Column Packing for Process-Scale Chromatography: Guidelines for Reproducibility

For maximum efficiency, high product yield, and purity, you must produce a homogeneous packed bed every time you perform a separation. Irregularities in packing cause uneven flow within the bed, resulting in band broadening, zone mixing, changes in flow rate, and subsequent loss of product yield and quality. Here we provide guidelines for reproducible column packing along with useful troubleshooting tips.

Because chromatography is an important and ubiquitous unit operation in downstream bioprocessing, FDA inspectors are showing increased interest in the reproducible packing characteristics of process-scale chromatography columns. Biopharmaceutical companies need to develop protocols for column packing and subsequent qualification of the packed column to ensure robust separations and to meet FDA requirements. Process-scale bioseparations often involve several columns, so packing and qualifying each column in the process is an important validation concern.

Currently, there are three different ways to pack process chromatography columns: flow pack, which uses constant pressure or flow; dynamic axial compression, achieved by axially compressing the media slurry; and pack-in-place, which incorporates column valves into the flow cells.

The newer hardware designs that allow for dynamic axial compression and pack-in-place methods minimize the reliance on operator technique, but the tradeoff is increased hardware costs.

Whatever the packing methodology, there are certain critical factors you must consider before packing a column.

Prepacking Factors

Media. Various media considerations will affect column packing: particle size and homogeneity; liquid or mobile phase used to pack the media; propensity of the media to generate fines; effect of temperature and buffers on physicochemical behavior of the media; hydraulic properties leading to unique compression factors; and cross-contamination or irreversible binding when various media are used in the same column (such as during campaigning).

The liquid portion of the media slurry is called the mobile phase. Selecting the proper mobile phase, which typically consists of a weak buffer or water for injection (WFI), minimizes potential problems with the pack. When selecting the mobile phase for packing, consider the following factors:

- chemical compatibility of mobile phase with media (for example, sulfopropyl chemistries do not demonstrate long term stability in WFI)
- shrinkage or swelling of media in mobile phase (ensure packing buffer and process conditions induce similar levels of matrix swelling)
- viscosity (high viscosity buffers may restrict flow rates because of equipment pressure limitations)
- pH stability and range
- mobile phase binding to resins (select mobile phases that minimize nonspecific adsorption; inclusion of .01–.5 M NaCl is usually effective)
- compatibility of mobile phase with column storage solution.

One of the best ways to remove media fines, or fragments, is to not create them in the first place. Avoid stirring media exces-

sively, particularly with soft gels, because stirring can shear the beads and generate fines. Fines can occlude the bottom screens of the column, and though the blockage does not contaminate the separation, it increases the back pressure, which can result in decreased and nonuniform flow through the column.

It is important to develop a protocol for removing fines from the media. Slurry the media in the packing buffer and decant the supernatant after the media has gravimetrically settled. Settling time will vary according to the medium. As an alternative procedure, pour the slurry into the column and pump liquid at a sufficiently low flow rate upward through the suspended bed and out over the rim of a column (wet floor area) or through an adjuster with the bed supports removed (to prevent plugging). At suitable flow rates, this efficiently removes fines with no media loss.

Column. Before use, clean the column and inspect component parts for damage. Follow the manufacturer's instructions to assemble the column and perform hydrostatic integrity testing as recommended. Consider providing isolation valves at the column inlet and outlet.

If the column was assembled in ambient temperature and subsequently moved to a cold room, or vice versa, check the fittings and seals because differential expansion of the component materials can occur. This usually affects the seals or induces stress or constriction in the structure.

Bed Supports. If you are using plastic bed supports such as polyethylene (PE), polypropylene (PP), polyamide (PA), or polyether ether ketone (PEEK), check for plastic fatigue and cracking before packing. Ensure there are appropriate wetting procedures in place for the specific plastic material you are using.

If sintered polyethylene bed supports are used, thoroughly degas them before fitting them to the top cell. Use completely dry sinters because partly wetted sinters are more difficult to degas.

Soak the sinter in alcohol (EtOH, IPA, MeOH), ideally at concentrations >80%, to degas (or wet). Exchange the alcohol with packing buffer after thoroughly wetting the sinter. Avoid denatured alcohols because certain secondary components can irreversibly swell some plastics.

If stainless steel bed supports are used, check for corrosion and make sure the use history of the support doesn't contraindicate its use (for example, don't use the same bed supports for anion exchange (AEX) and cation exchange (CEX) media). Be certain the bed supports are not plugged by the resin.

Locate pilot and production columns in their process environment before packing because moving a column once it's packed is not practical. You will require mechanical handling devices for moving the column and top cell assembly of larger columns (440 mm diameter and larger). Auxiliary systems, such as pneumatic feed or air lines for inflatable seals, add to the size of a production column.

Before packing, use a level or fill the bottom cell with mobile phase to ensure the column is level so the bed packs evenly.

Columns are designed to provide excellent flow distribution with very low-pressure drops derived from equipment geometry, which allows for optimal chromatographic performance. Your packing procedures can take full advantage of the pressure–flow rate characteristics of the media employed in the purification step.

System. Columns can be packed as part of an integrated system or not, but system design does have a significant impact on chromatographic separation. You may find that a perceived packing problem is nothing more than poor system design.

A well-designed system should have:

- a pump that accurately delivers desired flow rates against significant back pressure
- a bubble trap, which removes air and dampens pulsations
- an injection port or recycle loop for the mobile phase
- pipework that minimizes sample dilution effects (All pipework used for processing should be equivalent in diameter, have sensors close to column outlet, minimize holdup volume, and not contain any deadleg configurations.)
- protocols to ensure that all measurement equipment is appropriately calibrated.

Flow Pack

Flow-packing (constant pressure or flow) entails pumping mobile phase through the sealed column. This causes suspended particles to be packed more quickly and uniformly than gravity settling, reducing the tendency for larger particles to settle first. The higher packing flow rate (usually 30% greater than that used during operation) also reduces the likelihood of further postpacking bed compaction.

Flow packing is often the method of choice for soft gel media. With soft gel media such as dextrans, agaroses, and some celluloses, the particles can further compact during column operation, so it is recommended you have an adjustable end cell so any void (or headspace) that develops under the end cell can be removed.

You can use this method only if the total column volume and the adjuster length are adequate (otherwise filler or extension tubes may be required). This procedure requires higher packing flow rates than others, which can necessitate a larger pump. Flow-packing smaller particles is generally not recommended because it results in a bed less densely packed at top than bottom.

Packing flow rate determination. When using the flow packing method, pack the column at a higher flow rate than that used during chromatography. This minimizes further settling or "packing" later in the process that can negatively affect separations and require remedial actions. Use a column at least 10 cm in diameter to determine optimum packing flow rate and reduce wall effects (a mechanical supporting action produced by the column tube wall that is most pronounced within 30 particle diameters from the wall). Define bed height using a range (for example: 20+/- 1 cm). Once you have determined the bed height for the separation step, you can calculate the amount of gel needed for column packing, taking gel compressibility into account. Gel compressibility depends on the gel and can range from 0% to as much as 40%.

Identify the optimal packing flow rate by measuring the pressure differential across the column at incrementally increasing flow rates. For soft and semirigid gels, the pressure drop will typically increase dramatically upon reaching a critical flow rate (medium and column dependent), forming an asymptote in the pressure-flow curve. An optimal packing flow rate for the medium column combination is generally 65–90% of this value. This is especially important when scaling up because the increased pressure differences due to the wall effect plus lower column pressure ratings of larger-scale separations can affect the process (2).

Rigid, practically incompressible matrices, such as controlled pore glass and silica, exhibit a linear pressure flow relationship. Pack them at the highest flow rates possible to form the most densely packed bed.

During these flow excursions, be careful not to exceed the manufacturer's pressure rating for either the column or the chromatography media. As always, heed manufacturer's recommendations for column and medium, which often include recommended operating linear velocities, bed heights, and compression factors.

Packing procedure. Pack the column in a controlled temperature environment, ideally at the same temperature at which it will be operated. It is best not to pack the column at a lower than operating temperature because increases in temperature can release air from the mobile phase within the column, disrupting the packed bed and impeding flow distribution. Completely temperature equilibrate both the column itself and the media before packing.

The first step in packing a column is to fill the fixed end of the column with packing buffer to a height of 2–3 cm, and then evacuate any entrained air in this end. Then, totally remove the adjuster assembly from the column.

Next, slurry the matrix or resin to the manufacturer's recommendations, generally between 50% and 75% settled matrix. If the slurry is too thick (>75%), it retains air bubbles. Thinner slurry, approximately 50%, is better for pressure packing. Swell the matrix initially in degassed mobile phase or in mobile phase at an elevated temperature. As the mobile phase cools, gas bubbles dissolve. Ideally, the matrix slurry should be deaerated before packing. Deaeration is usually done by leaving the resin undisturbed for about 12 hours, which may not be practical on a large scale.

Manually transfer the media to the column by containers or by pumping into the open column using a slurry pump. The best approach is to pour the slurry into the column down the sidewall, avoiding the generation or entrapment of air bubbles. Ideally, the entire column contents should be poured all at once to aid uniform packing. If the slurry volume is greater than the column volume, a tube extension is required.

After all the media is transferred to the column, stir gently but thoroughly in situ to ensure it is a homogenous mixture. This critical step often takes longer than anticipated, especially with large columns.

Allow the slurry to settle under gravity or reinstall the adjuster assembly and pack the bed flow. Reinsert the adjuster into the column tube and lower to a point just below the surface of the slurry. By the time the adjuster is lowered to the surface, the uppermost layer of the slurry should be relatively media-free. By breaching the air–liquid interface with the adjuster, maximal air clearance (via seal bypass) can be achieved. Then, seal the adjuster (for example: O-ring, wedge seal, or inflatable seal), and lower the adjuster approximately 0.5–2.0 cm, venting any remaining air through the open top process port. Once all the air is vented, the column can be connected to the packing–processing system.

Next, flow-pack the bed downward at the predetermined flow rate or pressure. When the compacted bed height does not vary with time (for opaque column tubes, 45 min is adequate), mark the the height of the packed bed on the column tube. Stop the flow (it is very difficult to lower the adjuster of large diameter columns against significant back pressure) and ease the adjuster seal slightly.

Remove any remaining visible air by lowering the adjuster cell. Alternatively, you can lower the cell at a slight angle to aid in clearing the air trapped under the center of the bed support. However, the top cell must be leveled before it contacts the bed. If bubbles are trapped against the bed support fixing ring, you can insert a hypodermic syringe with stainless steel tubing down the side of the seal to aspirate the trapped air bubbles.

Lower the adjuster cell to a point approximately 2 cm from the mark (for opaque column tubes, lower to a height based on expected compression or until resistance is encountered). Tighten the adjuster seal and reapply flow. Repeat this process until the adjuster face contacts the bed's surface. At that point, lower the adjuster no more than about 0.5 cm into the bed.

Fixed bed height columns may require the use of a packing device to pack the bed. If a packing tube is used, drain the liquid to a level below the coupling after formation of

the bed. Then, remove the extension tube, and attach the top, fixed adapter. Reapply flow and continue as previously described.

Following completion of the final adjuster lowering step, equilibrate the column with two or more column volumes of packing or equilibration buffer at the prescribed processing flow rate.

Dynamic Axial Compression (DAC)

Dynamic axial compression (DAC) has several advantages over flow packing or pack-in-place procedures. Axially compressing the media slurry accelerates the packing process, reduces size segregation due to gravity settling, and provides a constant compressive force to the whole bed. The dynamic axial piston eliminates the formation of voids or channels in packed beds. This ensures a uniform distribution of the mobile phase across the entire bed. Only one packing step is required, compared with the multiple steps employed in flow-packing. The control strategies can automatically adjust for any post-packing bed compression. And, the process can be automated, which ensures better control and repeatability. DAC is particularly beneficial with small and rigid particles and is the preferred method of packing for pilot and production columns.

Interference of bed supports with the compressive force is a limitation of DAC. It results in a bed that is more densely packed immediately next to the piston end and less densely packed at the distal end. You can reduce this by packing as quickly as possible, because the fluid exiting through the distal end has the effect of flow-packing — it improves the bed compression and compensates for the lower axial compressive force at that end.

Packing procedure. Pour the slurry into the open column. Insert the adjuster and engage the seals, and clear entrained air by lowering the adjuster cell. If you are using mesh bed supports, the free passage of air through the bed support, as it is moved through the air gap to contact the buffer interface, facilitates free passage of air. Close the upper process port while opening the lower process port. Lower the adjuster so that a constant preset pressure is exerted on the bed until it reaches a preset bed height (calculated based on desired compression factor). Adjusters typically include a controller and employ a mechanical drive, pneumatic pressure, or hydraulic pistons. Prevent bead crushing or equipment damage using feedback from appropriate sensors monitoring the process. The final result is a homogenously packed bed that has good contact with the distributor plate. Total pack time is typically less than five minutes.

The DAC control system automatically compensates for any subsequent settling of the bed. If the adjuster cell is locked in place after DAC packing, it should be released if height compensation is needed later. Apply pressure to recompress the bed and lock the adjuster cell in its new position.

Pack-in-Place

By incorporating multifunctional integrated column valves into the flow cells, pack-in-place employs essentially the same approach as in flow packing but with the added advantage of not having to remove the adjuster cell from the column, provided a contained column filling operation is involved. This method facilitates packing in larger columns and in columns with fixed bed heights.

Pack-in-place methods involve pumping the media slurry into the column where the adjuster cell has been preset to a fixed bed height. The packing pressure is preset on the slurry transfer skid and the slurry pumped in via the fixed valve until the pump stalls, which indicates completion of packing. The packing pressure may be varied to match the characteristics of the media being packed.

Guidelines

- Column's fixed end should contain 2–3 cm of packing buffer before slurry transfer.
- Air should be removed from the fixed end before packing starts.
- Air permeability is highest when steel bed supports are dry.
- Optimization studies can be expedited when conducted on lab-scale columns. However, consideration should be given to wall effects and column design when scaling up.
- Column should always be level.
- Adequate media definition is necessary to prolong the life of bed supports and to ensure reproducible packs.
- Organic solvents may cause pronounced shrinking or swelling of synthetic matrices.
- Following completion of a pack, make sure the top flow distributor is always in contact with the bed. A gap will act as a dilution zone, resulting in band spreading, loss of efficiency, and substantial tailing.
- If air entrainment in the adjuster cell is a problem:

1. Ensure there is clear liquid immediately under the bed support (if an opaque column tube is present, flow downward and gently raise the adjuster 0.5-1.0 cm from the bed).
2. Isolate the column with valves.
3. Lower the adjuster slightly.
4. Quickly open the top adjuster port to vent. If necessary, reposition the adjuster to the predetermined packed bed height.

Qualifying the Column Packing

Once the column is packed, perform a series of standard qualification tests. The results of these tests will not predict the success of the actual chromatography step, but they are useful for determining column packing efficiency and reproducibility.

Qualification metrics must be appropriate for all the columns in the process. Typically, height equivalent to theoretical plate (HETP) and asymmetry (A_S) are selected for bed characterization, and a full pressure-flow curve may be the metric to establish the pressure drop for the processing flow rate.

Pulse injections. Qualification is usually done by determining bed characterization using pulse injections of a nonreactive marker. The marker can be an internal standard selected to mimic the protein to be processed. Alternatively, it can be a generalized standard such as acetone, p-aminobenzoic acid (PABVA), NaOH or NaCl, chosen for its ease of detection. Inject the marker at process flow rates if no internal standard exists. The marker can be detected by conductivity, absorbance, or refractive index. Sample volume is usually a percent of the column volume (1% is typical) or a standard column height independent of bed volume (5 mm is common).

Pulse injections are used to assess the uniformity of the packed column or bed integrity. Therefore the injection volume should be sufficiently large so the hold-up volumes do not dilute it. Conventionally, the metrics used to assess column packing efficiency are HETP and A_S.

Frontal analysis. You also can qualify columns by determining bed characterization using the frontal analysis of phase transitions. This technique is growing in popularity because of the presence of many of these transitions points during a process

Table 1. The following table provides some troubleshooting tips for common column packing problems.

HETP/Asymmetry	Potential Cause	Corrective Action
High/Acceptable	Bed not packed tightly enough column	Lower adjuster, level
High/Tailing	Injection (loop) not optimized	Check, modify loop
	Bed supports fouled	Inject in reverse direction to verify, clean, or replace supports
	Liquid gab between adjuster, bed	Lower adjuster
	Air entrainment in end cells	Purge air
	Solute interacts with resin	Alter solvent composition, changer tracer molecule
	Bed not packed tightly enough	Lower adjuster, level column
High/Fronting	Channeling in bed	Lower adjuster, repacking may be necessary
Acceptable/Tailing	Injection (loop) not optimized	Check, modify loop
	Dirty or worn bed supports	Inject in reverse direction to verify, clean, or replace supports
	Air entrainment in end cells	Purge air
	Bed no packed tightly enough	Lower adjuster, level column
	Solute interacts with resin	Alter solvent composition, changer tracer molecule
Acceptable/Fronting	Channeling in bed	Lower adjuster, repacking may be necessary
Other Symptoms		
Split Peaks	Extreme channeling in bed Peaks with Shoulders	Lower adjuster, repacking may be necessary
	Injection (loop) not optimized	Check, modify loop
	Plugged bed supports	Inject in reverse direction to verify, clean, or replace supports
	Massive air entrapment	Purge air
High Pressure Drop	Bed supports not fully wetted	Inject 30% EtOh if materially compatible
	Dirty bed supports due to fowling or fines	Clean or replace supports
	Media deterioration	Remove fines from media
	Contaminated media	Chemically clean media
	Media deformation (soft gels)	Reduce flow rate or repack
	Flow path restriction	Check valves, prefilters, increase pipework diameter

run (for example, regeneration to equilibration or equilibration to sample). An analysis of the pack can be made any time the mobile phase is changed to a solution with a different quantifiable characteristic. This assumes the marker does not interact with the column or media. The marker can be detected by conductivity, absorbance, or refractive index.

Frontal curves and pulse injection peaks don't look the same, but they can provide precisely the same data. If the frontal curve is uniform, it indicates the packing is of high quality, as would be the case when a narrow, symmetrical injection peak is produced. The frontal peak data can be manipulated to compare it with a pulse injection peak. Taking the first derivative of the frontal curve creates its analogous injection peak.

References

(1) Rathore, A. et al., "Qualification of a Chromatographic Column: Why and How to Do it", *BioPharm Int.* 16(3), 30–36 (March 2003).

(2) Stickel, J.J. and Fotopoulos, A., "Pressure-Flow Relationships for Packed Beds of Compressible Chromatography Media at Laboratory and Production Scale", *Biotechnol. Prog.* 17, 744–751 (2001).

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