PROCESS-SCALE CHROMATOGRAPHY

Tech note 0341

Packing Ca^{++}Pure-HA® from TOSOH Bioscience in the Verdot Ips² InPlace(™) column

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Summary

Hydroxyapatite (HA) has become one of the best characterized multimodal chromatography media available today, with valuable research and industrial applications. It requires special consideration during column packing, since it settles quickly and tolerates no compression, unlike most other chromatography media. Hydroxyapatite media is also sensitive to mechanical shear. Careless handling can lead to fracturing of particles and creation of fine particles of media which can decrease column performance.

VERDOT InPlace® columns offer a unique solution for packing media like Hydroxyapatite Ca++Pure-HA® from TOSOH. One of the key features of VERDOT's InPlace column is the InPlace slurry valve. The InPlace slurry valves 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 patented low-shear design of the InPlace valve minimizes damage to media such as Ca++Pure-HA. 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 best practices for maximizing the life and performance of Tosoh's Ca++Pure-HA in VERDOT's InPlace columns. These best practices were validated during a packing study with experts from VERDOT Ips² and TOSOH Bioscience using an InPlace column with interior diameter of 20 cm. The tech note addresses column and media preparation, media transfer into the column, column packing, the reslurrying in place and the unpacking operations.

Materials and Methods

Material and Equipment

The VERDOT Ips² InPlace column can be mounted with filters with ≤15µm absolute porosity, which is optimum for Ca++Pure-HA which has a 39 µm mean particle size. For automated operations, the InPlace column is fitted with an instrumentation package 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 InPlace Advanced Control Console was used for this packing study.

Slurry preparation

Slurry Preparation: Ca++Pure-HA arrives as a dry media. To achieve a uniform, dense packing in the column, Ca++Pure-HA

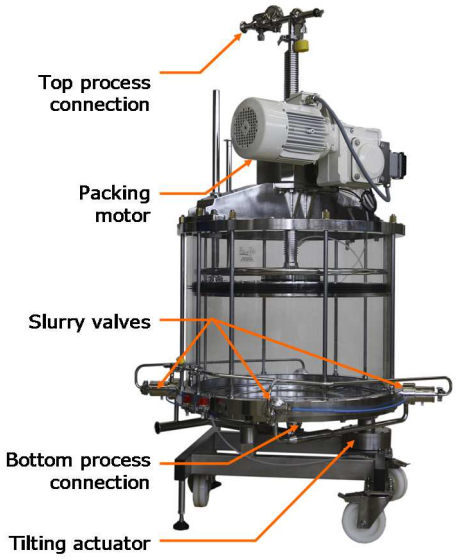


Fig.1. VERDOT Ips² InPlace column

is combined with 20mM Na2HPO4, 150mM NaCl buffer at a 50% (v/v) slurry, using a conversion factor of 0.63g dry media/ L packed bed.To account for potential material loss during slurry transfer, an additional 5% of the calculated media requirement is added to the total.

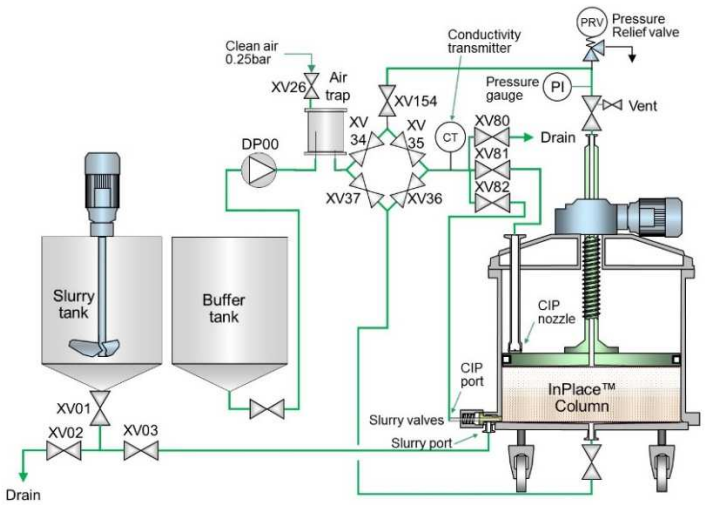
Media transfer in syringe mode

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

Column priming

When the InPlace slurry valves are closed, the Clean-In-Place (CIP) and slurry ports are open. Using pump DP00 at 100 LPH, the slurry valve manifold and transfer hoses were primed by injecting packing buffer through XV82 and flowing through XV03 and XV02.

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



With the top adaptor positioned 20 cm. above the bottom filter, the column was filled from the bottom inlet with 15cm of packing buffer flowing up from the bottom process connection. The slurry valves were cycled (opened and closed) several times to release any air present in the lines. The top adapter was lowered until the inflatable seal was fully submerged. The pneumatic cylinder on the column was used to tilt the column and remove any remaining air bubbles. The inflatable seal was inflated and the column was placed back into the level position. 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 300 cm/h for 5cm to prime the top process line. Once primed, the valves were immediately closed. The bottom process line was primed by opening the bottom process connection to the drain position (XV36-80) and lowering the top adapter at a speed of 300cm/h until it was positioned 5cm above the bottom frit. Once primed, the valves were immediately closed. At this point, the column has been effectively purged with air and all product-contacting areas are filled with buffer.

Media transfer via syringe method

Both the bottom slurry tank valve (XV01-03) and the InPlace slurry valves were opened. Media transfer from slurry tank to column was performed using the syringe method: The top adapter, initially positioned at a height of 5 cm above the bottom frit (with the seal inflated) was raised at a speed of 300cm/hr, which creates a negative pressure in the column and pulls the slurry into the column from the slurry tank. The ending position of the piston was chosen to transfer enough slurry for the target bed height:

$$Length\ of\ travel\ (cm) = \frac{\text{Packed height (cm)}}{\text{Slurry ratio}} + \frac{slurry\ line\ volume\ (L) \times 4000}{\pi \times ID\ Column(cm)^2}$$

Slurry line volume was the volume comprised between the tank outlet and the slurry valves, which starts with packing buffer and ends with slurry. The InPlace slurry valves were immediately closed after the slurry transfer.

Because the InPlace slurry valves and transfer lines contained residual slurry, approximatively 4L of water was pumped through the slurry valve cleaning ports (XV82-03-01) until no material was seen in the transparent transfer hose, ensuring 100% of the unused slurry was returned to the slurry tank.

Re-slurrying within the column after media transfer

Because the Ca++Pure-HA media was 70% settled after the slurry transfer, a re-slurrying in place with air sparging was done. The inflatable seal was deflated and the top adapter was moved to 8 cm above the liquid level. The inflatable seal was re-inflated and the top process valve was connected to drain (XV154-35-80). Compressed air at 0.25 bar, was injected through XV26 into the bottom of the column for 15 minutes. (extended to 30min for a 28cm bed in 55% slurry).

Figure 3 – End of slurry transfer in syringe mode

After resuspension, the air was shut off, the bottom distributor was filled again with buffer and the slurry was allowed to settle until a 4cm clear supernatant layer was observed. With the inflatable seal deflated, the top adapter was lowered until the inflatable seal was submerged into the buffer layer.

To refill the top connection with buffer, the top isolation valve was opened to the drain position (XV154-35-80) and the top adapter was lowered at a speed of 300cm/h for 3-5cm, immediately followed by the packing step.

Packing method -Dynamic Axial compression

Packing was accomplished using dynamic axial compression (DAC). DAC 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 was closed upstream of the pressure gauge (close XV154) and the bottom process isolation valve was opened to the drain (XV36-80).

This configuration allowed the bed to build from the bottom to the top while avoiding bed drying (refer to figure 4). The top adapter was lowered at 250cm/hr, until the top filter is 1mm above the packed bed. Only the filter screws, slightly protruding below the filter, touched the bed.

Figure 4 – during axial compression

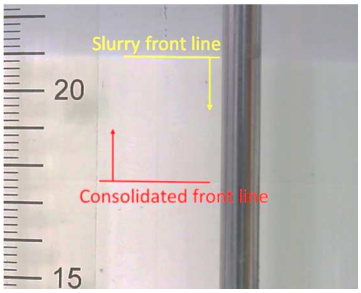
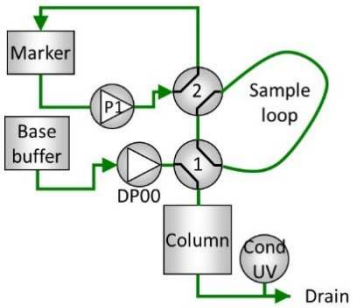


Figure 5 – Marker injection



Column equilibration and validation (HETP, As)

The installation described in figure 5, 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 PBS buffer with 3-5% of acetone was used as marker. While the column was equilibrated with 1CV of packing buffer in downflow, 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.

The following results were obtained during a campaign of several packings and unpackings of Ca++Pure-HA media in an InPlace D200mm column.

Test 4 was performed after an axial compression packing performed on a fully settled bed, a simpler method. The plate counts appeared to be lower than the others but still decent. All the other tests were performed with the method described in this technical note.

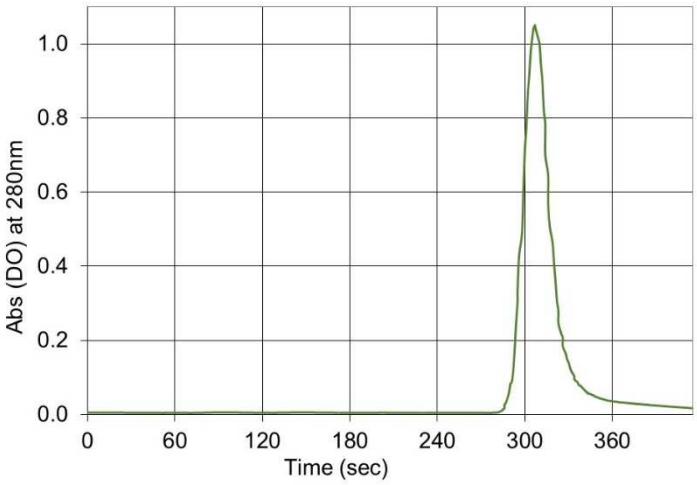


Figure 6 – example of HETP curve with test 5

		Test 1	Test 2	Test 3	Test 4*	Test 5
As=		1.31	1.98	1.71	1.92	1.87
N=	plates	1897	1070	1552	862	1605
bed height=	mm	205	157	194	191	275
N/m=	plates/m	9252	6814	8000	4513	5838
HETP=	cm	0.0108	0.0147	0.0125	0.0222	0.0171

After Test 1, the pressure drop across the packed bed was measured at different flow rates. Being proportional to bed height, the following curve is normalized for a 20cm bed.

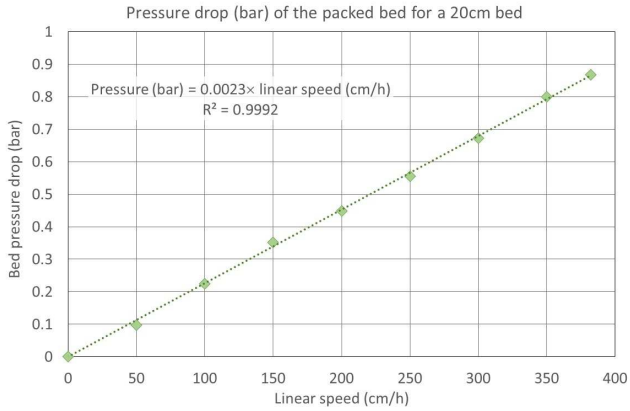


Figure 7 – Curve pressure drop vs linear speed with 20cm bed

Re-slurrying within the column

A 5cm headspace was created above the packed bed by injecting PBS at 200cm/h downflow while simultaneously raising the top adapter at 232cm/h, maintaining a net positive down flow. Once the headspace was created, the buffer flow direction was reversed to flow upwards from the bottom process connection. The top adapter was raised until the top adapter was one bed height above the settled media below. This ensures an approximate 50% slurry concentration when fully suspended. Note: Due to the height of the column tube, test 5 with a 27.5cm bed was limited to a 55% slurry. Injecting buffer from bottom process port broke up the packed bed every time.

Once the media bed was broken up, the inflatable seal was deflated and the top adapter was moved 5-8 cm above the liquid level. To fully mix the slurry in the column, the inflatable seal was re-inflated and the top process valve was connected to drain (XV36-80). Compressed air, injected through the cover of the bubble trap (XV26-37) sparged through the bottom frit at 0.25 Bar for 15 minutes with 15-20cm bed, and 30 minutes with 27.5cm bed for complete reslurrying.

Unpacking method

With air continuing to bubble up through the column, the top process isolation valve (XV154) was closed and the column pressurized to 0.25 bar. The InPlace column tilting feature was activated to create a positive slope and facilitate draining the media out of the column. A hose was connected from the outlet of the slurry valve located opposite the tilting device (the lowest point of the tilted column) to the slurry tank. When the InPlace slurry valve was opened, the slight positive pressure in the column pushed the slurry back into the slurry tank. This allows transferring of the media from the column to the slurry tank in a few minutes.

Air sparging was maintained during the slurry transfer to keep the media suspended above the bottom filter and push the slurry through the InPlace slurry valve and over to the slurry tank.

The compressed air pressurizing the column was shut off. The InPlace slurry valve was closed. The inflatable seal was deflated. A hose was connected from the top process connection to the drain. To remove the last visible particles of media, water was injected first through XV81 and the top spraying nozzle to rinse the column tube, then through the bottom process port (XV36-80) to a total depth of 1 cm of the column. The inflatable seal was inflated and air sparging was repeated for 30 seconds at 0.25 bar. The top process isolation valve was then closed, pressurizing the column to 0.25 bar. The column tilting device was re-activated. The slurry valve located at the low point of the tilted column was opened to transfer any remaining media. This rinsing operation was repeated three times, until no media particles were observed in the column.

Less than 2.5 column volumes of unpacking buffer are usually required to re-slurry the bed within the column, transfer the slurry back into the tank (at a 50% concentration) and completely rinse the column.

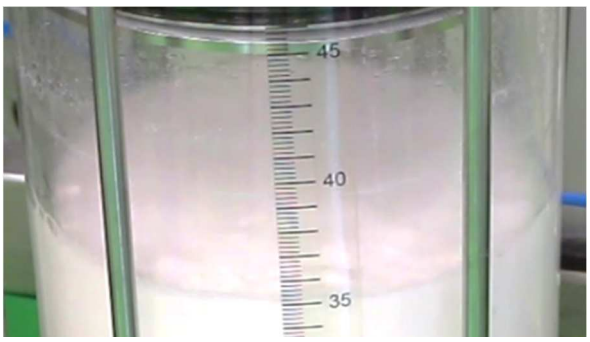


Fig. 6. Re-slurrying with air-sparging .

Conclusion

The VERDOT Ips² InPlace column is ideally suited for processes requiring non-compressible media, such as Ca++Pure-HA. 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 results. The InPlace column can be easily unpacked and cleaned in place in less than one hour using minimal equipment and buffer volumes.

TOSOH

VERDOT IPS²