Development of column packing methods based on pressure-flow measurements

Scale-up of a chromatography process might appear as simply increasing the column diameter to accommodate for the higher quantity of material to be processed, while maintaining the same bed height and flow velocity (i.e., residence time). In practice, however, additional aspects such as method of column packing, hydrodynamic pressure drop, and efficiency of liquid flow distribution need to be considered. A typical problem to be addressed during scale-up is the loss of wall support for the chromatography resin as a result of the large change in aspect ratio, causing larger compression of the resin and an additional hydrodynamic pressure drop. This paper provides a brief overview on the pressure-flow relationship for chromatography resins in packed and compressed beds, and the optimization of a suitable degree of bed compression when developing packing methods for scale-up.

Introduction

The backpressure generated when a liquid is flowing through a packed bed is influenced by factors such as flow velocity, liquid viscosity (temperature dependent), as well as the permeability and height of the packed bed. The permeability of the packed bed depends on the particle size of the chromatography resin that form the porous bed structure but also on the compressibility and actual compression of the packed bed. Column wall effects, heterogeneities in the bed structure, as well as the pressure loss over the bed support can also impact the overall permeability and pressure drop. When designing a process and characterizing a column setup, it is important to consider both the pressure drop over the packed bed itself and the pressure loss over the empty column and liquid conduits upstream and downstream of the column. While the pressure loss (pressure differential) over the packed bed is relevant for an optimal chromatographic performance, the additional pressure loss generated by the column and system needs to be considered in regard to the maximum pressure of the column and system hardware. The hydrostatic pressure in a column and system can be larger than the pressure differential over the packed bed as long as the specifications for pressure differential over the packed bed and the maximum pressure of column and system are not exceeded.

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

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

where

Δp = *pressure drop*

u = linearly flow velocity

μ = liquid viscosity

L = bed height

d_p = average particle diameter

ε = void fraction

k = constant, typically assumes a value between 150 and 180

As can be seen from the equation, there is a second order dependence on particle size. Further, the void fraction is of outmost importance for the resulting pressure drop. As the particle itself exhibits a certain degree of compressibility and compression and void fraction is not perfectly homogeneously distributed within the packed bed, the change in void fraction is difficult to predict. Hence, the change in pressure drop over the packed bed upon compression of the bed needs to be determined experimentally for semi-rigid and compressible chromatography resins.

During scale-up by increasing the diameter-to-bed height ratio, wall support by means of frictional forces between the particles and the column wall is reduced. A small-diameter bed (< 30 mm) is supported by the column wall to a greater extent than a wider bed. In practice, the effect of a diminishing wall support is reflected in a lower critical flow velocity. The critical velocity is the maximum flow velocity that can be applied to a column and packed bed before the bed collapses.

Determination of the specific pressure drop for systems and empty columns

An experimental column setup should be designed and characterized so that the information obtained on pressure and pressure loss can be used in an unambiguous analysis. It is therefore required to characterize the individual contributors to the pressure loss, including the pressure loss over the empty (liquid filled) column as well as liquid conduits upstream and downstream of the column. The characterization is usually performed by sequentially determining the pressure loss over individual or combined subsystems. For example, the pressure drop over the liquid conduit upstream of the column (between the pressure sensor and column) can be determined in a first step by disconnecting the column. In the second and third step, the liquid-filled column and the conduit downstream of the column can be connected and the pressure loss over these individual contributors can be determined.

The pressure sensor should be located in the same place during all pressure-flow measurements, preferably as close to the column inlet as possible. The use of an additional pressure sensor at the column outlet can simplify the procedure. Due to a potentially nonlinear behavior of the pressure-flow relationships, the analysis should be performed for a range of flow velocities, typically with some margin covering the flow velocities that will be used in the process. With the detailed information obtained on pressure loss over the included system components, the pressure loss over the packed bed can easily be determined when column packing is performed.

Compression and packing factors

Pressure drop over a packed bed is strongly dependent of the degree of (mechanical) bed compression. The degree of bed compression is optimized and the information is used to yield bed structures with good performance over a wide operating range. The degree of bed compression is equivalent to the volumetric compression that is calculated when comparing the final packed bed volume with the volume of uncompressed resin allowed to settle using flow or by gravity. Depending on the conditions for the uncompressed resin, either the packing factor (*PF*), when settling by flow, or the compression factor (*CF*), when settling by gravity, is used. Flow settling methods are preferred as a wide range of buffers can be used and the resin volume can be determined within a shorter time period (approx. 1 h) compared with gravity settling methods. Results between both methods are often comparable when the settled beds are at rest (no settling flow). When using *PF*, however, the consolidation velocity applied during the experiment should always be stated, as *PF* will vary with flow velocity, especially when the settling has not ceased. Commonly, consolidation velocities for agarose-based resins are between 30 and 60 cm/h. When working with dynamic axial compression packing methods, the *PF* can be determined by detection of the height of the consolidated (yet not mechanically compressed) bed when settling using flow. In this case, the packing method will use the *PF* as reference rather than the *CF*.

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)

Note that the selection of liquid for suspending the resin (slurry buffer) can have a significant impact on the settling and consolidation of the resin, and thus on the *PF* and *CF*. It is recommended to always compare the height of a consolidated or gravity settled bed in a buffer solution with corresponding value determined in pure water.

Open bed measurements for determination of critical velocity

To determine the maximum bed compression that allows for stable operation of the packed bed, pressure-flow measurements are run over an open bed, that is, a consolidated bed in a column where the top adapter is not in contact with the upper bed surface. Hereby, the relationship between flow velocity, pressure loss, and bed compression is determined for a specific combination of chromatography resin, column, 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 prior to collapsing of the packed bed. The analysis is performed by measuring volumetric compression of the bed and pressure drop over the packed bed as a function of the flow velocity. The experiments are suitably performed in see-through columns with transparent column tubes to enable monitoring of the bed height. The experiments are preferably carried out with water at room temperature (viscosity 1 cP at 20°C) with temperature being monitored and recorded so that the resulting data can be converted to other liquids.

After introduction of the chromatography resin suspension into the column, the bed is consolidated using a low flow velocity (< 60 cm/h) and the consolidated bed height is determined as a reference point. As this reference point is determined with settling using flow (as alternative to a gravity settling of the suspension), bed compression will be expressed as the *PF*, that is, the relationship of the bed height

at the reference point (settling using flow) and the bed height at the higher velocities applied during the test. As a next step, the flow rate is stopped and the top adaptor positioned slightly above the surface of the consolidated bed (1 to 3 cm). Thereafter, the bed is compressed in increments by stepwise increasing the flow velocity while recording bed height and pressure drop over the bed under stable conditions. In practice, typically six or more data points should be recorded between zero and the critical velocity for the chosen resin. The flow velocity at which the slope of the curve becomes infinite is to be defined as the critical velocity. Figure 1 shows the result of such an analysis.

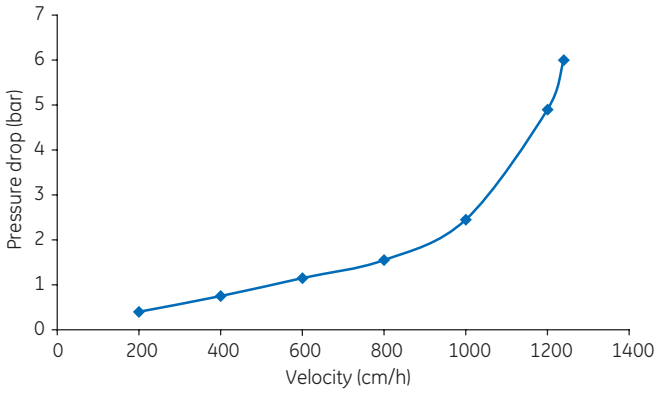

Fig 1. Example of open bed measurements for determination of critical velocity.

Application of critical velocity and maximum pressure drop for development of a packing method

Determination of maximum operating pressure

The maximum operating pressure can be established by plotting the pressure drop against the flow velocity to determine the flow velocity at which the slope of the curve becomes infinite, defined as the critical velocity (Fig 2).

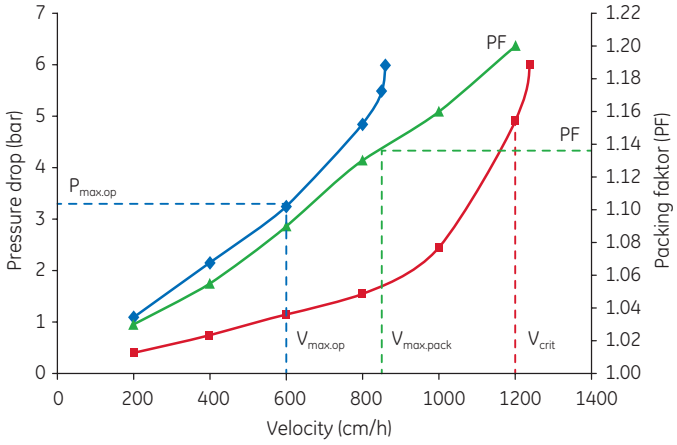

Fig 2. Example of packed and open bed measurements where the critical velocity (V_{crit}), the maximum velocity for packing ($V_{max.pack}$), and the maximum velocity for operation ($V_{max.op}$) are determined.

Sometimes, the flow velocity is instead plotted against the pressure drop. From such a plot, the critical compression pressure can be determined and the maximum packing velocity and pressure subsequently calculated. A common rule is that V_{max} and P_{max} is 70% of V_{crit} and P_{crit}. Similarly, the $V_{max.op}$ and $P_{max.op}$ should be 70% of the $V_{max.pack}$ and $P_{max.pack}$. The definition of the critical velocity is the maximum velocity at which the bed is at its maximum state of compression by flow settling and the slope of the curve becomes infinite. This is the highest achievable steady-state flow velocity for a packed bed at a given condition.

Pressure-flow measurements of packed column beds

Pressure-flow measurements of a packed column 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. A *CF* from 1.15 to 1.20 is commonly used for agarose-based resins. The main use of packed bed pressure-flow data is determination of the pressure drop during column operation. The data shows the flow velocity at which the column operation pressure will exceed the column's pressure rating or the flow velocity limit when the bed will start to compress to such an extent that a gap is created to the top bed support (resin rigidity limit). 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.

Conclusion

Chromatographic processes are typically developed for a specific scale. However, scaling of the process from the smaller laboratory scale to a preparative chromatographic process in larger scale involves consideration of aspects beyond the chromatographic separation process. To achieve a robust large-scale chromatography process, it is important to know the limitations of the included resin and how it behaves under the conditions it is going to be used. It is important never to exceed the maximum operating pressure. The maximum operating flow velocity can only be exceeded as long as the maximum operating pressure is maintained below its limit.

Reference

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)

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