Procedure

Develop your column packing methods based on pressure-flow measurements

Scaling up your chromatography process might appear as simple as increasing the diameter of your column while you keep the same resin bed height and flow velocity (i.e., residence time). In practice, however, you will also need to consider the method of column packing, hydrodynamic pressure drop over the packed bed, and efficiency of liquid flow distribution.

A typical problem during scale-up is the loss of wall support for the chromatography resin because of the increased column diameter. This causes larger compression of the resin and an additional hydrodynamic pressure drop. Here, we provide you with an overview of the pressure-flow relationship for compressible agarose chromatography resins in packed beds. We also describe how to optimize bed compression when developing packing methods for scale-up in pilot-scale columns that are traditionally flow-packed, like BPG columns.

Introduction

The pressure generated when liquid flows through a packed bed is influenced by factors such as linear flow velocity, liquid viscosity (temperature dependent), and the permeability and height of the packed bed. The permeability of the packed bed depends on:

- Particle size and porosity of the chromatography resin
- Compressibility of the resin particles
- Actual compression of the packed bed

Column wall effects, heterogeneities in the bed structure, and the pressure loss over the bed support also affect the overall permeability and pressure drop over the packed column. When you design a process and characterize the column setup, you need to consider both the pressure drop over the packed bed itself, the pressure loss over the column hardware, and the flow path upstream and downstream of the column.

In this guide, we show you how to obtain the parameters needed to develop a 2-step packing method including settling and axial compression for pilot- to large-scale chromatography columns such as BPG columns. Alternative methods such as 1- or 2-step flow packing with low or high settling flow can also be used. However, you can achieve improved robustness from a method with consolidation and mechanical compression depending on resin type (2–4).

The relationship between pressure and flow velocity is dependent of the structure of a chromatographic bed and is expressed in the Kozeny-Carman equation (1), *see* equation 1:

Eq. 1

$$\Delta p = \frac{kuL\mu}{d_p^2}\frac{(1-\varepsilon)^2}{\varepsilon^3}$$

where

Δp = pressure drop over the packed bed
u = linear flow velocity
μ = fluid viscosity
L = bed height
d_p = average particle diameter
ε = void fraction (bed porosity)
k = constant, typically assumes a value between 150 and 180

Pressure drop (pressure differential) over the packed bed is important for optimal chromatographic performance. The additional pressure loss generated by the column and system also needs to be considered.

The Kozeny-Carman equation shows a second order dependence on particle size. Furthermore, the void fraction is very important for the resulting pressure drop. The change in void fraction is difficult to predict as the particle itself exhibits a degree of compressibility. In addition, the compression and void fraction are not homogeneously distributed within the packed bed. Hence, the change in pressure drop over the packed bed upon compression needs to be determined experimentally for semi-rigid and compressible chromatography resins.

Compression and packing factors

The pressure drop over the packed bed is strongly dependent of the bed compression. The degree of bed compression is optimized during packing method development and the information is used to yield bed structures with good performance over a wide operating range. The degree of bed compression is calculated by comparing the final packed bed height with the height of the consolidated bed or the gravity settled bed. Depending on the conditions, use the packing factor, *PF* (when consolidated, settled by flow) and the compression factor, CF (when settled by gravity), *see* equation 2:

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Eq. 2

Packing factor, $PF = L_{cons}/L_{packed}$

Compression factor, $CF = L_{settled} / L_{packed}$

where

L_{cons} = consolidated bed height, that is, bed height measured after settling the resin at a given flow velocity (cm)

L_{packed} = packed bed height (cm)

$L_{settled}$ = bed height measured after settling by gravity (cm)

The bed height of a gravity-settled bed differs from the bed height of a bed settled at low flow (consolidated). Therefore, the compression factor (CF) should be separated from the packing factor (PF). In water, for example, the consolidated bed height (at 60 cm/h) is higher than the gravity settled bed height. For MabSelect PrismA™ chromatography resin, the *PF* would be 1.18 while the *CF* would be 1.10.

When you pack BPG and AxiChrom™ columns, use the *PF* in the packing procedure to calculate the packed bed height after the consolidation step. Use the *CF* in the resin preparation step to calculate the resin volume needed to pack a desired bed height.

For *PF*, the consolidation velocity applied during the experiment should always be stated, as *PF* will vary with flow velocity. Typically, consolidation velocities for agarose-based resins are between 30 and 60 cm/h.

Note that different slurry buffer/solution components may significantly affect the settling and consolidation of your resin, and therefore also affect the *PF* and *CF*. The settling/consolidation of different resins can be different in different buffers/solutions with the biggest effect on ion exchange resins and some affinity resins in ionic solutions. If you are measuring and packing resin in ionic solutions investigate their consolidation in the specific solution compared to water to ensure stable reading.

Determine the specific pressure drop for your systems and empty column

To understand how the pressure drop is affected the individual contributors to the pressure drop — pressure loss over the empty (liquid filled) column and the flow path both upstream and downstream of the column —should be characterized.

The characterization is usually performed by sequentially determining the pressure drop over individual or combined subsystems. When doing this, place the pressure sensor in the same location during all pressure-flow measurements, preferably as close to the column inlet as possible. You should use an additional pressure sensor at the column outlet to simplify the procedure. Because of the potentially nonlinear behavior of the pressure-flow relationship, perform the analysis for a range of flow velocities to give yourself a margin of error to cover the flow velocities that will be used in the purification process for the packed bed.

Open bed measurements to determine critical velocity

To determine the maximum bed compression that allows for stable operation of the packed bed, you run pressure-flow measurements over an open bed. An open bed is defined as a consolidated (low-flow settled) bed in a column where the top adapter is not in contact with the upper bed surface.

The relationship between flow velocity, pressure, and bed compression is determined for a specific combination of chromatography resin, column, packing buffer/solution, and target bed geometry (diameter and height). One objective of this analysis is to determine the critical pressure and velocity, which are the pressure drop and corresponding flow velocity (for the given liquid properties) that can be achieved. Pressure drop and flow velocity are measured until bed failure, that is, at a point before flow velocity cannot be increased further because pressure would be too high.

The analysis is performed by measuring compression of the bed and pressure drop as a function of the flow velocity (Fig 1). The experiments can be performed in columns with transparent column tubes to enable monitoring of the bed height.

The experiments are preferably carried out with the packing buffer at the temperature with which you pack the column and run it in your process. An alternative is to carry out the tests with water at room temperature (viscosity 1 cP at 20°C) while you monitor and record the temperature so that the resulting data can be converted to other liquids.

Procedure to determine open bed pressure flow curve in a BPG column:

1. Add the chromatography resin slurry into the column and mount the adapter at its top primed position. Gently prime the top mobile phase by forcing the liquid to pass up through the adapter while moving the adapter slowly downwards. The adapter seal should slide against the column tube.
2. The bed is consolidated using a low flow velocity (30 to 60 cm/h) — generally 30 cm/h for resin < 50 µm in bead size or 60 cm/h for resins > 50 µm bead diameter. Differences in initial settling flow exist and must be determined for each respective resin. The consolidated bed height where all resin has settled is the reference point. As this reference point is determined by settling using flow (as opposed to a gravity settling of the slurry), bed compression is expressed as *PF*. This considers the relationship to the bed height at the reference point and the bed height at higher velocities applied during the test.
3. While keeping the adapter at the top position, the bed is compressed in the next step in increments by stepwise increase of the flow velocity. Bed height and pressure drop over the bed under stable conditions are recorded. Typically, six or more data points should be recorded between the reference point and the expected critical velocity for the chosen resin. The flow velocity at which the slope of the curve becomes infinite is the critical velocity (red curve, V_{crit}, Fig 1).
4. The maximum operating pressure ($V_{max.\,op.}$) is established by plotting the pressure against the linear flow velocity to determine the critical velocity (V_{crit})for the open bed measurements.
5. The resin compression achieved when increasing the flow over the open bed is also plotted (Fig 1.) as a function of the velocity presented as *PF* in the curve. The *PF* is defined as:

$$PF_{open\ bed} = L_{start\ flow\ height} / L_{read\ height\ at\ set\ flow}$$

6. Packing parameter data from this measurement is used based on the principle that V_{max} and P_{max} are 70% of V_{crit} and P_{crit}, respectively. Also, V_{max}.op and $P_{max.op}$ should be 70% of the $V_{max.\,pack}$ and $P_{max.pack}$, respectively.

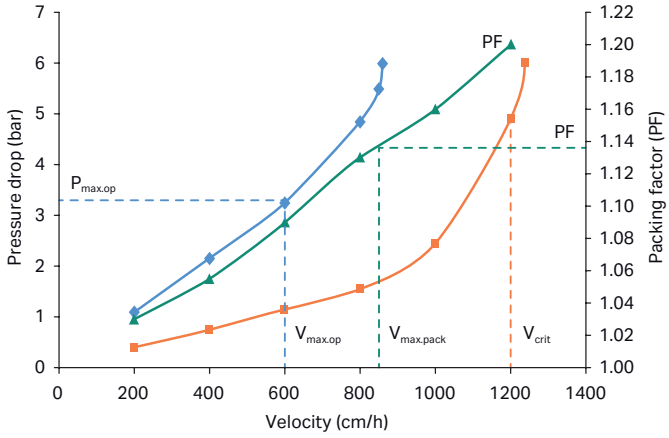

Fig 1. Example of how **packed** (blue curve) and **open bed** (red curve) for operation ($V_{max.op}$) are determined. The **PF** (green curve) is determined for $V_{max\ pack}$ (maximum flow velocity for packing the bed) and is used as a parameter for packing.

Determination of the *PF* for mechanical axial compression method

The parameters extracted from the curves in the open bed measurement are used to set the *PF* for axial compression for a packing at 30 to 60 cm/h depending on resin *PF* value for $V_{max\ pack}$. If the $V_{max\ pack}$ is approx. 830cm/h, this corresponds to approx. 1.14 in PF according to Figure 1. This means that your initial packing trials should be performed as follows:

1. Settle the resin at 30 or 60 cm/h depending on the resin bead (particle) size. Mark the level on the column when all resin has settled. Calculate with a *PF* of 1.14 (from above example) to where the adapter shall be moved for the axial compression, for example, bed height/*PF*, and mark this height.
2. Stop the flow rate, close the column top keeping the bottom open. Ensure smooth movement of the adapter without letting liquid to pass the adapter seals; For BPG columns, release the compression of the adapter O-ring so that the adapter can move down without letting liquid pass the adapter seal.

Note: step 2 is performed with a continuous adapter movement to ensure steady state of the packed bed. Stopping along the way down with the adapter might cause the resin bed to expand, which affects the final packed bed.

3. Move the adapter down at a steady continuous motion into the settled resin bed all the way down to the mark of the final calculated bed height, allowing liquid to be pushed out from the bottom of the column instead of past the adapter seal.

Note: Letting the liquid out through the bottom ensures that the consolidated bed is kept in place instead of being disturbed by upwards liquid motion.

4. When the adapter is at the marked final height for the set compression, the bottom valve is closed.
5. Before the packed column is subjected for performance evaluation, equilibrate the column in an upwards direction initially, which removes air trapped in the top of the column.
6. Evaluate the packed bed according to the *Column efficiency testing application note* (5) and evaluate bed robustness with blank runs and CIP procedures. A bed that is too compact bed will result in leading peaks and low theoretical plates (N/m), which can be resolved by decreasing compression. If the bed is too loosely packed, low N/m with peak leading and bed instability, which can be resolved by increased compression. Adjust packing parameters accordingly if required mainly focusing on axial compression level adjustment.

Pressure-flow measurements of packed column beds

Pressure-flow measurements of a packed column (blue curve, Fig 1) are performed to obtain data that represents the pressure-flow relationship of the column during operation. The data is used to predict the limit of the flow velocity and the limit of the operating pressure drop.

Compared with an open bed pressure-flow measurement, where the top bed support is not in contact with the bed, this measurement is conducted on a compressed bed. *CF/PF* from 1.10 to 1.20 is commonly used for agarose-based resins. The data shows either: 1) the flow velocity at which the column operation pressure will exceed the column's pressure rating; or 2) the flow velocity limit when the bed compresses to such an extent that a gap is created to the top bed support (*Vcrit*).

This analysis is performed by recording the increase in pressure drop as the velocity is stepwise increased. The analysis also shows whether it is the resin or the column that sets the flow velocity and pressure limits.

Conclusions

Chromatographic processes are typically developed for a specific scale. However, if you want to scale the process up from the smaller laboratory scale to a preparative chromatographic process in larger scale, there are a few additional factors to consider and we describe these factors in this article:

- To achieve a robust large-scale chromatography process, you need to know the limitations of the included resin and how it behaves under the conditions it is going to be used.
- Never exceed the maximum operating pressure.

References

1. Lars Hagel, L., Jagschies, G., and Sofer, G. eds., Handbook of process chromatography: development, manufacturing, validation, and economics, 2nd Edition, Academic Press, London (2008).
2. Article: Verified chromatography column packing methods for MabSelect PrismA™ resin. Cytiva, CY16029-20Jan21-AN (2021).
3. Article: How to pack Capto™ ImpRes resins using verified packing methods. Cytiva, CY20544-28Jun21-AN (2021).
4. Application note: Packing Capto™ S, Capto™ Q, and Capto™ DEAE in production-scale columns. Cytiva, 28925932 AC (2017).
5. Application note: Column efficiency testing. Cytiva, 28937207 AA (2010).

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