PROCESS-SCALE CHROMATOGRAPHY

Tech note 0351

Packing UniPMM S-60M from NanoMicro in a 1.6M diameter Verdot® InPlace(™) column.

Sébastien Lefebvre, VERDOT Ips², Riom, France.

Summary

Suzhou Nanomicro Technology Co, based in China, provides a broad spectrum of products including high performance chromatography media, solid phase extraction (SPE) sorbents, particle size standards, magnetic particles, LCD spacers, ACF conductive particles, and various other customized functional microsphere products.

UniPMM S-60M is a Polyacrylamide-based chromatography media with 500µm pores size, and -CH2SO3- functional groups. It contains rigid, spherical, monodisperse microspheres of 59µm mean diameter. Despite their hydrophilic coating, the beads are more hydrophobic than agarose and tend to adsorb air bubbles, forming a foamy layer. Preventing this phenomenon induces some constraints in the packing and unpacking method, which were addressed during the packing exercise described in the present technical note.

This tech note shares a packing and unpacking experience of UniPMM S-60M, in VERDOT InPlace column. The method proved its efficiency with good packing results.

Materials and Methods

Material and Equipment

The VERDOT Ips² InPlace column is mounted with filters with ≤25µm absolute porosity, which is adapted for the S-60M media with a monodisperse 59µm mean diameter. For automated operations, the InPlace column is fitted with an instrumentation package that includes a rotary encoder for precise positioning of the top adapter and a pressure sensor transmitter for monitoring packing conditions. An Advanced Control Console controls the speed and positioning of the top adapter.

The Piping & Instrument diagram (P&ID) in figure 2 shows a typical example of configuration for packing. The installation for unpacking is the same but the pump DP01 must be inverted. The valve numbering shown will be used later in this note.

Slurry preparation

The media is supplied in liquid form. The storage buffer is exchanged with water and the slurry is prepared in an agitated tank with low shear stress impellers. As media tends to adsorb air making a foamy supernatant, Ethanol is added until the slurry is free of foam.

A sample is transferred to a drip column for determination of the slurry ratio, resulting in a measurement of 76.9%.

With a target bed height of 248mm and a compression factor of 1.09, the volume to load is calculated at 546L.

Media transfer with pneumatic diaphragm pump (DP01)

The topside of the column top adapter is wiped clean to ensure that no debris or particulates are present. The column is leveled using leveling adjustments on the column.

Using pump DP01 supplied with water (XV02 open), the slurry transfer line is primed to the CIP port of the slurry valves, connected to the drain.

Fig.1. VERDOT InPlace column D160cm with stainless steel tube

One of the key features of VERDOT's InPlace column is the InPlace slurry valve. 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 number of slurry valves is correlated with column diameter so that the efficiency of packing and unpacking in place is kept across the entire range of dimensions. The slurry valve works as a 3 way valve: when the InPlace slurry valves are closed, the Clean-In-Place (CIP) and slurry ports are open.

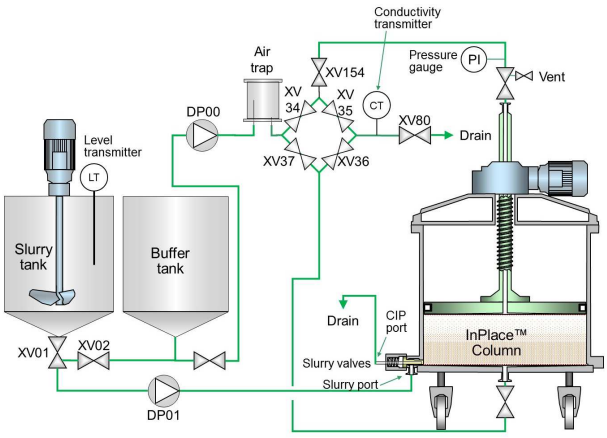

Fig.2. P&ID of the installation

With the top adaptor positioned at 50cm above the bottom filter, to be able to contain the full slurry volume, the column is filled with water from the bottom inlet until it overflows through the top process connection. The slurry valves are cycled (opened and closed) 10 times to release any air present in the lines. The inflatable seal is deflated and the top adapter is lowered until the inflatable seal is fully submerged. The InPlace column is equipped with a pneumatic cylinder which is used to tilt the column slightly to remove any remaining air bubbles. Once done, the inflatable seal is inflated and the column is placed back into the level position. The top process port is opened to the drain position (XV154-35-80).

Using the control console, the top adapter is lowered at a speed of 120 cm/h for a few centimeters to prime the top process line. Once primed, the valves are immediately closed. The bottom process line is primed by opening the bottom process connection to the drain position (XV36-80) and lowering the top adapter at a speed of 120cm/h for a few centimeters. Once primed, the valves are immediately closed. At this point, the column has been effectively purged with air and all slurry-contacting areas are filled with buffer.

Media transfer with DP01

The top process port is opened to the drain position (XV154-35-80), the bottom slurry tank valve (XV01) and the InPlace slurry valves are opened. Media transfer from slurry tank to column is performed using the pneumatic pump DP01 supplied at 2bar air pressure. Since the column is pre-filled with water, the volume of water flowing through the top process connection is equivalent to the volume of slurry entering the column. Because the media is sensitive to foaming, the transfer to column prefilled with water ensures that no foam is formed, as would likely happen if transfer was performed in an empty column. The volume of slurry is monitored by the level transmitter installed on the slurry tank. The volume of slurry to be transferred in the column equals the target volume of slurry calculated earlier (546L) plus the volume of the transfer line, as it starts filled with water and ends filled with media.

Once the correct volume of media is transferred in the column, the slurry valves are closed. The CIP port of the slurry valves is connected to a process system outlet so as to pump water through the CIP port down to XV1 and return the slurry to the tank before it settles in the slurry transfer line.

The column tube being stainless steel, it is not possible to assess if some air may be still present in the column. So, for safety, a purge operation is performed before continuing with the packing. With top process port still opened to the drain position (XV154-35-80), and bottom connection isolated, the top adapter is lowered at 75cm/h so bubbles can escape through the top filter. Once no bubbles can be seen through the translucent flexible hoses, the top adapter is stopped, and axial compression packing is started.

Packing in Axial compression

Packing was accomplished using axial compression. It involves lowering the top adapter at a constant speed to push the packing buffer down through the media and out the bottom process port, consolidating the bed.

The column's top process port is closed upstream of the pressure gauge (close XV154) and the bottom process isolation valve is opened to the drain (XV36-80).

This configuration allowed the bed to build from the bottom up to the top while avoiding bed drying. The top adapter is lowered at 100cm/hr, until the top filter is at 33cm height, thus approximatively 5cm above the settled bed. Figure 4 shows the evolution of the pressure drop during bed consolidation, indicating that the bed was consolidated from the moment when the top adapter reached 40cm above bottom filter, as the pressure drop was stable from this point. For finishing the packing and bed compression, the speed is lowered at 50cm/h, until the top adaptor reached the final height, i.e. 248mm.

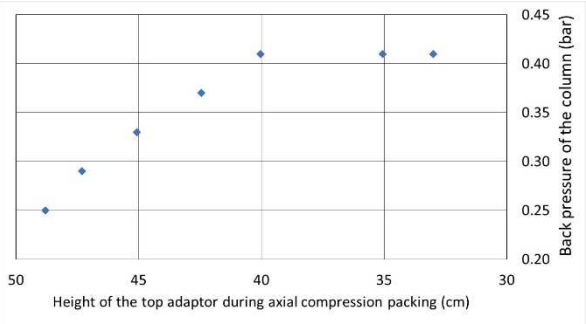

Figure 4 – Back pressure of the column during bed consolidation

Column equilibration and validation (HETP, As)

The column being equipped with a 3 way valve on the top connection, a pump and flexible hose is installed and primed with marker solution down to the 3 way valve. The marker solution used is 3% acetone v/v in water corresponding to 1% of the column volume, thus 5L.

The column is equilibrated with water for one column volume at 76cm/h in downflow. Then the pump of the skid is stopped, the 3 way valve switched to the marker line and a peristaltic pump is used for injecting the marker volume in the column. The 3 way valve is switched back to the process skid position and water is pumped again through the column at 76cm/h until the acetone peak elutes from the column. The UV absorbance curve can be seen in figure 6.

The plate count and asymmetry is calculated, as described in the technical note DP-MKT-338-00 HETP TEST:

N=4564pl/m
As=1.04
rHETP = 3.7

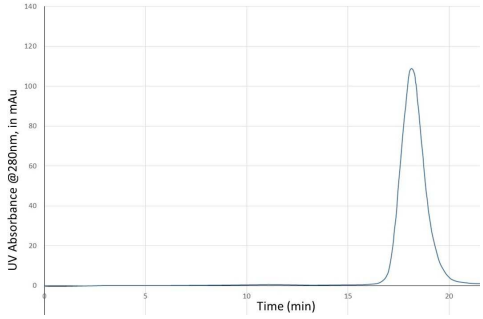

Figure 6 – Column validation (HETP, As)

Unpacking of the column

Because the media is sensitive to foaming, the use of air sparging for reslurrying is not appropriate. A more classical method is thus used. A headspace is created above the packed bed by injecting water for 5min at 120cm/h downflow while simultaneously raising the top adapter at 100cm/h, maintaining a net positive down flow. Once the headspace is created, the buffer flow direction is reversed to flow upwards from the bottom process connection, while continuing the raising of the top adapter. The direction of the flow is reversed every 5 min to collapse the bed. The pump and the top adapter are stopped when the top adapter has reached the height corresponding to 2x the column volume. This ensures an approximate 50% slurry concentration when fully suspended. A pneumatic diaphragm pump is installed downstream of the slurry valves discharging in the slurry tank. The slurry valves are opened and the pneumatic diaphragm pump is started, supplied with 2 bar air pressure to transfer the media from the column to the tank. When almost two thirds of the total volume (ie: 2 column volumes) is pumped out, the media transfer is continued while injecting water in upflow through the bottom filter at 50cm/h, so as to dissolve the eventual media chunks laying on the bottom filter. When 90% of the total volume is pumped out, the column is tilted and all slurry valves except the slurry valve positioned in the low position are closed. The media transfer is continued until the translucent flexible hoses are clear of media.

Conclusion

We were able to achieve excellent packing results in the large scale 1.6M diameter VERDOT InPlace column. The data indicate that the bed is uniformly distributed across the diameter of the column. The low shear design of the InPlace slurry valves ensures the integrity of the media during transfer. The dynamic axial compression capability of the InPlace columns provides quick and easy packing operations with a high level of reproducible performance. The InPlace column can be easily unpacked and rinsed in one hour despite the large scale of the column, using minimal equipment and buffer volumes.