PROCESS-SCALE CHROMATOGRAPHY

Tech note 0316

Packing GE Sepharose™ FF media in the Verdot Ips² InPlace™ column

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Summary

Testing was performed in order to evaluate the packing and unpacking capabilities of the Verdot Ips² InPlace column, using Sepharose™ media. A 14cm bed of GE Zinc Imac Sepharose™ Fast Flow media was packed in an 80 cm diameter Verdot Ips² InPlace column, using a compression factor of 1.15.

Two packings were performed. The initial packing was performed with an empty column, the second following the performance of re-slurrying within the column. Both packings were evaluated using HETP and Asymmetry tests and provided great results and reproducibility.

Prior to removal, air sparging and the InPlace columns tilting feature were used to re-suspended the media within the column in less than 20 minutes. Through the column slurry valves, all media was transferred into the slurry tank in less than 5 minutes.

Materials and Methods

Material and Equipment

The VERDOT Ips² InPlace column, (80 cm diameter, 60cm tube height) was equipped with an instrumentation package for semi-automated operations. Instrumentation package includes a rotary encoder and pressure transmitter. Column isolation valves were installed above the pressure transmitter and on the bottom process connection.

The Advanced Control Console was used for monitoring the bed height and packing motor functions.

An Endress & Hauser Coriolis massic flowmeter was utilized for measuring the volume of slurry transferred into the column.

Testing also included a chromatography process system, with:

- Column valve block, for configuring the fluid path in up flow/down flow/bypass
- Bubble trap with low pressure air inlet (0.25bar) for media slurrying

Slurry

Slurry Preparation: Several carboys of media stored in 20% ethanol were poured into a slurry tank and exchanged 1x with water. The packing solution was water.

Slurry percentage for the 170L was calculated at 52% by allowing slurry tank samples to settle in a graduated cylinder for 240 minutes.

Slurry transfer volume of 155.6L was calculated based on target bed height of 14cm and compression factor of 1.15.

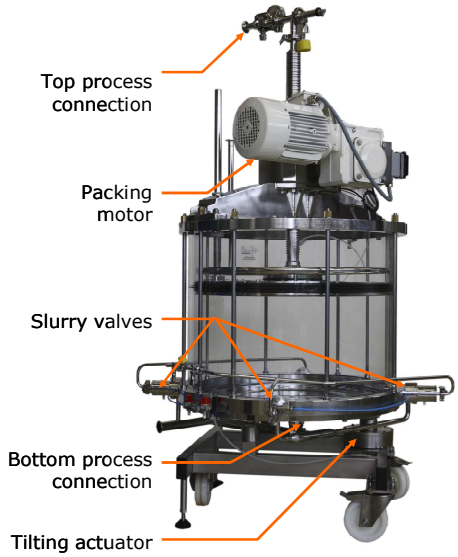


Fig.1. VERDOT Ips² InPlace column

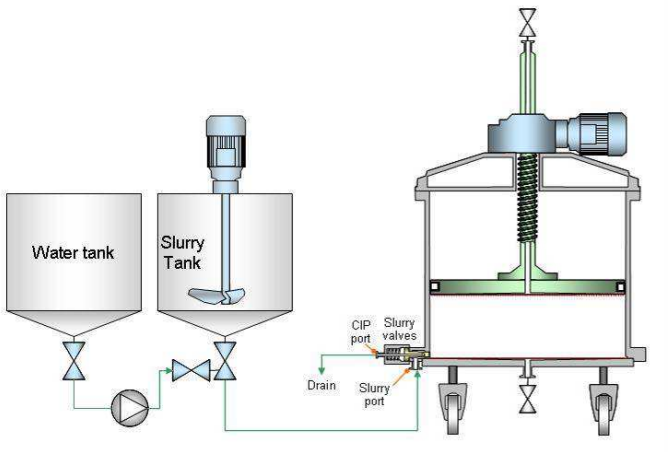
Media transfer

The topside of the columns top adapter was wiped to ensure that no debris or particulates were present. The column was leveled using leveling adjustments on column.

Column priming

The slurry valve manifold and transfer hoses were primed with water supplied through the slurry tank valve, entering the columns slurry ports and exiting the columns slurry valve CIP port. Priming operations continued until no air was observed in the solution flowing from the CIP port.

Fig. 2 Tank and column connections during priming



VERDOT

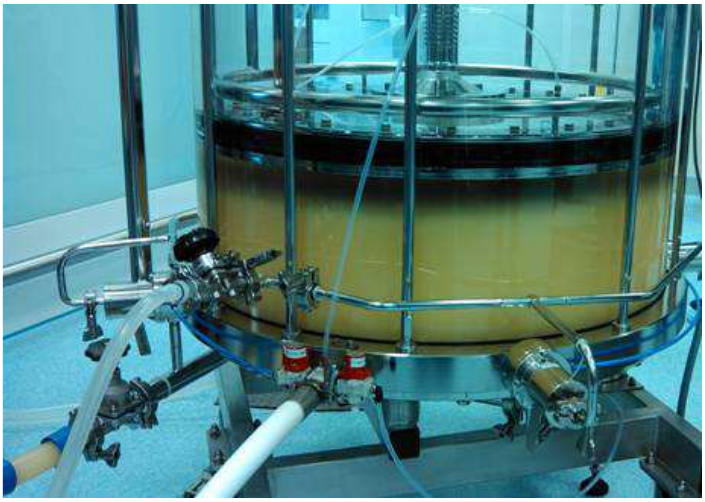
The slurry valves were opened and the column was filled with water to a height of 15cm. The slurry valves were cycled (opened and closed) to release any potential air that could be caught in the lines. The column was degassed by use of the tilting actuator and through introducing water into the column until the inflatable seal was fully submerged. The inflatable seal was inflated and the column tilting actuator was disengaged, placing the column back into the level position. The top isolation valve was opened to the drain position. Using the advanced control console, the top adapter was lowered at a speed of 200cm/h for 5cm in order to prime the top process line. Once primed, the top isolation valve was immediately closed. Conversely, the bottom process line was primed by opening the bottom process isolation valve to the drain position and lowering the top adapter at a speed of 200cm/h until it was positioned approximately 5cm above the bottom frit. Once primed, the bottom isolation valve was immediately closed.

Media transfer via syringe method

The bottom slurry tank valve was connected to the columns slurry manifold via a flowmeter (installed between two flexible hoses). Both the bottom slurry tank valve and the column slurry valves were then opened. Media transfer from slurry tank to column was performed using the syringe method. The top adapter, positioned at a height of 5 cm above the bottom frit (with the seal inflated) was then raised at a speed of 200cm/hr initializing slurry transfer via suction. With the advanced control console, the top adapter was raised until the required volume of slurry into the column was obtained. The flowmeter was used to confirm the slurry transfer volume, with less than 1% difference. The slurry valve was immediately closed after the slurry transfer.

Because the slurry valves and transfer lines contained residual slurry, approximatively 20L of water was pumped through the slurry valve cleaning ports to recover 100% of the unused slurry back to the slurry tank.

Figure 3 - Slurry transfer in syringe mode

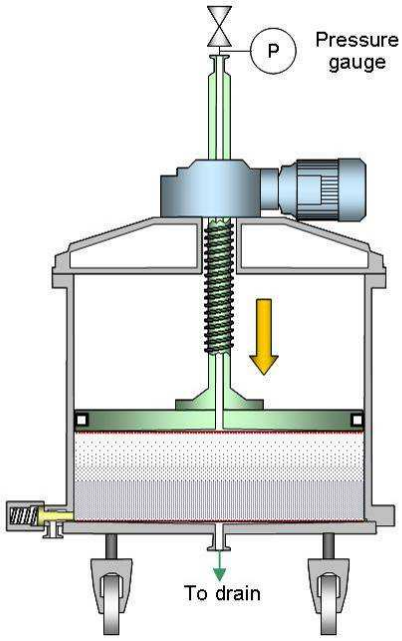


Packing method -Dynamic Axial compression

Packing was accomplished using dynamic axial compression. DAC involves lowering the top adapter at a constant speed to remove the packing buffer and consolidate the bed. The columns top process isolation valve was closed and the bottom process isolation valve was opened to the drain.

This configuration allows the bed to build from the bottom to the top while avoiding bed drying. The top adapter was lowered at 200 cm/hr until the bed was consolidated. The top adapter speed was then reduced to 75 cm/hr to achieve the final bed height of 14cm.

Figure 4 - Dynamic axial compression



Column equilibration and validation

The column was connected to chromatography process system. The hose connected to the columns top process port was purged and the column was then equilibrated with 5CV 0.5M NaCl. A 1% CV sample of 2M NaCl was injected for HETP and symmetry testing.

The following results were observed:

- Number of plates = 4270 plates per meter
- Asymmetry = 1.29
- HETP = 0.023cm/pl
- rHETP (= HETP / mean particle size) = 2.6

Re-slurrying within the column

A 5cm headspace was created above the packed bed by injecting water at 220cm/h while simultaneously raising the top adapter at 200cm/h to maintain a net positive down flow. Once the headspace was created, flow direction was changed to up flow and the top adapter was raised with the advanced control console. Up flow continued until the top adapter reached a height of 28cm from the bottom frit (corresponding to 2CV, for obtaining a 50% slurry). At this stage, the media bed was still intact. Flow from the skid was reversed (down flow) and the direction was alternated 2 times for 2 minutes between up flow and down flow to raise and lower the media bed until it collapsed. Once the media bed collapsed, the inflatable seal was deflated and the top adapter moved 8 cm above the liquid level. The inflatable seal was re-inflated and the top process valve was placed to drain. Compressed air, injected through the cover of the bubble trap sparged through the bottom frit at 0.25 Bar for 15 minutes. After resuspension, the air was shut off and the slurry settled until a 4cm clear supernatant layer was formed.

Re-packing of the column

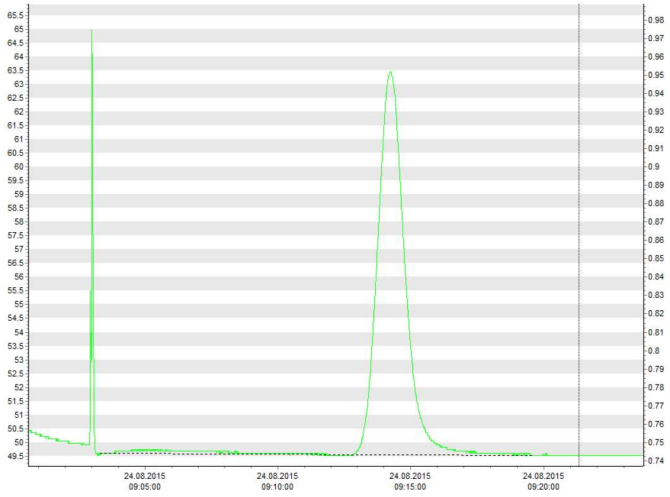
With the inflatable seal deflated, the top adapter was lowered until the inflatable seal was submerged in the water. The column was degassed with the tilting actuator. Once air bubbles were removed, the inflatable seal was inflated at 4bar and the tilting actuator disengaged.

Column re-packing was performed and tested utilizing the same methods as the initial packing.

The following results were observed:

- Number of plates = 4282 plates per meter
- Asymmetry = 1.21
- HETP = 0.023cm/pl
- rHETP (= HETP / mean particle size) = 2.6

Fig. 5. HETP and symmetry test



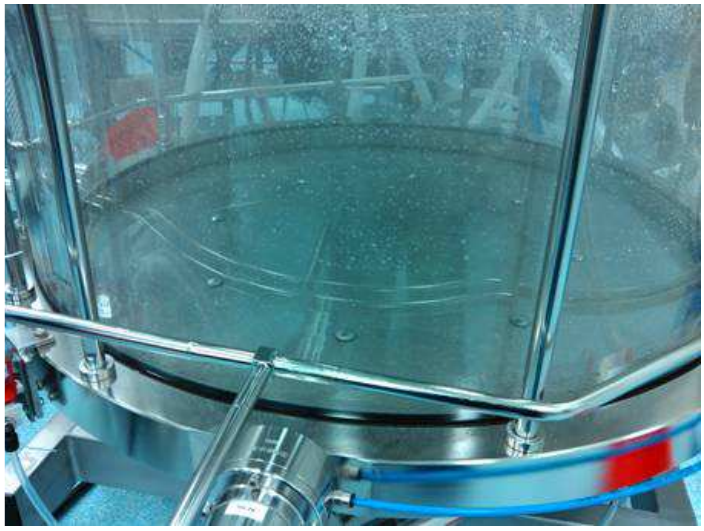
Unpacking method

The packed bed was re-slurried within the column as described previously. Following the 15 minutes of air sparging, the top process isolation valve was closed to pressurize the column at 0.25bar and the column tilting actuator was activated. The slurry valve located at the lowest point of the tilted column was opened to the slurry tank through a flexible hose. In less than 5 minutes, all slurry was removed from the column. Air sparging was maintained during the slurry transfer to keep the media suspended, maintain slight pressurization and help the media flow toward the slurry valve.

Small traces of media were removed by closing the slurry valve, disengaging the tilting actuator and opening the top process isolation valve. Ten liters of water were injected in up flow, followed with air sparging for 30 seconds. The top process isolation valve was then closed to pressurized the column at 0.25bar and the column tilting actuator was engaged. The slurry valve located at the low point of the tilted column was opened to transfer any remaining media.

Only 170L (approximatively 2.4CV) of water was required to reslurry the bed within the column, transfer the slurry back into the tank (maintaining a 50/50 slurry) and completely rinse the column, leaving no trace media.

Fig. 6. Column after media removal before final rinsing.



Conclusion

The VERDOT Ips² InPlace column is ideally suited for processes involving chromatography media, such as GE Zinc Imac Sepharose(TM) Fast Flow, or medias of the same matrix. The InPlace columns dynamic axial compression provides quick and easy packing operations with a high level of reproducible performance results. The column can be easily unpacked in less than one hour using minimal equipment (via air sparging) and minimal buffer usage.

(1) Sepharose is a Trade mark of GE HEALTHCARE BIO-SCIENCES