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CHROMATOGRAPHY COLUMN PACKING

BEST PRACTICES AND CONSIDERATIONS FROM LABORATORY TO MANUFACTURING SCALE

Cheryl Scott

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Chromatography Column Packing

Best Practices and Considerations from Laboratory to Manufacturing Scale

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Well-packed columns provide for the best chromatographic separations. Successful column packing ensures proper mobile-phase distribution and resin contact. Scaling up to the column size pictured here can introduce many more complexities. Manufacturers of chromatography media provide best-practice advice for packing their products. This eBook provides a starting point with key considerations for packing both traditional compressible and incompressible media.

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Back to Contents

Column chromatography is a powerful separation tool for biopharmaceutical research and industry, with applications ranging from bench-scale purification to process development and commercial-scale manufacturing of biotherapeutics. Ensuring the highest quality of separations depends on many factors, including the technique used for packing the chromatography column. Well-packed columns, in which the beds of media are homogeneous and continuous from top to bottom, provide for the best chromatographic separations. Successful column packing ensures proper mobile-phase distribution and resin contact, which helps to ensure desired yield, separation, and product quality. Poor-quality packing can lead to solute band broadening and compromise those parameters.

Manufacturers of chromatography media can provide best-practice advice for packing their products to help customers develop optimized methodologies for their own processes. This eBook provides a starting point with key considerations for packing different types of resins, including both traditional compressible and incompressible media.

Compressible and Incompressible Resins

Because chromatography resins vary in compressibility, users need to develop proper knowledge of the respective packing methodologies to ensure high performance and scalability. Table 1 compares the attributes used to determine packing protocols for both compressible and incompressible resins. The former present as spherical, porous particles based on cross-linked organic polymers such as agarose, polymethacrylate, and polyvinyl acrylamide. By contrast, incompressible resins are based on substances such as rigid, nearly spherical, sintered ceramic hydroxyapatite particles or irregularly shaped, porous glass particles (**1**).

Packing Incompressible Resins: You can determine the amount of incompressible resin needed to fill a column based on tap-settled density. For example, if 0.9 g of resin is needed to fill 1.0 mL of a 10-mL column, then 9 g of resin will be needed. Incompressible resins are denser and thus settle more rapidly than compressible resins do. Packing therefore must be done as quickly as possible to prevent larger beads from settling more rapidly than smaller beads, which leads to stratification of the bed. Incompressible resins also need a small head-space at the top of their column to prevent inadvertent compression that can cause particles to break.

Packing Compressible Resins: As Table 1 shows, packing compressible resins brings different considerations. To ensure proper packing, the

Table 1: Compression factors affect packing methodologies for incompressible and compressible resins (adapted with permission from Bio-Rad Laboratories, Inc.).

Base Matrix	Attributes	Compression Factor*
Cross-linked cellulose	Highly compressible, suspended	1.50–1.70
Polyvinyl acrylamide	Compressible	1.10–1.25
Polymethacrylate	Semicompressible	1.10–1.25
Agarose	Compressible	1.10–1.20
Ceramic apatite, silica	Incompressible, rapid settling	1.00
Controlled-porous glass	Incompressible	1.00

* Check your resin supplier's product manual for specific ranges.

compression factor — the ratio of the heights of a packed and a gravity-settled bed, which is typically 1.1–1.3 — must be known in advance. The *packing factor* is a ratio between the heights of a consolidated and packed bed. Once resin has settled at the bottom of a column, the beads can be compressed. Parameters such as compression factor, settling rate, and certain attributes of the base matrix need to be considered for packing compressible resins at process scale. That helps users adapt small-scale packing methods to develop simple and reproducible column packing procedures.

Whether using an compressible resin or incompressible resin, column packing comprises four main steps: slurry preparation, slurry transfer, packing, and evaluation and qualification. A buffer-based slurry eliminates air bubbles from forming in the resin bed, and the choice of solvent depends on the media and chromatographic chemistry being prepared.

Key Factors Affecting Packing Methodologies

Here are some other factors that affect packing methodologies for compressible and incompressible resins.

Compressible Resins

Amount of resin needed is determined by the compression factor (Table 1).

Additional compression needs to be applied in packing.

Most resins settle slowly, and once they are packed, there is no headspace.

Thus, rapid packing is not a significant factor.

Incompressible Resins

Amount of resin needed is determined by the tap-settled density.

Bed consolidation equals bed packing.

Resins settle rapidly and require a headspace once packed.

Best results are achieved with rapid packing.

Slurry Preparation and Transfer

Because incompressible resins typically come in dry-powder form, slurry preparation requires dispensing the correct amount of media based on tap-settled density, followed by rewetting. For compressible resins, the amount needed depends on the compression factor, as described above.

Note that to minimize mechanical damage, a low-shear impeller must be used for preparing slurries of incompressible resins — and often is recommended for shear-sensitive compressible resins as well. Excessive mixing can fracture incompressible-resin particles, as can the action of impellers other than low–mechanical-shear hydrofoil models. Fine particles resulting from such fracturing can increase column backpressures. Many compressible resins also are somewhat shear sensitive.

Incompressible chromatography media can be damaged by excessive physical force during transfer. Peristaltic and rotary-lobe pumps can fracture resin particles and thus should not be used. Instead, use pressurized slurry vessels or

Back to Contents

Key Considerations for Slurry Preparation

Here are some key considerations in slurry preparation for compressible and incompressible resins.

Compressible Resins

Most come as a slurry.

The amount of compressible slurry equals the bed volume plus the compression factor.

Low-shear impellers are recommended.

Incompressible Resins

Many come as a dry powder.

They require dispensing the correct amount followed by rehydration.

Low-shear impellers are necessary.

diaphragm pumps for media transfer, or use a syringe action to draw slurry into the column. Do not recirculate slurry through a diaphragm pump for mixing; experts recommend only a single-pass operation of well-mixed slurry when a pump is used for transfer.

The use of open- or closed-column packing also makes a difference. Open columns are commonly used for pilot- and small-scale manufacturing; in such cases, a resin–solvent slurry can be prepared in the column itself. The height of the column tube must be sufficient to contain the entire slurry volume, which is based on the dry weight of the resin and the volume of packing buffer needed.

The amount of slurry that can be used in an open process column depends on the functional column height, which is the height of the column with its top piston in place. For example, with a 50-cm tube height and a top piston that occupies 12 cm, only 38 cm of the tube remains to be used. Therefore, the maximum bed height that can be packed with a 50% slurry is 19 cm.

Large process-scale chromatography columns typically are closed systems into which slurry must be introduced through valves. It can be transferred into a closed column through suction, vacuum transfer, pumps, pressurized slurry vessels, and packing stations.

Suction or Vacuum Transfer: Some types of process columns with motorized pistons can introduce a media slurry without a pump. A top-mounted adaptor draws slurry into the column by suction in a syringe-style motion. Such transfers should be performed rapidly for incompressible resins because of their rapid settling.

Media Transfer Through Pumps or Pressurized Slurry Vessels: Media transfer stations can be used to transfer slurries from mixing tanks to empty columns. In such cases, determining the proper concentration is essential to ensuring efficient packing.

Media-Packing Stations or Stall Packing Through Pumps or Pressurized Slurry Vessels: Packing stations can be used to pack thin slurries from mixing tanks into closed columns through packing nozzles containing multiple small-diameter orifices. Experts recommend using relatively low concentrations and diaphragm pumps in such cases. Thinner slurries minimize shear forces and particle-to-particle collisions in piping, connections, and packing nozzles. Damage also can be minimized with a single pass of slurry through the pumps, piping, valves, and nozzle.

Column Packing

The overall goal of column packing is to prevent stratification of different-sized particles during consolidation of the bed by using appropriate flow rates and piston movement. Even compressible resins, which settle quite slowly, will stratify if not handled properly, with larger particles settling to the bottom and smaller ones remaining at the top of the bed. The “Packing Columns” box summarizes key differences in packing incompressible and compressible resins. For incompressible resins, the bed should have a headspace, so upflow bed conditioning or cleaning should not be

Packing a Compressible Resin

The following steps are used when packing a column with compressible resins. They should be handled according to their manufacturers’ recommendations, so this information is only an example.

To calculate the appropriate amount of resin to use, determine the desired final bed height. For example, that may be 40 cm. Assuming a compression factor of 1.15, the bed would be effectively 46 cm (40 cm × 1.15). The volume of resin required would be $\pi \times cr^2 \times bh$, where cr = column radius and bh = bed height. Using that calculated resin volume, calculate the total amount of resin slurry to be added using this equation: slurry volume = total volume resin ÷ percentage slurry needed. In this example, assuming an inner column diameter of 2.5 cm,

- total bed height = 40 cm × 1.15 = 46 cm
- volume of resin = $\pi \times 1.25\ cm^2 \times 46$ cm ≈ 226 mL resin
- slurry volume = 226 mL resin ÷ 0.5 (50% slurry) = 452 mL slurry.

An Online Resource

The chromatography conversion tool online at bio-rad.com/calculator provide a handy resource for calculating

- bed volume in liters based on bed height and column width (in cm)
- residence time in minutes based on bed height (cm) and flow velocity (cm/h)
- flow rate in either mL/min or L/h based on column diameter (cm) and flow velocity (cm/h).

The webpage also includes a calculator for determining the amount of buffer and media needed for packing columns with different types of ceramic apatite powder. Users enter column diameter and resin bed height in cm, slurry percentage (v/v), and packing density information (g/cm^3).

used. By contrast, compressible resin beds should not have a headspace and typically are conditioned and/or operated with a combination of upflow and downflow.

During **stall packing,** slurry is pumped into a column through a nozzle at the bottom, and column effluent exits through the top. With an incompressible resin, the pump stops when the space is filled. With compressible resins, pumping continues until a pressure limit is reached, indicating that the proper compression factor has been achieved. Figure 1 depicts several additional packing methodologies.

Packing Columns: Key Considerations

Here are some key considerations for packing compressible and incompressible resins. Preventing stratification of particle sizes is important for all types of media.

Compressible Resins

Optimal packing requires precise measurement of settled (consolidated) bed height and compression factor.

Packed beds must not have a headspace.

Typical compression factors range from 1.1 to 1.5.

Compressible packed beds typically conditioned with a combination of upflow and downflow.

Incompressible Resins

Neither precise measurement of settled bed height nor compression factor is significant.

Packed beds should have a headspace.

Compression factor is exactly 1.00.

Upflow bed conditioning should not be used during packing or subsequent cleaning.

With **flow packing,** once the slurry begins to settle by gravity and a 2-cm to 3-cm layer of clear supernatant is formed, the top adapter is lowered to that layer and sealed. A downward buffer flow is applied to consolidate the bed. Once it is consolidated, a piston is lowered to the bed height, leaving a headspace of 1–5 mm. It is important when using this approach to ensure that the flow rate is greater than the fastest particle-settling velocity.

Axial packing is an alternative method in which the entire slurry volume is introduced into a column by a pump or suction. Following that transfer, the piston is lowered, and bed consolidation starts with liquid displacement from the downward motion of the piston, which must be greater than the fastest particle-settling velocity. Axial compression stops when the desired packed-bed height is reached.

A **hybrid method** combines aspects of both flow and axial packing. Once the slurry is transferred to the column, the piston is lowered. A downward flow is used simultaneously with axial movement to consolidate the bed. As with axial packing, the total consolidation rate must be greater than the fastest particle-settling velocity.

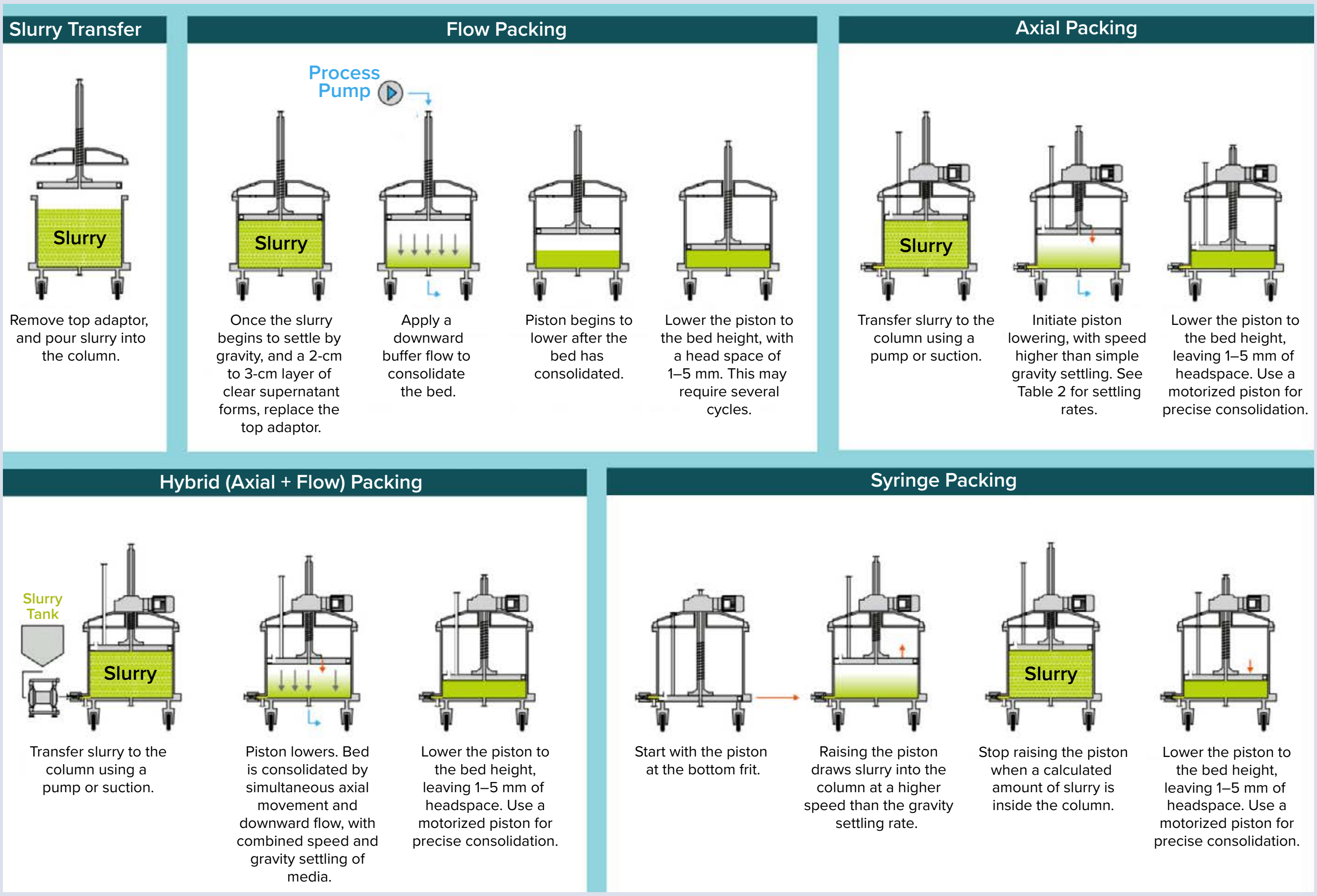
Syringe packing is another option. In this technique, the piston starts at the bottom frit of a column and then gets raised to draw slurry into the column. The piston stops once a desired amount of slurry is in the column and then gets lowered to the bed height, creating a headspace of 1–5 mm.

Evaluation and Qualification

The efficiency of a packed column typically is determined based on the height equivalent to a theoretical plate (HETP) and the peak asymmetry factor (A_s) (**2**). Both are easy to obtain by applying to a column a sample such as NaCl or acetone that does not interact

Back to Contents

Figure 1: Comparing column-packing methodologies (ADAPTED WITH PERMISSION FROM BIO-RAD LABORATORIES INC.)



with the column material. Then the column is eluted under isocratic conditions. The calculated plate number (N) will vary depending on test conditions and therefore should be used as a reference value only. It is important to maintain constant conditions and use the same equipment when comparing results. Changes in test-probe concentration, buffer, sample volume, flow rate, and liquid pathway all can affect experimental outcomes. If an acceptance limit is defined in relation to column performance, then a column plate number can be used as part of the acceptance criteria for column use.

HETP is calculated as L/N, where L = bed height (cm), $N = 5.54 (V_e/W_{1/2})$, V_e = peak elution distance, and $W_{1/2}$ = peak width at half peak height. The peak asymmetry factor is measured as b/a, where a = first-half peak width at 10% of peak height, and b = second-half peak width at 10% of peak height. For optimal results, experts I spoke with recommend that the test probe volume be 1–2% of the column volume. Flow rate can range 75–150 cm/hr but should remain consistent across tests.

Troubleshooting

Adhering to the guidelines provided by your resin manufacturer will help to ensure successful and consistent column packing. However, if unexpected results occur, you can explore the root

Back to Contents

identify possible corrections. Table 2 lists some guidelines and suggested corrective actions for troubleshooting column packing and evaluation methodologies. For more general information, search for "column packing" on the BPI website.

Table 2: Some troubleshooting tips for packing chromatography columns (adapted with permission from Bio-Rad Laboratories, Inc.)

Observation	Possible Cause	Correction
Settling of incompressible resins during slurry preparation	Mixing in a standard circular motion	Use a J-stroke for mixing, or use an orbital shaker.
High reduced height equivalent to a theoretical plate (rHETP)	Clogged column screen/frit; probe volume too big or unoptimized efficiency-test conditions; unevenly packed column	Clean screen/frit; modify injection loop to reduce probe volume; repack column.
Peak fronting	Channels in column; packing pressure and/or flow rate too high	Repack column; use a lower packing pressure and/or flowrate.
Peak tailing	Probe volume too high, or unoptimized efficiency-test conditions; air trapped under piston or column adaptor; space between resin bed and column adaptor or piston	Modify injection loop to reduce probe volume; eliminate air; adjust position of piston or column adaptor.
High column pressure	Clogged column screen/frit; presence of fine particles (of incompressible resins) due to excessive mixing; contaminated resin	Clean column screen/frit; use the recommended mixing method to minimize fines with incompressible resins (and do not repack the same slurry); clean or replace the resin.
Trailing shoulder peak during asymmetry testing	Plugged or contaminated resin	Clean or replace the resin.
Channeling when packing	Hardware configuration	Use a slower flow rate to consolidate the bed, then lower the adaptor to the desired bed height, followed by conditioning with a higher flow rate.

A Foundation for Success

Column chromatography is a foundational technique for downstream processing workflows in the biopharmaceutical industry. Consistency and performance of the method require properly packed columns and stable resin beds. A bed that is packed too tightly or too loosely can lead to costly losses of valuable biological products and ultimately to disruption and delays in production. Resin manufacturers provide detailed instructions for packing their products. It helps, though, to understand the differences between compressible and incompressible resins, basic strategies for packing open and closed columns, and different packing techniques to ensure success.

Back to Contents

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About the Author

Cheryl Scott *is cofounder and senior technical editor of* BioProcess International, *part of Informa Connect; 1-212-600-3429; cheryl.scott@informa.com.*

Back to Contents