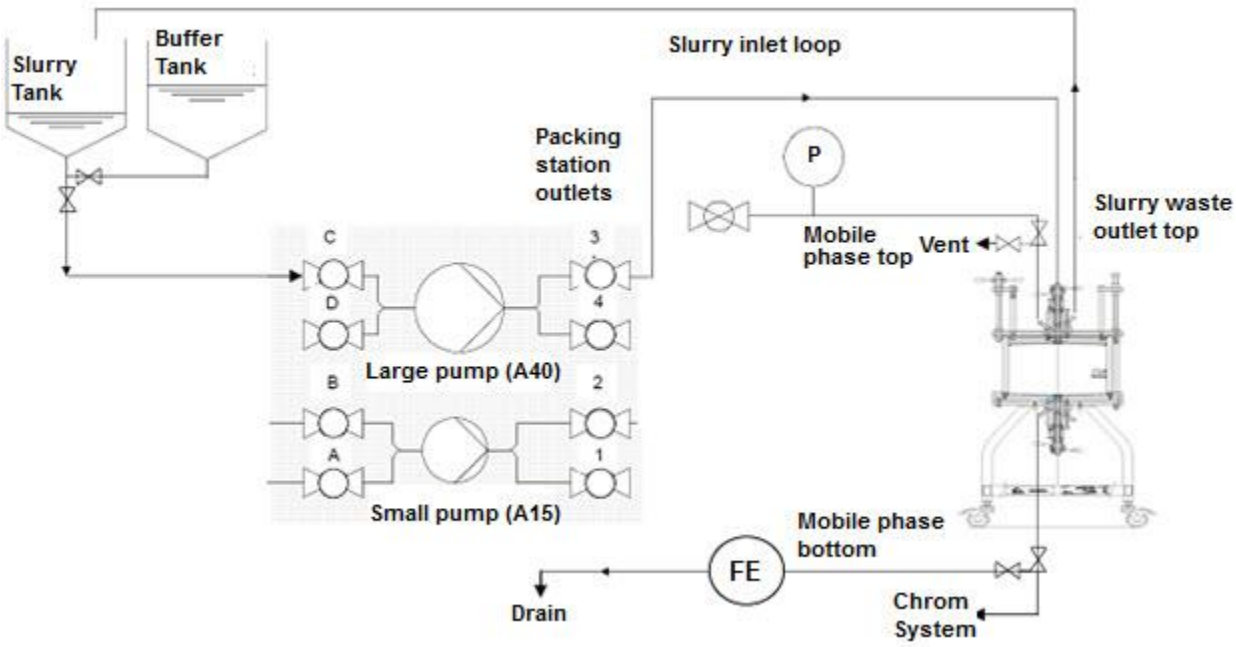
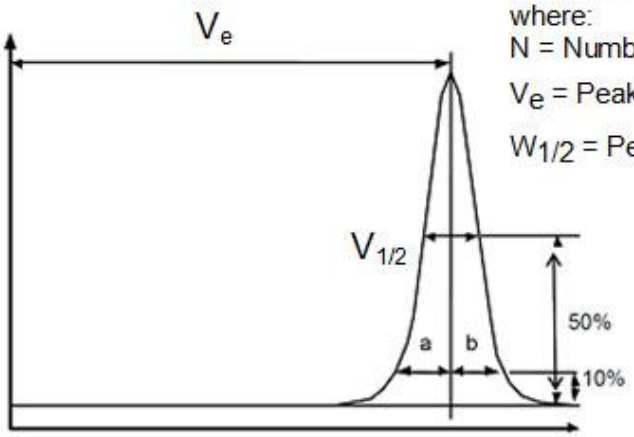


Figure 11-11. Diagram of pack-in-place pumped slurry packing of a column (courtesy Pall Life Sciences)

Testing of the newly packed column to qualify it as fit-for-use is most commonly accomplished by injecting a detectable probe and measuring the degree to which it becomes dispersed as it passes through the packed bed. A concentrated salt pulse with post-column conductivity monitoring is the most common means of accomplishing this (Figure 11-12).



$$N = (V_e/W_{1/2})^2 \times 5.54$$

where:
N = Number of theoretical plates active in the column
V_e = Peak elution volume or mm of chart
$W_{1/2}$ = Peak width at 1/2 height in volume on mm of chart

$$HETP = L/N$$

where:
HETP = Height of a theoretical plate
(units of L used for bed height)
N = Number of theoretical plates
L = Bed height (cm or mm)

$$A_f = b/a$$

where:
A_f = Peak asymmetry (at 10% peak height)
b, a = see figure above

$$h = HETP/dp$$

where:
h = Reduced Plate Height
HETP = Height of a theoretical plate
(units of L used for bed height)
Dp = Particle diameter

Figure 11-12. Qualifying the packed bed efficiency and integrity/fitness for use in production

Once processing begins, it is conventional to periodically analyze recorded conductivity transitions (frontal analysis) as well as protein elution profiles within a process cycle's chromatogram; this is performed in order to identify changes that can correlate with possible loss of bed integrity. Without such in-process analysis, it can be difficult to predict packed bed lifetime before experiencing a rapid loss of separation performance and unacceptable product yield decline (Figure 11-13).

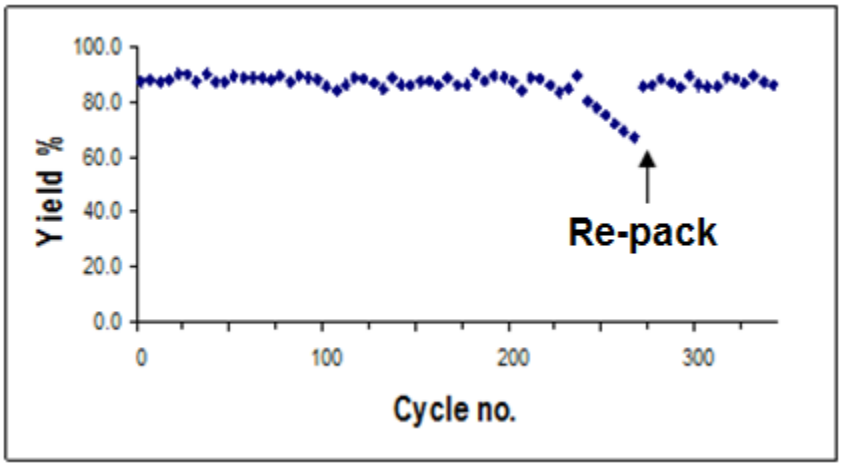


Figure 11-13. Loss of column performance showing decay and dictating a need to re-pack the column

Chromatography resins possess a wide range of physical and chemical characteristics, resulting in:

- differing degrees of compressibility
- porosity in packed form
- sedimentation densities
- susceptibility to shear forces
- tendency to arrange in unstable particle-to-particle bridging arrangements (especially rigid granular particles)
- elasticity

Some resins can be derivatized with highly reactive surface chemistries to such extents that they can affect the sorbent's tendency to swell and shrink in different buffers or solvents; they can even exhibit particle-to-particle or surface-to-surface physical forces solely as a result of that chemistry. As a result, the properties of any one sorbent may vary in different bed geometries and liquid environments; all but the most absolutely rigid particle sorbents show their own characteristic changes in shape and bed porosity under varying hydrodynamic forces of velocity and viscosity.

To better address these varying characteristics, there has been gradual progress toward more mechanization and, in recent years, toward the automation of the column packing and column qualification events. The ultimate goal is integrating the handling/mixing of the particle slurry and delivery to the column; flow conditioning of the consolidating bed with packing mobile phase; and pulse injection (HETP and peak asymmetry) testing, all directed and performed by automated systems (Figure 11-14). While not yet a part of the unit operation batch recording, this too is likely only a short time from being realized.

Figure 11-14. Integrated system for packing and qualifying a chromatography column of 80–100 cm diameter—approx. 50–150 liter scale (courtesy Pall Life Sciences)

Process Economics and Optimization

For a typical scale mAb downstream processing train, the initial investment in equipment and consumable filtration membrane/chromatography resins can cost several million dollars (Table 11-6). Even at moderate scales of production—grams to a few kilograms per year, as for human clinical trial material—the downstream process consumes an enormous volume of liquid, most of it in the form of costly sterile Water for Injection (WFI). The average cost of WFI can be approximately two–three dollars per liter; and with its formulation into processing buffers, that cost can increase to as much as seven–eight dollars per liter. The total cost of processing liquids consumed can become quite significant.

Added to the cost of capital equipment and consumables are significant staffing costs (salaries and fringe benefits), real estate or construction fit-out, and ongoing facility operating costs (utilities, transportation, maintenance, etc.). While rent/utilities and some personnel costs can become fixed for certain size facilities, some costs can vary greatly. These are tied to the cost of producing a particular drug product and can include personnel training and ongoing labor/overhead dedicated solely to its production.

Table 11-6. Typical costs for clinical manufacturing filtration/purification equipment and media

Step	Unit Operation	Major Capital Equipment Expense	Consumables Expense per year
1	**Depth filtration** single-use depth filters in 2-stage series	2 Holder/chassis: $30K manual/auto system: $300K **Subtotal hardware: $320K**	primary and secondary stages each 10 m^2 @ $1,500/$m^2$ = $15K per harvest x 10 harvests / y **Subtotal consumable: $150K**
2	**Sterile filtration** direct flow cartridge filters	in-line with chromatography system	integral single-use tubing and sterilizing grade 0.2 um membrane cartridges **Subtotal consumable: $15K**
3	**Affinity capture chromatography** protein A 150 L bed volume used for 20 cycles	column: 1.0 meter dia.: $180K manual/auto system: $550K **Subtotal hardware: $730K**	150 L resin @ ~$10K/L replace 1x per 2y = $750K / y **Subtotal consumable: $750K**
4	**TFF-1diafiltration** membrane cassettes: 10–15 m2 membrane surface area	manual/auto system: = $400K **Subtotal hardware: $400K**	$45K per change-out x2 change-outs per y = $90K / y **Subtotal consumable: $90K**
5	**Sterile filtration** direct flow cartridge filters	in-line with chromatography system	integral single-use tubing and sterilizing grade 0.2 um membrane cartridges **Subtotal consumable: $15K**

Table 11-6. Continued

6	**Cation-exchange chromatography** S-CEX 60 L bed volume used for at least 20 cycles	column: 80 cm dia.: $120K manual/auto system: = $400K **Subtotal hardware: $400K**	60 L resin @ ~$1k / L used 20x so replace 1x per 2y = $30K / y **Subtotal consumable: $30K**
7	**TFF-2 concentration + diafiltration** membrane cassettes: 10–15 m2 membrane surface area	manual/auto system: = $400K **Subtotal hardware: $400K**	$45K per change-out x2 change-outs per y = $90K / y **Subtotal consumable: $90K**
8	**Sterile filtration** direct flow cartridge filters	in-line with chromatography system	integral single-use tubing and sterilizing grade 0.2 um membrane cartridges **Subtotal consumable: $15K**
9	**Anion-exchange chromatography** Q-AEX 60 L bed volume used for at least 20 cycles	column: 80 cm dia.: $120K system: use cation-exchange system when idle **Subtotal hardware: $120K**	60 L resin @ ~$1k / L used at least 20x so replace 1x per 2y = $30K / y **Subtotal consumable: $30K**
10	**TFF-3 concentration + diafiltration** final concentration: 5 m2 membrane surface area	system: use TFF-1 system when idle	$35K if changed out 2x per y **Subtotal consumable: $35K**
11	**Sterile filtration** **direct flow cartridge filters**	peristaltic pump, semi-auto Subtotal hardware: $20K	integral single-use tubing and sterilizing grade 0.2 um membrane cartridges Subtotal consumable: $15K
Grand Totals/one year of production		**Hardware: $2.39 Mil *** * excluding tanks, hoses, and other ancillaries	**Consumable: $1.24 Mil **** ** excluding water for injection and buffers – buffer and water can double consumable expenses

Given the nature of the production operating expenses, it is apparent that the yield of purified drug must be maximized to maintain economic viability of a drug candidate as it progresses through the approval process and matures into a commercial product. Several sources of product loss and some of the common methods for addressing them are listed below, the methods helping to reduce the overall cost of goods produced. Common sources of product yield losses are:

- drug substance degradation from sources of shear and/or heat—encountered both within high speed pumps used to load product feed streams onto a chromatography column and during recirculation of the feed/retentate side of a Tangential Flow Filtration cassette
- liquid hold-up within the pipework and on the retentate of in-concentration diafiltration/ultrafiltration TFF cartridges; in the collection portions of normal flow microfiltration cartridges; or in eluted fractions from either flow-through or bind/elute chromatography steps
- inadequate flushing of pipework in systems for filtration or chromatography, as in the concentrate/retentate tank of an ultrafiltration unit operation

Following are some measures to prevent yield losses:

- in-line heat exchangers on board TFF systems, especially on feed/retentate recirculation loops
- tulip-shaped TFF retentate tanks
- shortened processing and hold times to minimize product degradation
- small volume flush/push and/or collection of target fractions via system drain port

Other means of optimizing process economics include:

- process compression/intensification: reduces the number of processing steps, as each contributes to recovery losses (Figure 11-15)
- shortened processing times (e.g., through increased productivity) to conserve product stability and reduce labor
- concentrate to reduce working volumes—save on tank volume and floor space, as well as processing time and labor
- reduce the potential for errors and employ automation where possible
- time steps to allow completion within single shifts and minimize disruptions that can result from errors on hand-over without continuity of personnel

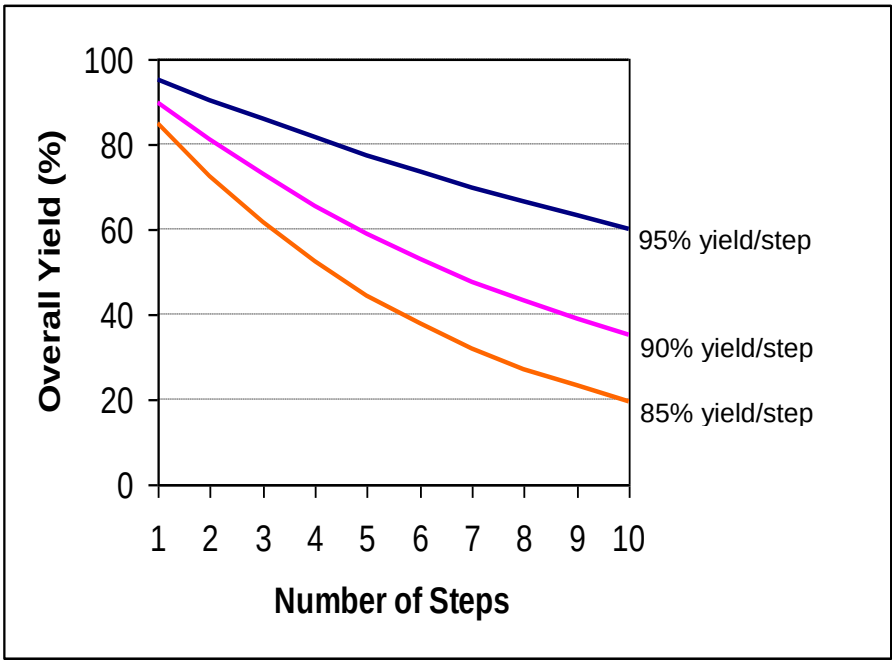


Figure 11-15. Overall yield as a function of cumulative step yields

Equipment Design Specification and Procurement

As the process is scaled up, it is the responsibility of an organization's Process Engineering personnel to establish the scale of the purification train and to detail each unit operation's requirements. A series of engineering documents are typically prepared to guide those responsible for procuring equipment and media. Among these planning documents, three of the most critical ones are described below.

General Process Description: a comprehensive overview of the downstream purification train, including (but not limited to) the number and class of each major system or media component and their specific throughput capacity requirements

Equipment Data Sheets: standardized summary sheets for each hardware component, with some required parameters defined for the vendor and others left blank for the vendor to provide, such as the specifics for usable flow rate of volume ranges, hardware weights, dimensions, etc., which all begin to clearly define equipment specifications

Vendor Documentation Requirements: a list of required design specifications, engineering calculations, general arrangement drawings, material certifications, project schedules, operator and maintenance manuals, test records, etc., that the chosen vendor must provide as elements of the scope of supply for sign-off by the purchasing party

Using the Process Description and Equipment Data Sheets, the requisitioning party invites equipment vendors to prepare commercial proposals or bids for supplying products that comply with each of the specified requirements. For example, given the extreme purity requirements of human therapeutic agents, all parties must prove that any materials supplied meet regulatory agency standards. These standards aim to prevent the introduction of unwanted leached or extractable material into the drug product.

For all but some single-use tubing sets and containment vessels, there are stringent requirements for demonstrating that all surfaces can be fully sanitized between uses and that all internal areas are adequately isolated from external sources of contaminants and carryover of product or contaminants from batch to batch.

Equipment Preventive Maintenance

Due to the cumulative costs associated with each step, the later that an API resides in the downstream train, the more valuable it becomes. Thus it is essential that equipment operates properly during processing. Equipment maintenance is an integral part of preventing or minimizing process interruptions. Any interruptions can dramatically increase costs and product loss due to thermal or chemical instability. An example is a long, unexpected hold that could result in product degradation.

Both equipment maintenance and operator/technician/maintenance training are critical to any drug production facility. In certain cases, such as for very high value drugs or those related to public health disaster management programs (e.g., pandemic flu), it is not uncommon for the production facility to acquire and maintain redundant hardware and systems trains to assure continuous process operations. In extreme cases this could mean a complete parallel train; oftentimes, however, the redundancy is limited to a packed, qualified chromatography column or ready-to-go set of membrane cassettes and holder assembly.

In order to avoid major delays or disruption, the installation of a process should always be accompanied by the establishment of local inventories and supply chains for all in-use materials and consumable elements of each unit operation. This includes the consumable media and processing fluids (membrane cartridges or cassettes, chromatographic sorbents, buffer salts, WFI, etc.), as well as the hardware assemblies and components critical to the proper functioning of those systems. Any routinely replaced components must be kept on hand at the facility or at local supplier warehouses. These include:

- column bed supports
- elastomer seals and gaskets
- filtration and chromatography system detectors/sensors
- valves
- pump internal mechanisms (diaphragms, rotors, etc.)

Some well-established commercial sites choose to purchase entire subassemblies for columns and systems as well as maintenance or service contracts. These agreements often specify a firm response time (e.g., 24–72 hours) for service personnel to be onsite.

Another common way of assuring minimal process down-times is for organizations to provide training programs for their internal facilities or maintenance department personnel. Adequate training at all levels for all involved personnel (e.g., internal staff, vendors, etc.) is vital given the high cost of processing downtimes or equipment issues.

Figure 11-16. Performing column preventive maintenance (without overhead hoist/crane) enabled by the column's integrated hydraulics (courtesy Pall Life Sciences)

Single-Use Disposable Systems

There is an increasing use of pre-sterilized bio-containers and dedicated tube/hose sets with in-line cartridge filters used in medium-scale production. Sterilization is typically achieved through Gamma irradiation. The most common reasons for selecting such processing trains is short set-up times and side-stepping the need for lengthy pre-sanitization or steaming of piping trains, filter housings, and other hardware.

Most every stage of a downstream purification train can be constructed from single-use components, with the exception of the packed bed chromatography column. Relatively large filtration and simplified chromatography systems are now designed to accept pre-fabricated disposable tubing sets; this is enabled by the emergence of automatable pinch valves, disposable pump heads, and a growing number of single-use sensors.

Smaller scale, short-term production sites (e.g., campaign-based) can handle multiple processes in the same production areas, although at different times. For example, the set-up of small scale contract manufacturer sites can be suited to easily change flow trains. While an initial concern for potentially higher outputs of solid waste could discourage this practice, many comprehensive environmental impact analyses have shown these systems to have less impact than those that rely upon the manufacture of large quantities of stainless steel/steel alloy-based flow systems.

Figure 11-17. Examples of single-use disposable bio-containers with integral hose/filter sets (courtesy Pall Life Sciences)

Key Trends in the Biomanufacturing Industry

Despite being a heavily regulated industry, and thus relatively conservative, there are constant innovations in the production of biopharmaceuticals. One primary reason is an increased pressure to reduce development and production costs and speed the introduction of this clinically-effective class of products to market. The trend is towards greater levels of productivity and higher levels of expression of the target compound upstream. This is to reduce the volume required for bioreactors and much of the hardware, plant space, and all consumables used upstream. In cases where this can increase stress on the cells, it could result in qualitative and quantitative changes in the profile of the contaminants. This, in turn, challenges the performance effectiveness of traditional downstream purification processes and methods.

These dynamics, along with continuous technology changes from an array of new materials and microprocessing capabilities, ensure that the study and practice of biomanufacturing methods will continue to rapidly evolve.

Check Your Knowledge

1. What step of downstream processing involves the elimination of trace contaminants and impurities? _____

2. Direct Flow Filtration is associated with which of these processing methods?

   a. clarification
   b. capture
   c. purification
   d. both A and C

3. Linear _____ is defined as the volumetric flow rate divided by the column cross-sectional area.

4. What is the most widespread method of viral inactivation in plasma fractionation processes?

   a. a solvent
   b. a detergent
   c. S/D treatment
   d. none of the above

5. For mAb processes, a very effective viral inactivation step (for enveloped viruses in particular) is to conveniently hold the solution to a low pH of:

   a. 1.25–1.5
   b. 2.5–4.0
   c. 4.5–6.0
   d. 7.0–9.0

6. Cell debris and colloids are removed during downstream processing using which of these methods?

   a. Ultrafiltration
   b. Chromatography
   c. Microfiltration
   d. Centrifugation

7. Frontal analysis is the periodic analysis of recorded _____ transitions.

8. Which of the following is a critical engineering document?

   a. General Process Description
   b. Equipment Data Sheets
   c. Vendor Documentation Requirements
   d. all of the above.

Activities

1. Research the processes of Direct Flow Filtration and Tangential Flow Filtration and write a two-page report on each. Describe configuration, use/application, benefits, drawbacks, etc.
2. Research bind-elute and flow-through methods of chromatography and create a PowerPoint presentation comparing and contrasting them. Present it to your class.