PROCESS-SCALE CHROMATOGRAPHY

Tech note 0343

Packing Sephacryl® S200 HR in the Verdot Ips² InPlace(TM) column

Sébastien Lefebvre, VERDOT Ips², Riom, France.
Martin Page, Sacha Müller, Nicolas Fouque, Merck,
Manufacturing Science and Technologies, Aubonne, Swizerland.

Summary

Size exclusion chromatography, also called gel filtration, separates molecules according to their size. While commonly used for purification at laboratory scale, this technique is also unavoidable in some applications at process scale for fine separation of impurities from molecules of interest. Such separation is very demanding in terms of packing performance (HETP, As) as it directly influences the purification yield and purity. Uniform and reproducible packing of the preparative column is thus essential.

VERDOT InPlace columns offer a unique solution for packing media like Sephacryl® S200 HR. One of the key features of VERDOT's InPlace column is the packing motor that can be configured for running at very low speed as required by this media. Another important feature is the InPlace slurry valves which enable transfer of media into the column in a syringe mode, and removal of under slight positive air pressure. The unique placement of the InPlace slurry valves around the perimeter of the column guarantees no interference with the distribution of liquid in the column or flow dynamics. The InPlace column also offers capability to combine air-sparging and tilting of the column during packing and unpacking to minimize the amount of buffer needed for bed reslurrying and unpacking.

This tech note shares a method for packing the Sephacryl S200 with high performance results. It was based on a packing study performed in collaboration with Merck Aubonne (Swizerland) with a packed bed between 33 and 35cm in a Verdot Ips² InPlace column Ø44.6 cm diameter.

Materials and Methods

Material and Equipment

A VERDOT Ips² InPlace column (44.6 cm diameter, 60cm tube height) was mounted with filters with ≤15µm absolute porosity, which is optimum for Sephacryl® S200 HR a 50 µm mean particle size. For automated operations, the InPlace column was fitted with a set of instrumentation that includes a rotary encoder for precise positioning of the piston and a pressure sensor transmitter for monitoring packing conditions.

The Piping & Instrument diagram (P&ID) in figure 2 shows a typical example of configuration for packing and unpacking. The valve numbering shown will be used later in this note.

In addition to this, a Basic Control Console or an automated InPlace Advanced Control Console controls the speed and positioning of the piston. The VERDOT Ips² Basic Control Console was used for this packing study.

Slurry preparation

Slurry Preparation: Sephacryl® S200 HR arrived in carboys combined with storage buffer. A volume of 79.7L was reslurried and poured directly in the column for being exchanged with 0.15M NaCl, used as packing buffer, by circulation of 2CV of packing buffer in downflow until the conductivity of the mobile phase exiting the column was stable. The settled bed was measured at 38.2cm after an overnight settlement in the packing buffer.

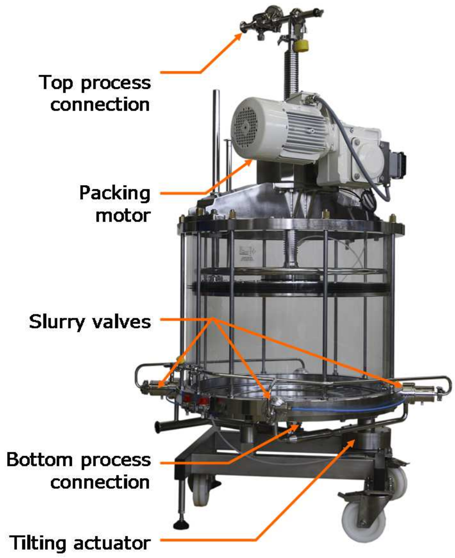


Fig.1. VERDOT Ips² InPlace column

The column was leveled using leveling adjustments on column.

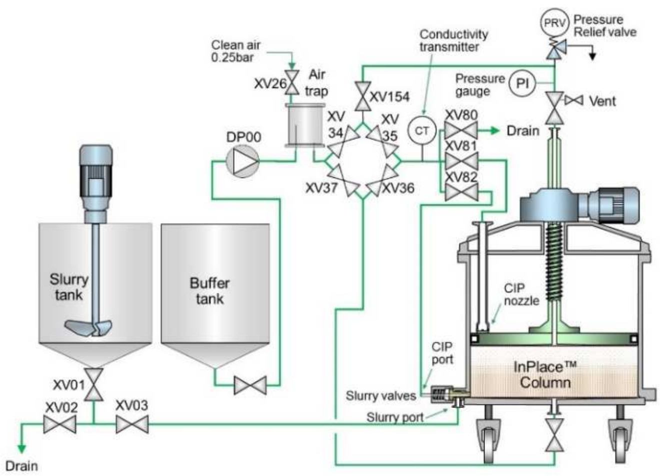
The column tube of 600mm long was offering insufficient height for reslurring in place using air sparging. Thus the top adaptor was removed, the packing buffer was injected through the bottom process port until the level of liquid in the column was at the maximum height authorized for re-installation of the top adapter. This corresponded to a slurry ratio of 75%. The slurry was homogenized using a paddle.

Column packing

Column priming

Packing buffer was added on the top of the slurry to form a supernatant. The top adaptor was remounted on the column, plunging in the clear supernatant. The pneumatic cylinder on the column was used to tilt the column and remove any remaining air bubbles.

Fig. 2 P&ID of the installation for packing and unpacking



The inflatable seal was inflated and the column was placed back into the level position.

The topside of the column top adapter was wiped clean to ensure that no debris or particulates were present.

The top process port was opened to the drain position (XV154-35-80). Using the control console, the top adapter was lowered at a speed of 15cm/h to prime the top process line until no bubble was visible through the transparent flexible hose.

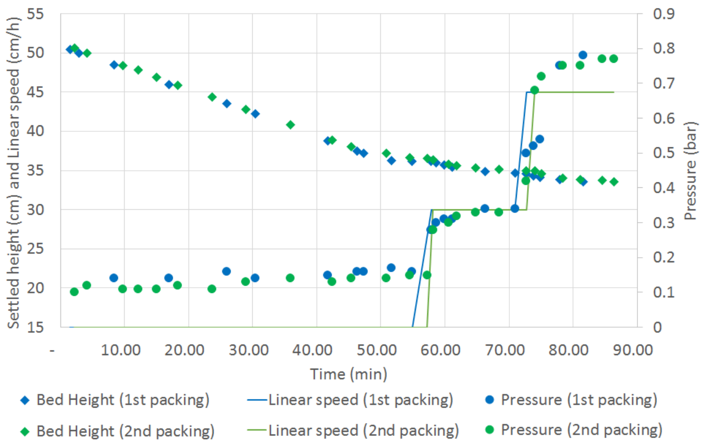
Column packing 1 & 2 with flow and axial compression

Using the process system, the packing buffer was injected through the column in downflow at 15cm/h. Once the media level settled at a stable height, the buffer injection with the system was stopped, and the piston was immediately lowered at 15cm/h using the packing motor so as to maintain the bed under a packing flow of 15cm/h. This transition from flow packing to axial compression packing allowed to move away most of the supernatant between the piston and the settled bed.

When the piston reached 2cm above the packed bed, the packing motor was stopped and the system was started immediately to inject the packing buffer through the column in downflow at 15cm/h. This linear speed once reached was increased to 30cm/h. Once the settled media height was reaching its new stable height, the flow was increased to 45cm/h until the settled bed height reached its new stable position. The buffer injection with the system was stopped, and the piston was immediately lowered at 45cm/h using the packing motor until the piston compressed the bed 3mm below the settled bed.

The here-after graph shows the evolution of the settled height and of the column pressure during the packing operation.

Figure 3: settled height and pressure evolutions during packing



Column equilibration and validation (HETP, As)

The column was equilibrated with packing buffer at 30cm/h in downflow for 1CV. For the HETP test, the installation depicted in figure 4, is ideal for the HETP test. It allows injection of a sharp marker peak while maintaining a constant flow. 1 and 2 are 4-ways valves.

The sample loop is a flexible hose with a volume corresponding to 1% CV. Please refer to our tech note DP-MKT-338 HETP test for more details on column qualification. Packing buffer was used as a baseline and 3% of acetone in packing buffer was used as marker. While the column was equilibrated, the pump P1 primed the loop with marker (valve 2 configured as valve 1 on picture). The loop was isolated by switching valve 2 as on picture. After column equilibration, valve 1 was switched as valve 2 on picture so as to push the sample in the column and the flow continued until the peak, measured with UV probe at 280nm, eluted from the column.

In the Fig 5, rHETP stands for reduced HETP = $\frac{\text{HETP (cm)}}{\text{Particle diameter (cm)}}$

Figure 4: marker injection layout

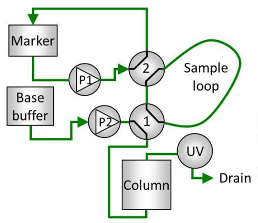
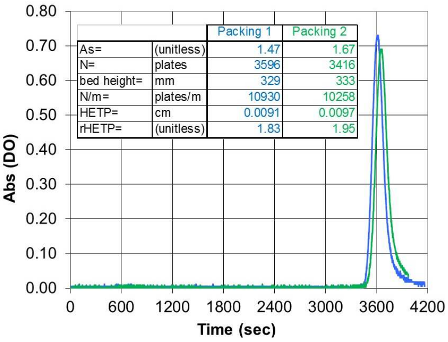


Figure 5: HETP curves during packing 1 and 2



Conclusion of the packing 1 and 2

The important success factors found during the packing campaign were the followings:

- No flow interruption during the packing: the transition between pump flow and axial compression must be smooth and almost instantaneous. Flow interruption, inducing pressure release and bed rebound, tends to reduce the plates number;
- No contact between the piston and the bed until the final compression.

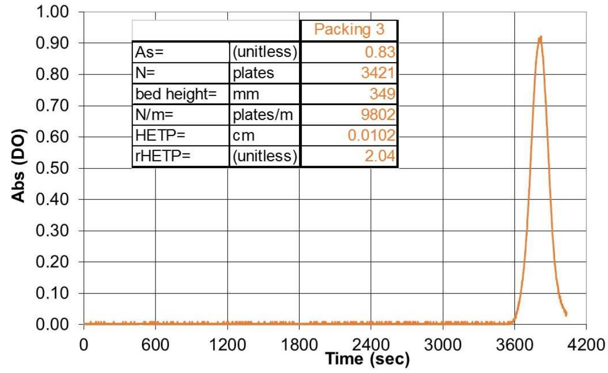
After the packing 2, the column was submitted to cycling with some steps in upflow and some other steps in downflow. It was found that the piston being not fully filled with media by lack of compression, the HETP increases after the cycling. As conclusion, the packing procedure was applicable when the bed is used only in one direction (upflow or downflow) but not alternatively in both directions.

For application which need to run the column in both direction, a 3rd packing was made with a slight evolution.

Column packing 3 with flow and axial compression

First, some more media was added in the slurry in order to obtain a final bed around 350mm. Otherwise, the method used was exactly the same as packing 1 & 2 except the final compression: the piston was lowered at 45cm/h using the packing motor until the piston compressed the bed 11mm below the settled bed, instead of 3mm during packing 1 & 2.

The equilibration and the validation were performed with the same method as after packing 1 & 2 and provided the following results:



Conclusion of the packing 3

Although having a slightly lower plate count, and some fronting instead of tailing for the Asymmetry, the column performance remained stable after the cycling with steps alternatively in downflow and upflow. This method is thus preferable for ensuring a bed stability in both directions.

With the special packing technique described in this document, the VERDOT InPlace™ column was a conclusive technology for obtaining a high performance packing at large scale.