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Chromatography for the Bioprocessing Industry

Developed and Written by BPE Subject Matter Experts

For names of contributors refer to pages 7 and 8

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ABSTRACT: Liquid chromatography is a critical step in the purification of nearly all biopharmaceuticals. Since the manufacturing and purification of proteins is often the foundation of bioprocessing, an understanding of chromatography mechanisms and the various equipment involved in the process is a necessity to those involved in process-scale chromatography.

This paper will touch on those types of chromatography systems that are pertinent to industrial bioprocessing, focusing on Liquid Chromatography and its varied designs. It will discuss in some detail the chromatography column and how it functions. The discussion with guide the reader through various chromatography processes such as: Ion Exchange, Hydrophobic Interaction, Affinity, Mixed Mode, Gel Filtration, and a mention of other types.

Key Words: Chromatography, column, ion exchange, hydrophobic, affinity, mixed mode, gel filtration, bioprocessing, liquid chromatography, mobile phase, stationary phase.

A BPE Focus Group
List of Contributors at the End

1.0 PURPOSE AND SCOPE

The purpose of this document is to familiarize the reader with the concepts of process-scale chromatography and associated equipment. The scope of this document is liquid chromatography for biopharmaceutical production.

2.0 OVERVIEW

Chromatography is a common unit operation for purification of biopharmaceutical products. Chromatographic separations exploit differences in molecular properties between the desired product and impurities that may be present in the process solution. Multiple chromatography steps may be implemented and are often used in conjunction with other separation techniques to isolate desired products to achieve purity requirements.

3.0 CONCEPTS

Every chromatography operation consists of a *stationary phase* and a *mobile phase* which work together to separate components in the feed solution. As the name implies, the stationary phase is generally immobilized and does not flow during normal operations. It may be a continuous solid or semi-solid structure or a mass of fine solid or semi-solid particles. In the latter case, the particles are often packed into a *bed*. The mobile phase flows through and/or over the surfaces of the stationary phase. The mobile phases and their composition vary according to the chromatography method, steps of the chromatography process, and the physical/chemical properties of the product and impurities. The planned and controlled interactions between the constituents of the mobile phases and the stationary phase during the chromatography steps form the fundamentals of process-scale chromatography.

4.0 EQUIPMENT

Most chromatography operations include two primary pieces of equipment: (1) the *column* or *membrane adsorber* and (2) the *system*. The stationary phase is confined to the column or membrane adsorber. The mobile phase flows through the stationary phase. The motive force is usually provided by pumping equipment integral to the *system.* The system that handles liquids comprising the mobile phases is often referred to as a *liquid handling module* (LHM).

5.0 COLUMNS

5.1 Construction

A typical chromatography column (Figure 1 following page) is comprised of a cylindrical shell (the tube) closed at each end. The space between the ends of the column contains the stationary phase. The mobile phase enters and leaves the column through hygienic connections at each end of the column. This is the most traditional configuration.

Chromatography columns are always used when the stationary phase consists of fine particles packed into a bed, as described above. Bed supports at each end of the tube retain these particles within the column interior. Each bed support is a porous plate, usually a rigid, finely woven mesh or a porous material. Behind each bed support is a flow distributor. This device aids in the uniform distribution of the mobile phase through the bed. During operation, the mobile phase flows axially through the column. Therefore, at the downstream end of the column, the flow distributor actually collects the liquid to a common point. Each distributor is typically supported by a rigid plate, which includes the hygienic connections for interface with the system.

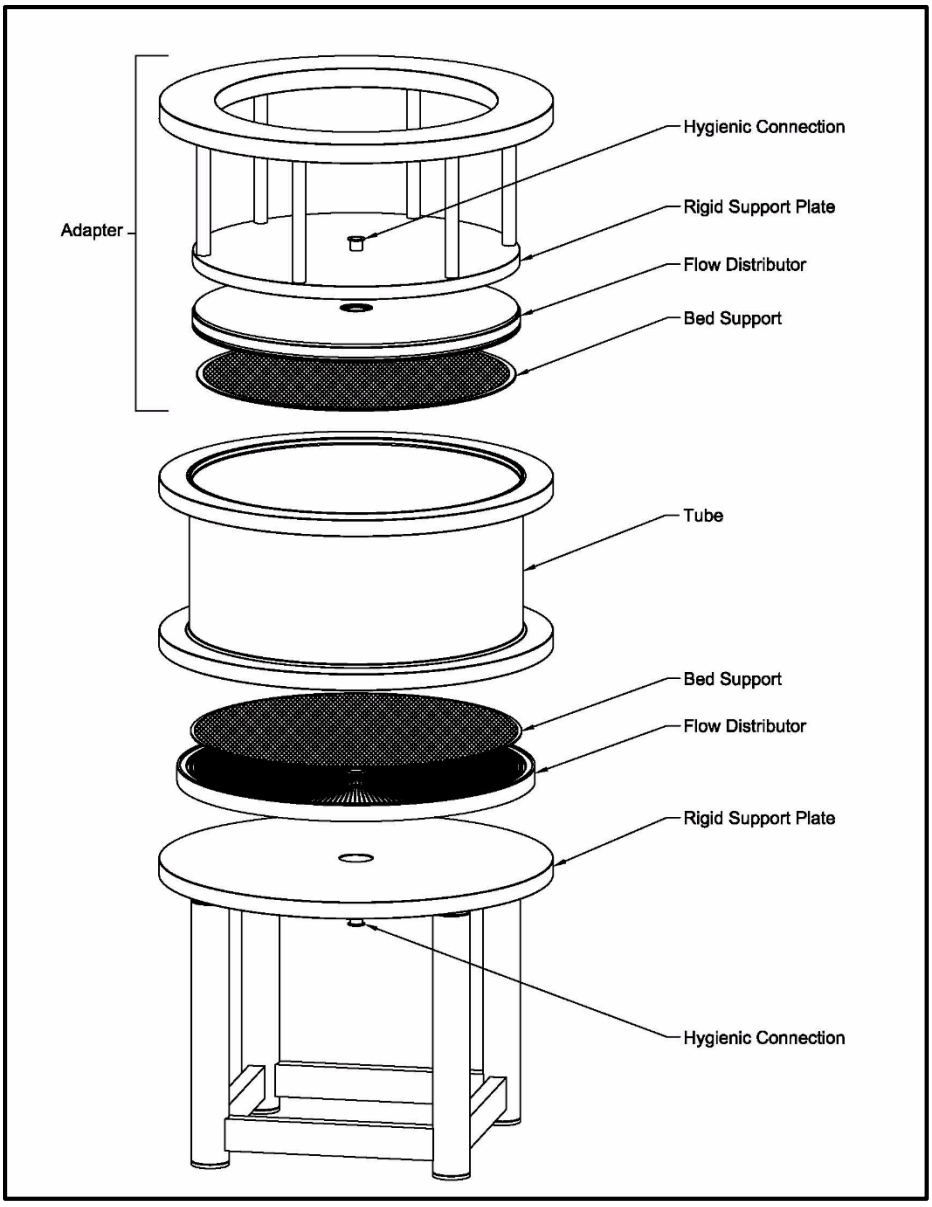

Figure 1 – Typical Chromatography Column

Usually, one end of the column is designed to be inserted into the column tube rather than sealing at the end (much like a syringe), allowing a column's internal volume to be varied. This assembly is referred to as the *adapter*, as it allows the column to adapt to different bed heights. A column is traditionally installed with its axis oriented vertically and the adapter positioned at the top. Therefore, the adapter is commonly called the "top" of the column. However, from a process perspective, there is no "top" or "bottom" of the column.

Chromatography columns may include additional mechanical features such as the ability to add and/or remove the stationary phase without opening the column (by removing the adapter in most cases) and the ability to mechanically compress the bed.

5.1.1 Operational and Cleaning considerations

Since the stationary phase is comprised of a packed bed of fine particles, its integrity requires that it be maintained in a wetted state. Air bubbles or other disturbances to the bed often result in discontinuities in the stationary phase that are adverse to the performance of the chromatographic separation.

Cleaning and sanitizing chromatographic columns differs from CIP operations for vessels and many other operations used in biopharmaceutical applications. Turbulent flow through the narrow inter- and intra-particle channels is practically impossible. Therefore, a packed column may only be cleaned in place by flowing, cleaning and sanitizing agents through the stationary phase, which are usually pumped through the same paths as the mobile phase. Exposure of all interior surfaces of the column and stationary phase to cleaning and sanitizing agents is ensured by the same flow distribution hardware that is used to deliver the mobile phase to the column. Cleaning protocols must be developed with these limitations in mind. Since it cannot be drained and dried, a packed column is commonly filled with a sanitizing agent (e.g. 20 percent ethanol or 0.1 M sodium hydroxide) to control bioburden when not in use.

5.1.2 Design considerations

Early column designs required that the top or adapter be removed so that a slurry of the stationary phase and buffer could be poured into the column. Most modern columns permit the stationary phase to be delivered to the interior of the column through a special nozzle which permits the slurry of particles and buffer to bypass one of the bed supports. Such a design reduces the probability of contaminating the interior of the column or the stationary phase.

Due to the large diameter of the column compared to the mobile phase connection at each end of the column, the flow profile will change from a turbulent to laminar regime as the mobile phase enters the column. Part of a column's performance often relies upon the quick and efficient distribution of the mobile phase before

reaching the stationary phase. A longer residence time of the mobile phase between the inlet nozzle and the stationary phase permits axial mixing that can reduce resolution of the separation. Minimizing the volume between each of the mobile phase nozzles and the bed supports helps to reduce this residence time.

The design considerations thus far have addressed axial flow only. However, columns may also be designed for radial flow of the mobile phase through the packed bed. In such a column, the mobile phase enters at one end of the column and is directed to the outer perimeter of the stationary phase. The mobile phase flows radially from the outer perimeter of the bed to the center where it is then directed out the other end of the column. Since most industrial chromatography columns use the axial flow design, the focus of this document is on axial flow columns and some of the considerations and descriptions may not apply to radial flow columns. Figures 2a and 2b illustrate the operational differences between axial flow (2a) and radial flow (2b) columns.

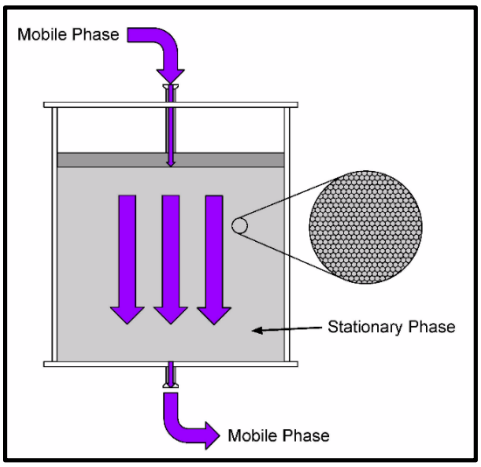

Figure 2a – Axial Flow Column

The column tube is both a wetted and pressure retaining component. Columns tubes are typically made from glass, stainless steel, or plastic and are most commonly packed by the biopharmaceutical manufacturer. Requirements for vessels operating under pressure are defined in ASME

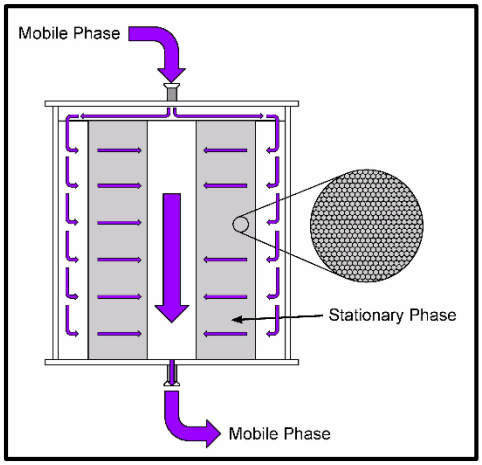

Figure 2b – Radial Flow Column

Bioprocessing Equipment standard (BPE), Part GR. UV stabilizers, if used in an acrylic tube, should comply with BPE Part PM. The manufacturer must be informed of the normal and abnormal operating conditions to which the column may be exposed. The manufacturer is responsible for ensuring the column will operate safely under said conditions.

A column's performance is also affected by how uniformly the mobile phase flows through the stationary phase. Ideally, the mobile phase should flow through the stationary phase as a plug of liquid with parallel profiles at the leading and trailing edges. Figures 3a and 3b illustrate desirable (3a) and undesirable (3b) flow profiles in an axial flow column.

5.2 Systems (Liquid Handling Modules)

The purpose of the chromatography system or liquid handling module (LHM) is to convey the mobile phase through the column and control the overall chromatographic process. A chromatography system is typically an assembly of pumps, valves, various instruments and devices, and a control system. The LHM systematically delivers the mobile phase to and from the column to isolate and purify a product of interest.

An example configuration for an LHM is presented in Figure 4 (following page).

5.2.1 Operational and Cleaning Considerations

The on-board pumps are sized to optimize the chromatography process. While it is possible to achieve turbulent flow within a chromatography system, sizing the on-board pumps to achieve such flow rates could limit capabilities for normal chromatography steps.

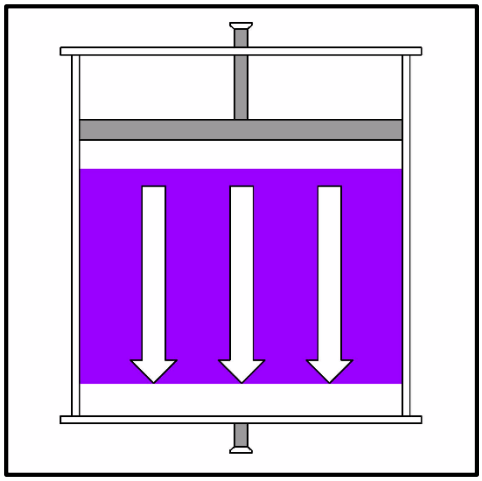

Figure 3a – Desirable Profile

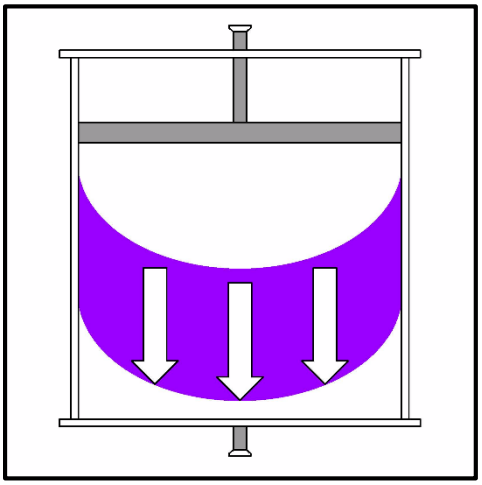

Figure 3b – Undesirable Profile

The chromatography system is typically used to deliver cleaning, sanitizing and conditioning agents to the column. Therefore, such systems

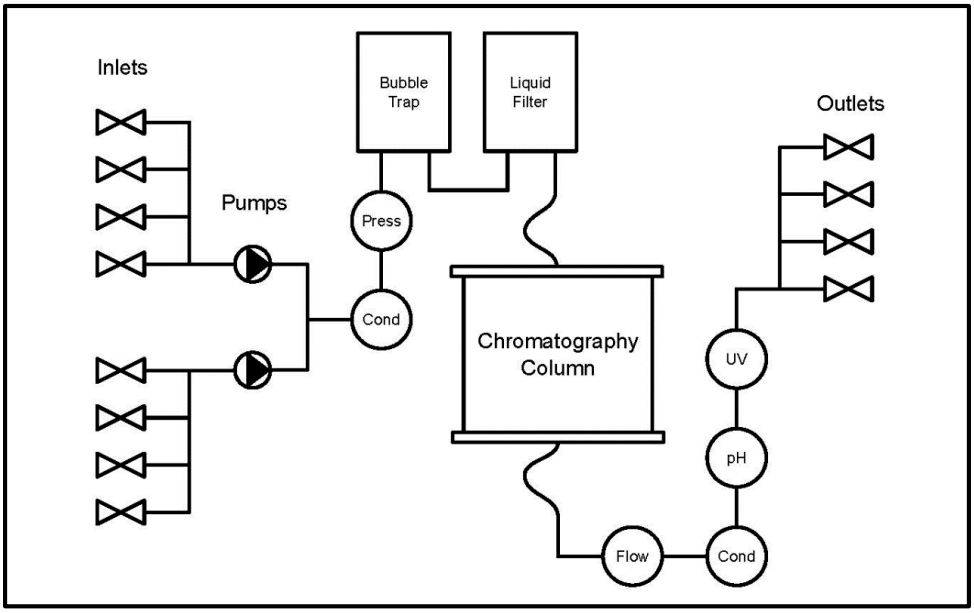

Figure 4 - Liquid Handling Module (LHM) Process Flow Diagram

usually rely on the same cleaning methodology applied to columns. The unique role of chromatography systems generally precludes the need for drainability. Because such a system is nearly always connected to a chromatography column, which cannot and should not be drained when the stationary phase is to be reused, there is little reason to maximize its own drainability.

5.3 Design Considerations

Generally, chromatography systems should be designed and built according to the BPE, Part SD. However, where drainability is concerned, chromatography performance should take priority. The system should be designed to isolate as much as possible the desired product from the other liquids that will be conveyed through the system, thereby maximizing purity.

6.0 OPERATING PRINCIPLES

6.1 Operating Pressures

Most chromatography processes are operated at relatively low pressures (typically < 10 bar), referred to as Low Pressure Liquid Chromatography (LPLC). The low operating pressures are due primarily to the relatively high permeability matrices (e.g., large particle size chromatography resins). With the high permeability matrices, high flow rates can be achieved with relatively low pressure drops, which is desirable if the intended separation can be achieved with this approach.

Occasionally, a separation that requires much higher resolution than can be achieved with a high permeability matrix is required. In this case, a smaller particle size is often needed, resulting in higher pressure drops through the chromatography column. In these less common cases where much smaller particles are required, high performance (a.k.a. high pressure) liquid chromatography (HPLC) or medium pressure liquid chromatography (MPLC) operations may be used (typically 10 to 30 bar).

6.2 Mobile Phase

The *mobile phase* is used to describe any liquid that is conveyed through a column during any chromatography step.

The vast majority of bioprocess chromatography separations are conducted using aqueous process solutions and buffers. Salt concentration and type (pH), and occasionally the concentration of other water-soluble compounds (such as urea, propylene glycol, non-ionic surfactants, etc.) are common solution property variables. Cleaning is frequently accomplished through the use of acidic or alkaline solutions, and the selection of cleaning agents is often dictated by resin compatibility.

6.3 Stationary Phase

The *stationary phase* (also "matrix", "Gel, or "Bed") refers to the medium by which the chromatographic separation is affected. In the case of a chromatography column this is usually a resin or gel that is specifically selected for the chromatography process. For membrane adsorbers, the stationary phase refers to the membrane itself. The stationary phase remains in place during all steps of the chromatography process.

For most bioprocess applications, a relatively hydrophilic matrix is used to ensure low non-specific adsorption and also to reduce the risk of denaturing the product. Examples of commonly used matrices include natural polymeric supports (e.g., cross-linked agarose), synthetic polymeric supports (e.g., methacrylate-based beads), or inorganic supports (e.g., controlled pore glass/silica).

Most bioprocess applications use relatively large particle size porous beads to ensure both good flow properties and high capacity. Since selectivity is generally accomplished through selective adsorption and desorption techniques based on careful process design work, it is less common that small particle size matrices are required for high resolution separations.

Recently, pre-packed chromatography products have been introduced so that chromatography columns can be purchased in a "ready to use" format.

Membrane adsorbers are typically supplied in a plastic cartridge that is ready to use with an inlet and outlet to allow connection to a chromatography system.

7.0 Common Types of Chromatography

Most chromatography modes used in bioprocess applications are adsorptive separations, including ion exchange, hydrophobic interaction and affinity chromatography. The following sections are a list of chromatography modes commonly used:

7.1 Ion Exchange Chromatography

In ion exchange chromatography, molecules are separated based upon their ionic charge—i.e. their attraction to a charged surface.

In anion exchange chromatography, a positively charged chromatography matrix is used to adsorb anionic compounds preferentially from the process solution. Cationic compounds do not bind to the matrix and flow through the column. The adsorbed anionic compounds are then eluted by increasing the ionic strength or changing the pH of the mobile phase. As an example, elution of a specific product can be achieved by increasing the salt concentration of the mobile phase so that species which are adsorbed less strongly are eluted first from the matrix.

Cation exchange chromatography operates by the same principle as anion exchange chromatography except that a negatively charged matrix is used.

7.2 Hydrophobic Interaction Chromatography (HIC)

Hydrophobic interaction chromatography is similar to ion exchange chromatography except that adsorption is based on preferential interaction of hydrophobic compounds in a high salt environment (equivalent to a "salting out" phenomenon). In this case, a chromatography matrix with a moderately hydrophobic surface is used to adsorb protein molecules from solutions containing high salt concentrations. Elution is accomplished by decreasing the salt concentration of the mobile phase.

7.3 Affinity Chromatography

In affinity chromatography, a ligand having a highly specific interaction with the target molecule (or a target impurity) is bound to particles comprising the chromatography matrix. This matrix (called an "affinity chromatography" matrix) is then used to "capture" the desired product from the solution. The highly selective nature of the interaction can enable affinity chromatography to deliver a highly purified product in a single step. Affinity chromatography can be used as a concentration step. An example of affinity chromatography is the use of a Protein A matrix to purify monoclonal antibodies.

7.4 Mixed Mode Chromatography

Another mode of chromatography involves so-called "mixed mode" media, which incorporates combinations of charged groups, hydrophobic groups and/or other functionalities to impart unique and multi-modal selectivity through mechanisms such as hydrogen bonding. One such approach has been to develop mixed mode ion exchange ligands with enhanced binding strength for the key impurities, typically through the addition of hydrophobic functionality. Another category of mixed mode resins are based on hydroxyapatite, made from calcium phosphate, which has both positively and negatively charged particles and interacts with proteins through multiple mechanisms.

7.5 Gel Filtration Chromatography

In gel filtration chromatography, molecules are separated by size (*Size Exclusion Chromatography*). A matrix with a well-defined pore size distribution is used. Larger molecules are excluded from the pores and therefore have a lower effective cross sectional area to flow through. As a result, these larger molecules travel through the column faster than the smaller molecules, which are included in the pores. Unlike all of the other separation modalities described above, gel filtration chromatography does not require a change in mobile phase conditions to effect the separation.

7.6 Other Chromatography

There are other forms of chromatography, including reversed phase chromatography. Although the specific nature of the separation will vary with different chromatography modes, most separations operate on similar general principles to the adsorption or isocratic chromatography modes described above.

8.0 MODES OF OPERATION

8.1 Batch Chromatography

Most chromatography separations conducted in biopharmaceutical process applications are currently operated in batch mode using a column packed with chromatography resin. In batch mode, chromatography separations on a "batch" of material are achieved by applying different buffers or process solutions to the column in a series of steps. These steps will always include the loading of the process solution to be purified onto the column and the elution and collection of purified product from the column.

Variations of conventional batch chromatography that are also used in bioprocess applications include the use of membrane adsorbers or monoliths

instead of chromatography columns to achieve an ion exchange or other type of separation.

8.2 Multi-column Chromatography

Multi-column chromatography techniques such as simulated moving bed (SMB) allow for semi-continuous operation of a chromatography process. These approaches are widely used in other process industries and are beginning to be considered for biopharmaceutical processes.

8.3 Expanded Bed Chromatography

In expanded bed chromatography, feed material is pumped into the bottom of a loosely packed chromatography column, forcing the particles to become fluidized (i.e., for the bed to "expand"). This allows crude materials such as unclarified cell culture supernatants to be directly loaded onto a chromatography column without fouling, as might happen in a packed bed. The benefit to this mode of operation is that clarification and initial purification can be combined into a single step.

9.0 SUMMARY

A solid foundation in the basic concepts of chromatography is crucial in specifying the correct equipment configuration from the available technologies and methods. As a universal solution does not exist in chromatography, a breadth of knowledge, coupled with detailed understanding of the process and separation requirements is required to select an optimal solution.■

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References

None.

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