Innovative Features of Bio-Rad® InPlace™ Chromatography Columns Simplify Packing Procedures for Any Media Type

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Introduction

The Bio-Rad InPlace process chromatography columns are designed for flexibility to suit packing needs at pilot and manufacturing scale. Every type of media from soft, highly compressible resin to rapidly settling, rigid, noncompressible beads benefit from the unique InPlace column design.

The packing procedures for the Bio-Rad InPlace column are similar to those for open column systems that are often used at laboratory scale and pilot scale. The packing protocols are directly scalable because the media bed is consolidated uniformly using axial compression packing, flow packing, or a combination of both. With alternative pack-in-place chromatography columns using a central nozzle, the bed often forms unevenly, with a dense region at the walls of the column and a less compact area near the center of the bed. In some cases the reverse is observed, with a very tightly packed bolus of material forming very close to the nozzle as the packing pump reaches its stall point.

The motorized piston of the InPlace column allows for precise and reproducible bed consolidation and packing based on optimized compression factors. Generally, the compression factor of a given media is defined as settled bed height divided by packed bed height and is influenced by the attributes of the media's base matrix.

With the Bio-Rad InPlace column, packed bed compression is regulated by the axial movement of the piston and can be programmed with the Bio-Rad InPlace control console independently of flow rates and pressure endpoints. Therefore, reproducible column performance is ensured at any diameter from 100 to 2,000 mm. Stall packing, which requires a pressure endpoint, can be unreliable if smaller-porosity frits are used or if fines have been introduced, and is sub-optimal for many types of noncompressible media.

The InPlace column slurry transfer valves are situated on the column wall rather than directly in the flowpath, which allows for better flow distribution with no areas of delayed flow due to centrally placed packing nozzles. The slurry transfer valves and manifold piping are designed for minimal shear of media beads during slurry transfer, creating fewer fines. The cleanability of the InPlace column has also been validated with bacterial challenge studies (Lefebvre et al. 2011).

Packing Various Media

Bio-Rad Laboratories has extensive experience packing columns with different types of chromatography media that require unusual or innovative packing techniques due to parameters such as compression factor (ranging from 1.0 to 1.7), settling rate, and attributes of the base matrix (Table 1). In most cases, packing methods are based on proven packing experience that can be applied to the attributes of the chromatography media's base matrix. For some challenging media types, packing methods can be adapted to obtain the easiest and most reproducible packing.

Table 1. Properties of different types of chromatography media.

Chromatography Base Matrix	Attributes	Compression Factor	Peak Asymmetry	RPH*	Bed Height, cm	Column Diameter, mm
Cross-linked agarose	Compressible	1.1–1.2	1.15–1.2	3.3–4.7	20–27	200–1,200
Cross-linked agarose (HP)	Compressible, suspended	1.05–1.20	1.0–1.4	3.5–4.5	20–36	450–800
Epoxide	Compressible	1.15–1.20	1.0–1.25	2.5–3.7	20–25	200–450
Methacrylate	Semi-compressible	1.1–1.25	0.8–1.4	2.5–3.5	15–28	200–1,300
Hydroxyapatite, silica, etc.	Noncompressible, rapidly settling	1.00	0.9–1.4	1.5–4.0	15–40	200–1,200
Cross-linked cellulose	Highly compressible, suspended	1.5–1.7	1.0–1.5	3.0–4.5	13–20	200

*Reduced plate height. This value, which is independent of bead size, is defined as the number of beads per theoretical plate. Optimal values for this parameter are generally below 5.0.

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Bio-Rad InPlace Column Packing Procedures

There are two steps to packing a process scale column: slurry transfer and bed consolidation/compression. For each of these steps, the Bio-Rad InPlace column offers several options, depending on the manufacturing environment (spatial constraints, equipment limitations, etc.) and the physical properties of the media (settling rate, compression factor, etc.).

Slurry Transfer

As shown in Figure 1, two proven methods for transferring slurry into the Bio-Rad InPlace columns are recommended:

- Pump transfer, using diaphragm pumps of the Bio-Rad media transfer device (MTD)
- Piston transfer, using the motorized piston as a large syringe

The method selected will depend on the column packing environment and media considerations.

Pump Transfer Method

The pump transfer method is preferred when it is desirable to transfer the entire volume of slurry into the column, eliminating the need for an overage of media. Using this method, it is necessary to calculate precisely the amount of media required for the target bed height, including the compression factor if appropriate. Knowing the exact slurry percentage is not critical as long as the appropriate amount of media is prepared in the slurry tank and the total volume of the slurry does not exceed the maximum working volume of the column.

Using the MTD is also advantageous for media that settle rapidly (for example, ceramic hydroxyapatite) and for procedures using air sparging for mixing in the slurry tank. Figure 1A shows the schematic of the setup.

The MTD uses a diaphragm pump for transferring the slurry; therefore, the slurry concentration should be 50% (v/v) or less to prevent stalling the pump. Priming of buffer lines or process inlets is unnecessary. To prepare the column for slurry transfer, the piston should be in its uppermost position, with the seal deflated. The slurry valves should be in the open position.

When all of the slurry is transferred from the tank into the column, the tank and transfer lines can be rinsed with reserved packing buffer. The volume of the rinse is usually less than 10 L. It is not important if air is introduced at this point, as the piston seal is not inflated. After the final rinse of the slurry tank and transfer lines, the slurry valves are closed and the media are allowed to settle briefly.

Once a supernatant layer of 2–5 cm develops, the piston is lowered into the buffer layer, the seal is inflated, and the entrapped air is removed through the upper process inlet. The column is then ready for bed consolidation and packing.

Piston Transfer Method

With the piston transfer method, automated loading is possible using the software in the Bio-Rad InPlace control console. The piston rises to a predetermined height to transfer the required volume of slurry into the column to obtain the final packed bed height.

The syringe-style method for slurry transfer is ideal for media that settle slowly (for example, high performance media with small bead size) and for slurries that may foam during transfer through a diaphragm pump (for example, some types of methacrylate-based beads). The piston transfer method is also useful for thicker slurries (> 50% v/v). Piston transfer often requires an excess of media and a known slurry concentration. The setup of this method is described in Figure 1B.

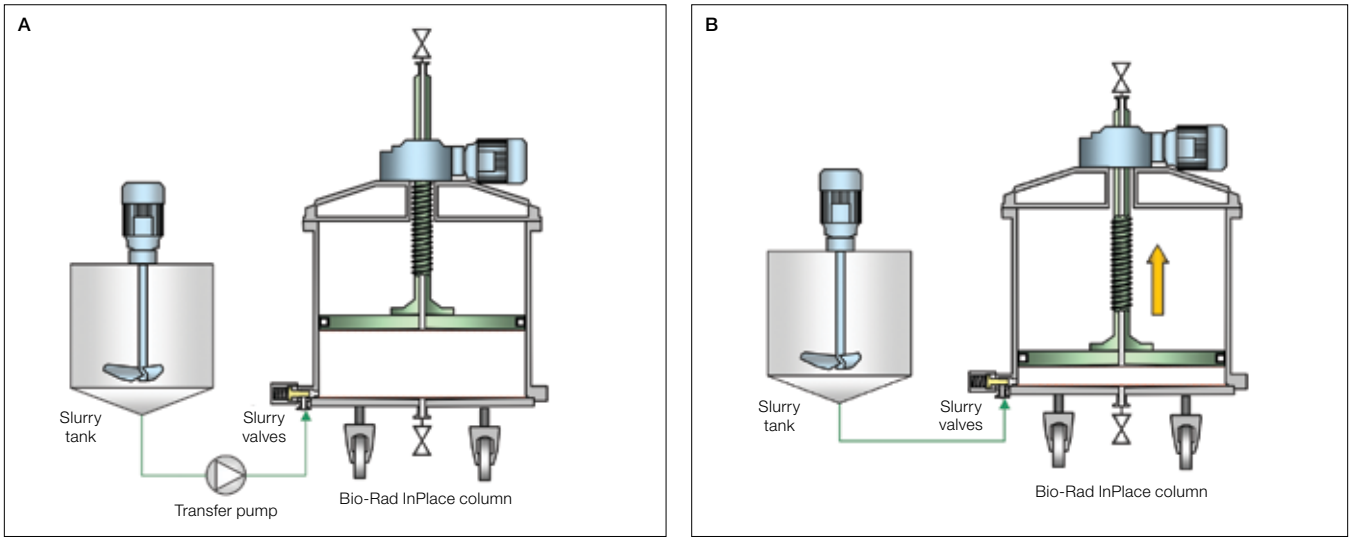

Fig. 1. Slurry transfer methods. A, pump-mediated transfer using diaphragm pumps of the Bio-Rad MTD; **B**, vacuum-mediated transfer uses the motorized piston as a large syringe to move slurry from the tank to the column by suction.

The piston is positioned at a height of approximately 5 cm from the bottom frit with the seal inflated and the column filled with packing buffer and the internal volume free of any air bubbles. The slurry manifold and slurry transfer lines should also be primed with packing buffer. When all lines are sufficiently primed, the slurry valves are opened and the piston is raised at 200–300 cm/hr to transfer the slurry via suction. The piston is moved upwards until the precise volume of slurry has been transferred; then the slurry valves are closed and the packing procedure can be initiated.

Bed Consolidation and Packing

Once the slurry is transferred into the column, the consolidation and packing procedures can be modified according to the media manufacturer's recommendations or the customer's specifications. Consolidation is the point at which beads suspended in the slurry have all migrated to the bottom of the column under the influence of flow and/or piston movement. It does not necessarily represent the final bed height since further compression is often applied.

As shown in Figure 2, there are three modes to consolidate the bed:

- Axial compression
- Flow packing
- Combination of axial compression + flow packing

Axial Compression Packing

This mode is used in cases where a process skid is not available in the column-packing area. The speed of the piston movement can be precisely set to consolidate the bed to reach the final height and, if necessary, further compress the bed (Figure 2A).

Flow Packing

This mode (see Figure 2B) may be used when a precise compression factor is of importance, allowing variations of the packed bed height within a certain range. Flow is provided by the process skid until the slurry is consolidated into a bed. At this point, the flow is stopped and the bed is allowed to rebound without any application of pressure. For most chromatography media, the rebound height is equivalent to the settled height and can be used to calculate the final packed bed height based on the recommended compression factor. If the rebound height is not equivalent to the settled bed height, then direct settling or centrifugation of smaller amounts of media may be necessary to determine the correct value. Flow packing is then followed by axial movement of the piston to the final bed height.

Combination of Axial Compression and Flow Packing

This mode offers the ease of axial compression with the flexibility of flow packing and can be used for any type of chromatography media. It is especially useful for media requiring a high compression factor, since the flow rate applied to the bed is the total of the axial movement plus the flow applied from the process skid. The process is shown in Figure 2C.

For all three consolidation methods, the compression factor and final bed height can be accurately regulated with the Bio-Rad InPlace control console.

Reslurrying and Unpacking

Unpacking in the Bio-Rad InPlace column can be completed with less than 2 column volumes (CV). By using air sparging to form a homogeneous slurry, less buffer is needed and there is no risk of mechanical shear to the media.

Reslurrying

After transferring the buffer into the preferred unpacking solution, the piston is raised and downflow from the process skid is initiated at a slightly higher flow rate to maintain a net positive downflow. When a headspace of 5–10 cm has formed, the flow direction is changed to upflow and approximately one column volume of buffer is introduced. If bed collapse does not occur within several minutes, flow

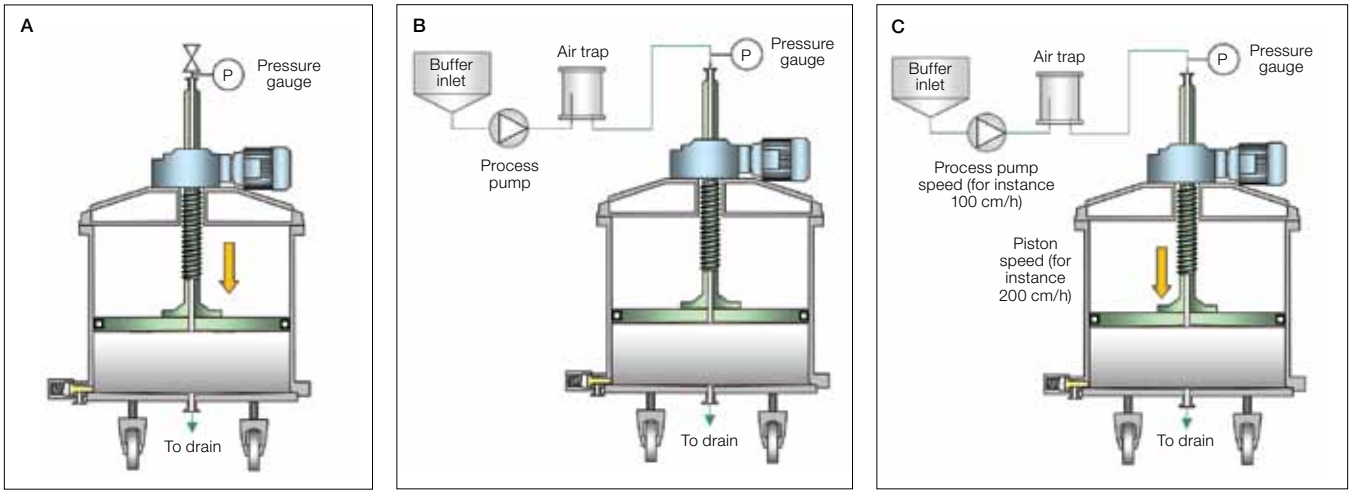

Fig. 2. Bed consolidation and packing methods. A, axial compression; **B**, flow packing; **C**, axial compression and flow packing. In this example, packing speed is 300 cm/h (process pump speed + piston speed)

from the skid is reversed again and the direction is alternated between upflow and downflow to raise and lower the bed until it collapses. This process may take 2–4 cycles.

At this point, the seal is deflated and the piston is moved to its uppermost position. Compressed air is applied to the bottom process valve and air is sparged through the bottom frit at 3 psi (0.2 Bar). The sparging continues until a uniform slurry is formed.

If repacking is required, the bed can be consolidated immediately following the procedures for pump transfer of slurry, without removing the slurry from the column.

Unpacking

Once the slurry is homogeneous, unpacking can be started by simply opening the slurry valves and using a diaphragm pump to transfer slurry from the column to a storage tank. With the addition of only 1 CV of buffer, the majority of slurry will be removed from the column.

In order to extract the residual media that coats the surface of the column walls and bottom frit, additional buffer can be pumped through the top spray nozzle to wash the sides of the column. Less than 0.5 CV of additional buffer is required to remove all visual traces of chromatography media.

Conclusion

The Bio-Rad InPlace column's innovative features offer great flexibility to meet the packing requirements of various media.

The motorized piston for axial compression provides reliable and robust bed compression without the necessity for high operating pressures during packing. With alternative pack-in-place columns, high flow rates and packing pressure endpoints are required to achieve the desired compression factor for a selected media. This can lead to inconsistencies in the amount of media packed into the column and in the actual bed compression if there are any changes to the buffer system or column connections, or if fines are introduced. Because the bed height and slurry transfer volume can be directly measured in the Bio-Rad InPlace column, the packed bed compression is consistent and reproducible from packing to packing.

With the proven scalable design principle of Bio-Rad InPlace columns, column packing protocols can be transferred from lab to pilot to process with minimal changes. With simplified slurry transfer and bed consolidation methods, even the most challenging chromatography media can be packed into a well-distributed and stable bed. Packing methodologies have been developed for virtually all types of chromatography media, and adapted solutions can be provided for unique procedural and environmental challenges.

References

Lefebvre S et al. (2011) Sanitization of a packed bed in the Bio-Rad InPlace process chromatography column. Bio-Rad bulletin 6061.

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