Choosing the Right Equipment for Process Chromatography

An overview of trends in process chromatography; how new innovations have improved production; and a guide to manufacturers involved in selecting chromatography equipment.

Process chromatography is universally applied in the downstream processing of biotherapeutics because it provides the most powerful means of achieving the levels of purity required for human therapeutic use. As with all other areas of biopharmaceutical production, manufacturers are critically concerned about scalability, validation, and ultimately, process economics in choosing the right chromatography equipment for their purification requirements. Just as the biopharmaceutical industry has matured, so too have chromatography methods and equipment to meet the ever increasing capacity challenges facing the industry.

Over the past 15 to 20 years of biopharmaceutical manufacturing, several trends are apparent. Not unexpectedly, the number of biotherapeutics in both commercial production and in development are growing more rapidly. A notable trend not so fully anticipated is the dramatic shift in the past few years in the percentage of biotherapeutics in the antibody-based drug class. Therapies targeted by this drug class require large doses and lengthy treatment programs. This, combined with their direction toward diseases with relatively large patient populations — cancer and autoimmune disease, for example — have created unprecedented demand for increased mass throughput, productivity, and economy. Although the rate of regulatory approvals will largely determine production growth rates, the motive to amplify production capacity by enhancing process efficiency and product yield is unlikely to disappear soon. The structure-function commonalities among this class of compounds allow increasingly generic purification schema, helping to speed the implementation of new manufacturing processes.

Improved Process Control

Despite the trend toward splitting batches for processing at more manageable scales, batch volumes have expanded to keep pace with growing throughput requirements. This drives the demand for more very large chromatography columns (>200 L in volume and >1,000 mm in diameter). New designs of construction may be necessary as column diameters expand beyond today's available 2-m range and to handle pressures produced in operating smaller particle, higher efficiency media or media tolerating very high flow velocities (>500 cm/h). Column packing systems are becoming more automated. New technologies are paving the way for more accurate monitoring and control and improved packing reproducibility, ensuring more consistent packed bed performance. The ultimate goal and result of these advances are improved process control.

Selecting the right column and related equipment for chromatography operations is ultimately as critical as choosing the media that goes in the column because they enable and preserve the carefully developed function of the packed bed media. The most important performance factors are packing reproducibility, maintenance of hygiene, and linear scalability from methods-development columns to ultimate production-scale installations.

True Linear Scalability

True linear scale-up is one of the most important manufacturing goals for chromato-

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Figure 1. One-meter chromatography column constructed of stainless steel

graphic unit operations because it determines the ease with which a drug product is transitioned from early-stage clinical production to full commercial manufacture. Assurance of the future manufacturability of a drug product is critical in what can be a competitive environment (for example, candidate compounds targeting a common clinical indication). A commercial advantage can be lost if scale-up complications impede efforts to meet expanding demand for a drug. The best column design should maintain all operational parameters at a constant level through various sizes.

Today's columns range in size from a few centimeters to two meters in diameter (see Figure 1). Some manufacturers have envisioned column diameters larger than two meters. At such scales, problems involving pressure limitations, irregularities in media packing, and improper flow distribution are likely to arise. Whatever the scale of production, there are always concerns regarding product purity, yields, and batch-to-batch consistency, all of which can be negatively affected by reduced separation resolution or adsorption capacity through nonoptimized flow distribution.

Predictable scale-up requires that pressure versus flow velocity for both feed material (sample) and the media slurry (when using automated packing methods) remain constant through process scale-up. This provides a consistent rate and quality of bed formation for all column diameters at the same bed height, and a similar back pressure at the same mobile phase velocity in actual operation of the column in process.

To achieve identical pressure versus flow velocity curves through the diameter range, it is critical that a single design principle be followed across the entire diameter series. This includes the specific geometries of all components of the process flow path and slurry delivery mechanism, including the mobile phase and slurry conducting paths through the column packing valves, the inlet and outlet flow distributors, and all external pipe work.

Slurry packing stations must also follow the same conserved design principle across the range of systems and be proportionally matched to the column size range.

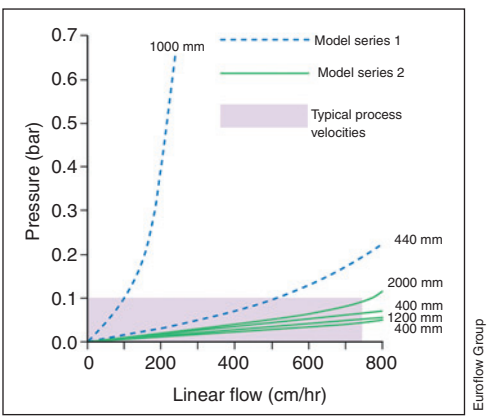

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Figure 2. Pressure versus flow for columns ranging up to two meters in diameter. The solid green curves illustrate true linear scalability.

Figure 2 provides pressure flow curves for different chromatography columns on the market. The columns for this comparison were filled with water for pressure drop measurements at a range of process flow rates. The column model series, whose pressure versus flow curves are shown as solid lines exhibits identical pressure flow performance which is linear up to approximately 800 cm/hr, at which the pressure is below 0.1 bar. Other column designs exhibit higher — and more significantly — unmatched pressure versus flow curves at different column diameters, which indicates design variations among different column diameters within the same supplier's model series (see dashed curves in Figure 2).

Column Construction and Materials

Columns and components must be constructed from non-leaching materials to be suitable for CGMP manufacturing of biotherapeutics. These materials must also exhibit a high level of chemical resistance.

Glass is an excellent material for several reasons. It is inert, exhibits chemical compatibility with most bioprocessing streams, and is optically clear, allowing operators a partial visual check of the condition of the packed bed during use, albeit just the first 1–2 cm from the inner wall of the column tube.

For pilot-scale applications, glass is the preferred material for column tubes. This was initially the case for manufacturing or process-scale columns as well. However, as requirements for larger scale production emerged, glass became an impractical material because tube suppliers could not maintain the close tolerances with glass that are needed for reliable sealing of column distribution end cells. Also, because of the relatively low material strength-to-weight ratio of glass, it is difficult to form and to handle at dimensions needed for typical column operating pressure requirements. Those pressure requirements have been increasing further with the emergence of the smaller particle (higher back pressure) high-resolution media class. As a result, beyond a certain diameter (in most cases 400 mm), glass is no longer a viable option.

Plastic. Manufacturers are increasingly using plastics of various compositions — the most common being various acrylic formulations — as a safe and practical alternative to glass. Acrylic is roughly half the weight of glass, shatter resistant, and offers high impact resistance to tolerate greater pressure and other mechanical forces. At 92% optical clarity, acrylic is somewhat clearer than glass, and in most cases it can be formed to precise tolerances for the critical sealing requirements of chromatography columns. Traditionally, acrylic's main drawbacks have been the existence of harmful plasticizers, and a lack of chemical resistance to organic solvents. For example, acrylic has posed a challenge when using solutions with high concentrations of ethanol, a common media shipment and storage agent.

New acrylic resins, however, have virtually no leachable plasticizers and offer increased solvent resistance. This further enhances the desirability of acrylic as a material for process chromatography columns. Selected commercially available acrylic columns are compatible with 20% ethanol solutions. They also have demonstrated biocompatibility with other relatively active process liquids such as NaOH (2M), urea (8M), and HCl (to 1N) to name a few.

Stainless steel (316L) is also viewed as a preferred material for process chromatography columns, both as a tube material itself or used in combination with acrylic in those portions of the column that provide structural support (tube flanges, tie rods, and lifting fixtures, for example). Steel can handle high pressures and offers a wide chemical resistance profile. It is subject to corrosion in some cases, especially at very low pH or with agents yielding free Cl^- ions. Solutions with high concentrations of sodium chloride or hydrochloric acid, for example, can cause

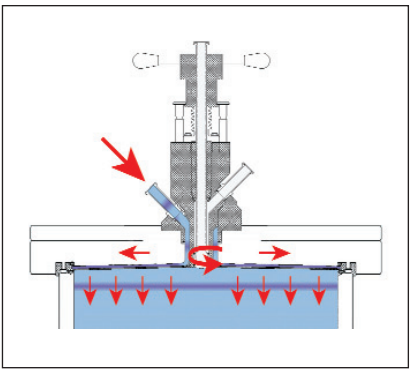

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Figure 3. Cross-section of the nozzle valve and flow distribution system.

mild corrosion on long-term exposure to stainless steel.

A column's bed supports, or frits, are particle filter stages that contain the media within the column bed. Their porosity allows the passage of liquid from the column inlet into the bed and from the bed to the outlet. Bed supports are typically made from woven stainless steel (typically configured as a multilayered, fine-wired mesh), sintered plastics (such as polyethylene or polypropylene) or in rare cases woven plastics. Typical process media range from approximately 30- to 300-micron (µm) average particle size, with the majority between 34 µm and 90 µm. Bed supports are microporous and come in various nominal pore sizes for compatibility with the particle diameter of a given media, and generally range from 10 to 100 µm.

It was not uncommon several years ago to provide a water-filled outer jacket to control the packed bed temperature for smaller scale separations, but this has not been widely applied to process columns. With the faster throughputs of modern media, the liquid feed (or sample) and mobile phase or buffers pass through the column in less than 10 minutes. Therefore, for the relatively rare process operations conducted outside of ambient conditions, more effective temperature control can be applied outside the column by using in-line heat exchangers or chillers.

Column manufacturers must specify that all materials of construction for those components in the wetted flow path be compliant with CGMP production of human therapeutics. Materials contacting the process fluids must be checked for chemical compatibility with every component of the feed and other process buffers and solvents. The manufacturer also should supply full traceability of all component materials and parts comprising the column systems and accessories to aid drug manufacturers in their regulatory submissions. Technical documentation, including an operator's manual, packing methods, and test results should be included, as well as the procedures for installation qualification, operational qualification, and column maintenance.

Optimal Flow Characteristics

Achieving optimal flow distribution within the column is the primary design goal for ensuring that a chromatography column is fit for use. A column's flow profile should ensure that the mobile phase enters the column bed equally over the whole column cross section. This promotes even distribution of the sample throughout the entire packed bed volume and full use of the separation medium.

A column flow distributor with a radial ribbed pattern helps channel flow evenly from the single central column inlet, through the bed axially, and then again toward the center of the column before exiting the outlet (see Figure 3). Combined with a well-packed bed of homogeneous density, the flow profile is formed as near as possible to a "plug-flow" pattern — that is, constant velocity across the entire cross-section of the tube — with minimal solute dilution. This results in the delivery of optimal throughput without reducing the resolution of individual feed components; it maintains the most effective isolation of target products and their resulting purity.

The channels between radial ribs of the flow distributor are typically sloped from the

center inlet to the outer perimeter of the packed bed face. This narrowing of the channels helps to accelerate liquid toward the perimeter, ensuring that it enters that portion of the bed at the same time as at the bed center (that is, it approaches "plug flow"). The frit, or bed support, also is often mildly sloped or dished, following the same pattern of the tops of the radial ribs it lies against. This slope is intended to facilitate the liquid priming of the column as the air travels to the highest point in the column and is vented through the slurry valve before slurry packing. A visible valve flow path enables confirmation that formerly trapped air is cleared from the mobile phase inlet.

Sanitary Design

As for any pharmaceutical processing equipment, a sanitary column design is critical to achieving effective flow-through clean-in-place (CIP) of the packed bed. To prevent product or contaminant retention, the column should have a fully flushed flow path with no dead space around the adjuster seal(s) or in the fixed cell seal arrangement, or at inlet and outlet clamp terminations.

Eliminating unswept areas also improves chromatographic performance, as monitored by column efficiency (theoretical plates) and peak symmetry determinations. Areas with limited or no flow can cause peak tailing and band broadening because they become small reservoirs in which soluble components pool and from which slow wash-out ensues.

Because process chromatography columns are also pressure vessels, column systems often are certified to meet regional design codes such as those set by the American Society of Mechanical Engineers (ASME) in the United States and the Pressure Equipment Directive (PED) of the European Union (EU). A particular design element required for compliance with the EU's PED is pressure relief safety valves installed somewhere within the pressurized train (on the slurry delivery instrument, for example).

Column Packing

The goal of any column packing process is to form an efficient and stable packed bed, with confirmed fitness for use. It is essential to the drug manufacturing process that the column performance duplicate previous packs.

Manufacturers develop a separation method and corresponding column packing methods using small columns at a pilot scale, then transfer this method to larger columns of increasing diameter at the same bed height for scale-up. Therefore, it is equally critical that the column packing and performance reflect that of the smaller development or pilot column. This highlights the importance of linear scalability between columns of increasing size. Packing consistency and separation process performance on scale-up can be severely compromised if the column design varies with increasing diameter. Inconsistent or ineffective scale-up can result in unacceptable increases in labor costs and production delays. Possible failure mechanisms can include density inconsistencies or local voids in the bed, leading to flow aberrations and irregularities, and even to bed instability. In the worst cases, beds can crack or form a moving void as feed is processed, effectively ruining the batch in process.

Column packing methods differ among different column designs and manufacturers and are always specific to the media being employed. As a result of this diversity, the level of operator training and experience can dramatically affect success rates in column packing. The more manually intensive the packing method (that is, the greater the requirement for operator intervention), the greater the chance of variability in the packing results. Traditionally, column packing has been a manual process, highly dependent on the experience of technicians.

Manual packing methods are commonly referred to as "traditional" or "conventional." One of the earliest methods employed was called dry packing or tap packing. In this method, a technician would add dried resin to the column, then agitate the column to help the media particles settle to a stable pack. The technician would then hydrate the media directly in the column.

Eventually, most resins were supplied hydrated, and flow packing became the conventional approach. In this method, operators pour the media as a dilute slurry directly into the column from a supplied container or conditioning tank. The operator stirs the media suspension to ensure homogeneity, then inserts and seals the column top end cell before applying pressurized liquid flow to cause resin particles to settle. Considerable manipulation of the end cell is required to ensure there is no air trapped above the bed and the bed surface remains undisturbed. These methods can be effective, but given the number of manual manipulations, reproducibility of the packing procedure in a CGMP environment is typically beyond the skills of even moderately experienced operators.

Automated Packing

Over the past decade, automated packing methods have been developed that reduce variability and largely eliminate the "human factor." This has transformed large-scale column packing techniques from a skilled craft to more of a science, based on the mechanics of the equipment and the physical properties of the media. For a given chromatographic media, there is an ideal rate at which packing should be performed to provide optimum media compression within a homogenous bed for maximizing installed column performance.

An automated pack-in-place method was first introduced to the market in 1994. Because automated methods vastly improve consistency and reproducibility over manual methods, they have clearly become the industry preference. Today approximately 70% of all new process chromatography operations use automated packing technology.

By eliminating manual handling of column operations, packing parameters in an automated packing system can be established and applied within a standard operating procedure, vastly reducing operator variability and column failure, and thereby enhancing productivity in the manufacturing facility.

Fully Contained Packing Operation

A pack-in-place system offers users a fully contained column packing operation, enabling packing and unpacking to occur with the column fully assembled. An adjustable or fixed end cell is already in place at the ultimate bed height, and operators form the bed by pumping in the slurry and simultaneously exhausting excess slurry liquid. Operators have greater process control, which leads to an overall improvement in manufacturing.

Because all packing and CIP procedures occur without removing the top column assembly, the risk of operator contact with hazardous materials is minimized, and product exposure to potential external contamination is reduced. The result is a safer and more hygienic column operation.

Central to the pack-in-place operation is a nozzle valve located on the top and bottom of the column. Media slurry can enter and exit the column through either of these nozzles, depending on the characteristics of the medium and the packing method used. The top and bottom nozzle valves should be identical so the flow profile is the same in either direction. User preferences are met and convenience can be added by the availability of either manual or pneumatically assisted operations.

There are three functions of the nozzle valve in a pack-in-place operation: packing, running or "process," and unpacking. This

can be achieved with a two- or three-position valve. One example of nozzle valve design functions as follows:

In the packing position, the top nozzle is extended part way (mid position) into the column, and the bottom nozzle is fully retracted. Slurry enters the column through the top nozzle, and excess liquid exits through the bottom mobile phase outlet. After packing, the slurry lines and paths within the packing valve are isolated from the mobile phase and can be cleaned independently from the rest of the column. If packing with upward flow, the bottom nozzle can be extended part way to the mid position into the column. The top nozzle is fully retracted, slurry is pumped into the bottom, and the media bed is formed on the top bed support.

In the running position, both nozzles are fully retracted. Mobile phase enters the column through the mobile-phase port, flows through the bed support, and then through the packed bed and bed support at the other end of the column. It then exits through the opposite mobile-phase port.

For column unpacking, the top and bottom nozzles are fully extended into the column. This makes the slurry waste ports (top and bottom) continuous with the column interior. Media can exit through these channels as it is resuspended with buffer pumped in through the slurry inlet ports during unpacking. Cleaning solution can be pumped through the nozzle and sprayed into the column. In this way, media is completely removed from the column interior, and the column can be easily and effectively cleaned without dismantling the column and exposing the interior or the medium to the external environment.

Slurry Packing Systems

A pack-in-place method requires an integrated and scalable slurry packing system or station (SPS). The function of the SPS is to deliver the media slurry to the column during packing and unpacking (see Figure 4). The SPS also must be capable of delivering functional packing flow rates at pressures matched to appropriate media packing limits (for example, to 3.0 bar for the majority of today's high-flow semirigid media). Its output capacity should be matched to both packing and unpacking flow rates as column diameter is increased. To effectively remove all media from a column during unpacking, it is necessary to increase flow rates over those used during packing. An SPS should offer simple and convenient control to simplify training and reduce the burden and delays of transferring packing methods during scale-up. Ideally, flexibility should be designed in to allow packing of multiple media, multiple column sizes, and multiple methods.

An SPS with PTFE-lined diaphragm pumps and integrated pulse dampening has been shown to deliver a smooth and controlled flow of media slurry at high transfer rates, even toward the end of the packing cycle. This, together with properly selected components at all scales, ensures reproducible packing conditions at all column

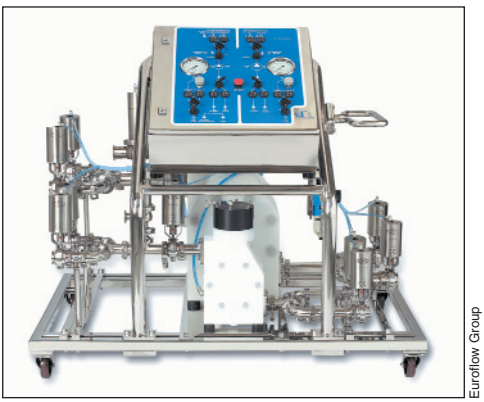

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Figure 4. A slurry packing system delivers media to a column during the packing process. Separate from the column, it delivers media through flexible hosing.

diameters and significantly reduces methods validation issues. A pneumatically actuated, fast-acting control valve can provide excellent control during critical stages of the packing process.

Chromatographic media require various, and often unique, packing methods. Packing an efficient column requires a comprehensive understanding of the media characteristics and a packing system with adequate flow capacity and control. A packing system should accommodate a wide range of media types and column configurations, for example, axial compression techniques used in combination with normal pumped slurry packing.

An efficiently designed SPS should offer two pumps to deliver a range of flow rates for optimal packing of any sized column with any type of media. The system should also be able to meet higher flow rates for hard-to-unpack media types, such as small-particle beaded and certain angular rigid media. An SPS is connected to the actual column through transfer hoses. It is important that these hoses and terminations be correctly sized and of optimal length to minimize pressure drops and media losses. The slurry tank should include a mixing device to maintain the homogeneity of the slurry during packing operations.

Media Compatibility

Chromatography resins generally fall into three general categories: carbohydrate-based such as agarose, dextran, and cellulose; polymeric resins, typically composed of methacrylic or styrenic derivatives; and rigid packings, which include silica and controlled pore glass. Each of these media types possess unique characteristics that are critical in determining packing requirements. For example, once slurry has been pumped into a column and excess liquid evacuated, certain media benefit from the application of measured compression (this can be accomplished with a series of carefully controlled hydraulics).

Chromatography media for one column step can vastly outweigh the costs of the column and systems containing and managing the separation. In the extreme case (specific affinity media, for example), the contents of a $200,000 specially engineered column may be valued at several million dollars. This demonstrates the importance of ensuring that the longevity of the content media is not compromised by column packing systems or methods. This is assured by using a combination of low-shear diaphragm pumps and flow paths and by developing packing methods that reduce the number of passages through the media.

Considering the Whole Design

Efficient chromatography processing is not simply about selecting the right column or right packing method, or even about employing the right technical staff. All aspects of this equation are important for effective processing, and they cannot be considered in isolation from each other. The goal is to get the best performance as consistently as possible, and delivery of performance depends on a series of factors, from the media being used to the column design, flow distribution, packing methods, and so on.

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