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Engineering Innovations Improve Process Column Chromatography

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Over the past decade there has been a steady increase in the number of biotherapeutics requiring high doses and long term administration. Most notable among these are monoclonal antibodies (MAbs) and fusion proteins comprised partially of antibody molecules. Column chromatography is a commonly applied purification method for downstream processing of biotherapeutics, and there is considerable pressure to process much greater volumes at a faster rate. For recombinant proteins and MAbs, a variety of chromatographic methods are employed, including affinity, ion exchange, hydrophobic interaction, and to a lesser extent, immobilized metal affinity and gel filtration.

Improving process control for chromatography operations is essential for biopharmaceutical manufacturers to process larger volumes and overcome capacity shortfalls. As the past ten years have seen increasing volumes of MAb-based drugs, there have been significant innovations to address growing productivity requirements. Dominant among these has been high throughput media capable of isolating product at faster rates than previously achievable. Another contributor to improved productivity is better plant utilization in the form of semiautomated, highly reliable methods of packing chromatography

A two-meter, 1,000 liter capacity process chromatography column fitted with hydraulic control of axial packaging.

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media into large chromatography columns. Before these innovations, manual methods required highly skilled users to ensure consistent methodology. However, even with the best-trained personnel, manual packing increases the likelihood of variability in packing results. Poor reproducibility and poorly packed columns can result in drift in key performance parameters such as product purity and yield (therefore, productivity), and in extreme cases, can directly result in costly loss of batches. Any increase in the frequency of column repacking increases the need to suspend production and thereby reduces plant utilization and productivity.

In 1994, a considerably less manually intensive *pack-in-place* method was introduced. Today, approximately 70 percent of all new chromatography operations utilize this more reproducible method. Pack-in-place relies on a separate packing station to suspend and then pump media slurries into columns at a controlled rate and pressure. Packing and unpacking occur with the column fully assembled. With an adjustable or fixed end cell set at the desired packed bed height, media slurried in buffer is pumped into the column to form a bed while the excess buffer is simultaneously exhausted.

Improving Process Control

The goal of any column packing process is to form an efficient and stable packed bed, with confirmed fitness for use. It is essential in a cGMP environment that column packing and chromatographic performance be fully reproducible. This is a key requirement for the manufacture of any regulated product, especially therapeutics intended for human use. For processes designed to achieve very high throughputs by rapid, successive cycling of multiple or split batches, finite media lifetimes dictate that column repacking occurs with greater frequency. This places added pressure on column packing methods to perform with a high degree of consistency to avoid process downtime.

The pack-in-place technique offers a fully contained system, isolating operators from the media to ensure a safer, more hygienic column operation. Packing, unpacking, and clean-in-place (CIP) procedures occur without removing the top column assembly. This is beneficial for all media since it prevents contamination during processing. It also eliminates the risk of operator exposure to potentially biohazardous materials such as those possibly encountered in blood fractionation and viral vaccine processing.

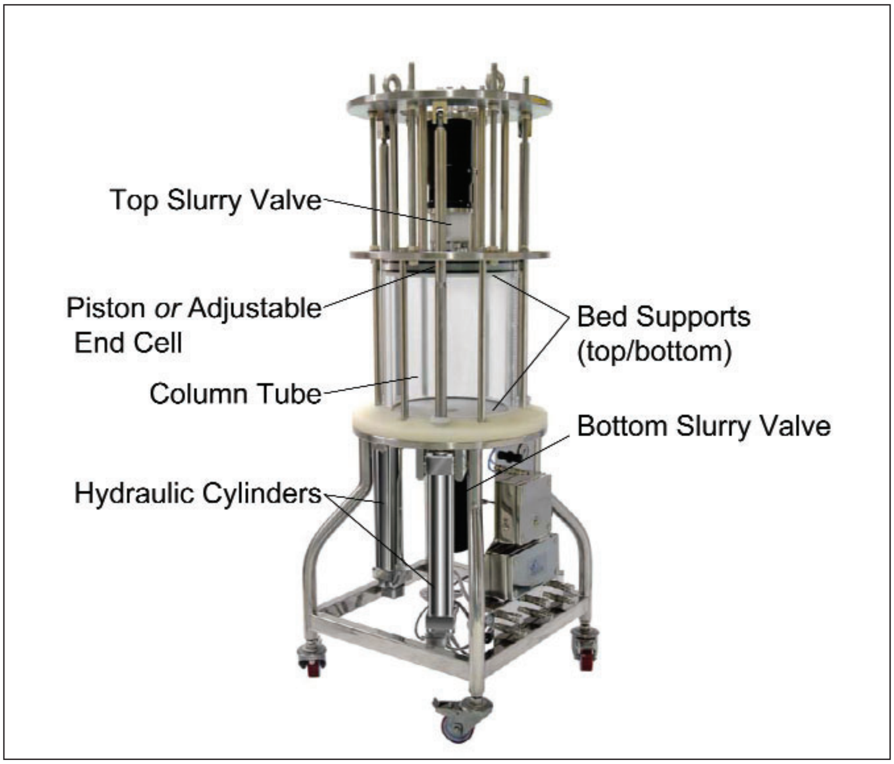
Lack of homogeneity of the packed bed is the main cause of poor column efficacy. This is due to flow aberrations and irregularities which can cause bed instability. For example, in the worst cases, beds can crack or form a moving void as feed is being processed, effectively ruining the batch in process.

Proper column packing improves chromatographic performance by controlling the space between particles (interstitial volume) to reduce the diffusion of migrating product bands during the separation. This ultimately leads to better resolution of product from contaminants and recovery of eluted product in a more concentrated state. It also ensures bed stability, providing a greater degree of assurance that the separation process remains consistent from batch to batch, an absolute requirement for overall process control.

Packing Procedures Vary by Media Type

Three categories of media are used for chromatography: carbohydrate-based media such as agarose, dextran, or cellulose; polymeric resins, usually composed of methacrylic or styrenic matrices; and fully rigid packings which include the inorganic matrices such as silica or controlled pore glass. Each media has characteristics that influence how packing should proceed (for example, how much axial pressure to place on the media.) Some resins are quite forgiving, while others are more sensitive to slight differences in the packing method.

Four different packing procedures accommodate most commonly used media. The first, *variable flow and pressure packing*, is typically used to pack moderately compressible media such as the lightly cross-linked agaroses e.g., Sepharose™ 4 Fast Flow. The pressure and bed height are allowed to increase slowly, and packing is complete when the hydraulic pressure within the bed — monitored at the mobile



Euroflow Resolute™ 400 mm Dynamic Axial Packing (DAP) column phase inlet port — drops noticeably.

Below are representative results for a 400-mm diameter column packed with Sepharose 4 Fast Flow by the variable flow and pressure method:

Af	Plates/m	Reduced Plate Height
1.30	6460	1.72
1.2	6260	1.77
1.33	6000	1.85
0.93	6000	1.84

The second packing method is *constant pressure packing*, which is typically used to pack the more rigid Sepharose 6 Fast Flow media. Packing pressure is maintained at 3 bar throughout the packing process, until the bed meets the bed support.

Below is a chart summarizing packing results of constant pressure packing for a 400-mm diameter column packed with Sepharose 6 Fast Flow:

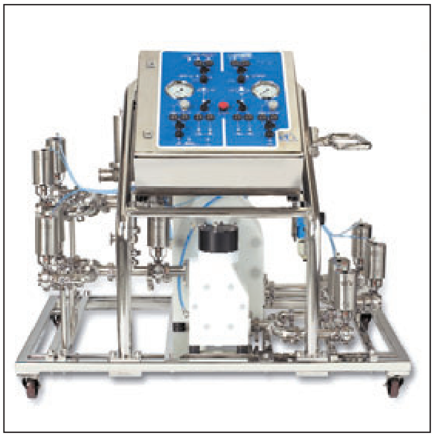
Af	Plates/m	Reduced Plate Height
1.17	4670	2.2
1.25	4230	2.4
1.35	3950	2.5
1.11	3920	2.6

The third packing method, termed *flow-compression packing*, maximizes the performance of specific media. The piston is set at a higher bed height than with other packing procedures, as slurry is pumped in. After the slurry has settled, it is flowed at 3 bar for 15 to 30 minutes. The piston is then moved onto the top of the bed by a system of hydraulics or by manually winding it down.

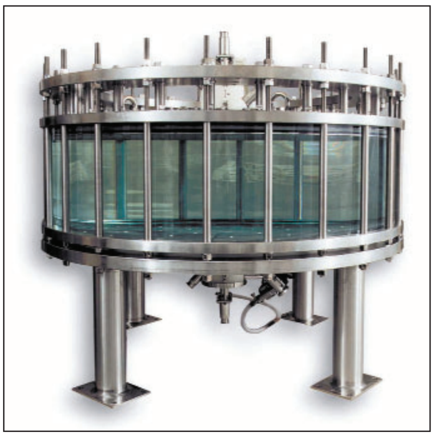
The fourth packing method is *fixed media volume packing*, which can be used to pack Sepharose Big Beads and controlled pore glass (CPG) based media. When using this method, bed volumes must be completely settled before slurry can be packed into columns. Exiting slurry can be recycled to wash the contents of the slurry tank back into the column. Conditioning is normally required for this procedure. This method is useful for costly media, such as protein A affinity packings, because it ensures full recovery of media from the dispensing vessel.

Hydraulic Control of Axial Packing

An important new development in low and medium pressure column chromatography is the incorporation of hydraulic linear actuators that carefully apply compressive force to a formed media bed to ensure optimal bed density. This is an important benefit for packing those media for which the flow compression packing method is indicated. Previous hydraulic column designs have focused on lifting and separating the adjustable cell and column tube from other elements of the device (i.e., "positioning hydraulics") to enable access to



Chromatography slurry packing station

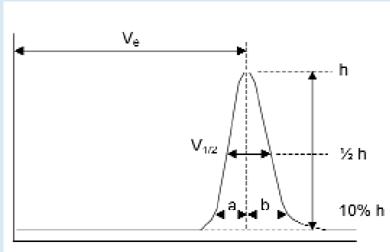


A 2000-liter chromatography column

Determining Packed Column Fitness for Use:

A packed column's fitness for use in process is often established by determining (1) the chromatographic ***Efficiency*** of the packed bed and (2) the symmetry of a representative elution peak profile expressed as ***Asymmetry Factor*** (A_f). Chromatographic Efficiency measurements quantify the resolving ability of a column by tracking the degree of band broadening or diffusion of a representative solute as it migrates through the bed. Peak symmetry is a second measure of solute migration or distribution in the column. Very low efficiency or relatively high asymmetry can be predictors of poor performance in process use, or worse, of packed bed instability.

Calculating Chromatographic Efficiency and Asymmetry (A_f):



The conventional method for determining efficiency is the ***Theoretical Plates*** (N) calculation, performed as follows:

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

where: V_e = retention time, $V_{1/2}$ = width of the peak at half height

Efficiency is typically normalized to column bed height by expressing it as ***Theoretical Plates per meter*** or **N/L** where **L** = bed length in meters.

The reciprocal of N/L provides the dimension of each theoretical plate, also referred to as the Height Equivalent of a Theoretical Plate or HETP:

HETP = L/N

where: L = column bed height, N = theoretical plates.

Efficiency is further normalized to account for different media particle diameters by establishing the number of particles per theoretical plate or the ***Reduced Plate Height*** (RPH). RPH is a unit-less number calculated as:

RPH = HETP/dp

where dp = particle diameter in the same units as HETP.

Exceptionally well packed columns produce an RPH of 2. Typical acceptable values for process chromatography columns range from 2–4.

Asymmetry Factor (A_f):

A_f = b/a (at 10% peak height)

where b is the width of that portion of the peak following the peak apex and a is the width of that portion ahead of the peak apex, both measured at 10% of peak height.

A_f >>1 can be indicative of fouled bed supports and A_f<<1 can, in severe cases, indicate the presence of a void or fissure in the bed.

Chromatography Comes Slowly to Blood Fractionation Operations

Pack-in-place column chromatography is widely used for biopharmaceutical manufacturing, and is now set to enter what has traditionally been a conservative segment of the biologics market: blood fractionation to process blood products. Blood fractionation involves the separation of approximately 20 protein factors from blood, ranging from human albumin and gamma globulin to clotting factors and other proteins.

The blood fractionation industry came into existence as a protected government activity in the United States during World War II, when there was a desperate need for various plasma expanders. As such, it generally was not subject to the rigorous regulations applied to the biopharmaceutical industry. A number of purification protocols were developed that would not be tolerated in normal commercial enterprises. The industry standard for purifying blood has been Cohn fractionation, a purely chemical method in which different proteins are selectively precipitated under varying chemical pH conditions and alcohols.

One major concern with Cohn fractionation is that processes are usually not contained, raising the possibility of contamination. Now that most blood fractionators are commercial enterprises, they are subject to regulations that require more stringent guarantees of safety, especially in today's climate of security.

Modern pack-in-place chromatography is the preferred method of extracting impurities, including rare components. Pack-in-place chromatography offers an enclosed processing system and a more advanced method of separation. The time is right for industry to invest in new systems of chromatography to meet purity challenges.

bed supports and seals for installation or replacement. Both functions are of significant utility at the large scale. Packing performance and, subsequently, process performance, as well as ease of use all contribute to efficiency in the production facility.

In the *flow and compression packing method*, media is pumped into the column using the pack-in-place system. The formed bed is then conditioned by flowing with buffer delivered by an external pump and then the piston is lowered in a controlled fashion. The pump action is seamlessly replaced by liquid displacement by the end cell movement, so the rate of this movement must be carefully controlled. The end cell must then capture the formed bed before it is allowed to relax and expand. In this way, the bed density is consistent throughout the column. The controller must also dictate the end state by stopping the piston when it contacts the top of the bed and before undue compression has been applied. This occurs at the same degree of compression each time the column is packed, thus improving pack-to-pack reproducibility. This method offers the added benefit of packing consistency when the operator is unable to see the forming bed, such as is the case when packing steel opaque columns.

Using Ultrasound to Monitor Packing

Monitoring column packing procedures has always been an elusive science based more on experience than objective measurements. One emerging technology in late-stage development involves the use of ultrasound to monitor densities of packed beds in columns.

Although ultrasound technology has been available for years as a critical diagnostic tool for other industries, it has not been applied to process chromatography. Theoretically, ultrasound can offer operators a measure of precision in determining the success of a column-packing event. There is an ideal rate and pattern by which a bed builds in order to perform optimally. Ultrasound waves have been able to monitor the building of beds as medium is pumped into columns. Ultrasound detectors are sensitive to bed compression, mobile phase composition, and the presence of soluble components such as acetone, albumin, casein, and monoclonal antibodies in the chromatography bed. Ultrasound enables visualization of these components as they migrate through a column.

Ultrasound technology can detect "real-time" bed formation, which offers immediate, accurate, and recordable verification of the integrity of a packed column before it is used. When used in conjunction with dynamic feedback control of the packing station, it can help ensure consistency by removing the "human factor" from the packing process. This provides true reproducibility of column packing with potential savings by avoiding costly repacking labor and plant downtime.

Once commercialized, ultrasound could offer great value to manufacturers by demonstrating that columns are packed the same way every time. It would also eliminate the need to perform intrusive tests to monitor the condition of the bed since ultrasonic monitoring takes place outside the column.

Optimizing Chromatography

In the face of pressures to speed production and maximize throughput, efficient and reproducible chromatography processing systems are essential to the overall process. Innovations to packing technologies and monitoring capabilities, while of tremendous benefit, are only some of the factors that need to be considered. Successful chromatography operations depend on a series of factors, from media selection, mobile phase composition, flow distribution within the column, and column ease of use. To pack columns successfully requires a comprehensive understanding of a media's physical characteristics, and the column systems best suited for that media and process scale. In the future, packing systems will need to be flexible to handle an expanding range of media and column configurations.