PROCESS-SCALE CHROMATOGRAPHY

Tech note 0339

Packing Ceramic Hydroxyapatite in the Verdot Ips² InPlace(™) column

Sébastien Lefebvre, VERDOT Ips², Riom, France.

Summary

CHT Ceramic Hydroxyapatite requires special consideration during process scale chromatography packing. This is primarily due to the high specific gravity and rapid settling rate of CHT, moreover for the CHT 80µm that has a free-settling velocity of 125-275 cm/hr. Additionally, as the CHT media is sensitive to mechanical shear, it can lead to fracturing of particles and creation of fines.

VERDOT InPlace columns offer a unique solution for packing media such as CHT. One of the key features of the InPlace column is the slurry valves. The slurry valves can be used to transfer media into the column in a syringe mode, or to transfer media out of the column under slight air pressure. The valves being situated on the column wall, do not interfere with flow dynamics. The low-shear configuration of the valves minimizes damage to media such as CHT. The other key feature of the InPlace column is the possibility to combine air-sparging and tilting of the column for minimizing the amount of buffer needed for bed reslurrying and unpacking.

This tech note gives information on the best practices of using CHT in InPlace columns. These best practices have been collected dating from a time when VERDOT was a part of Bio-Rad Laboratories(1), up until today. This information has been obtained from several packing studies performed with columns ID45cm, 80cm and 1.2m. The tech note addresses the column and media preparation, the slurry transfer, the packing, the reslurrying in place and the unpacking operations.

Materials and Methods

Material and Equipment

The VERDOT Ips² InPlace column, is to be equipped with filters with 15µm and 25µm absolute porosity for CHT40µm and CHT80µm respectively. For automated operations, it is to be equipped with an instrumentation package that includes a rotary encoder and pressure transmitter.

The Piping & Instrument diagram (P&ID) in figure 2 shows a good example of configuration for packing and unpacking. The numbering seen in the diagram will be used later in the instructions' section. The process systems are usually more equipped than seen on this P&ID.

In addition to this, a Basic Control Console or an automated Advanced Control Console will be required to drive the packing motor. The VERDOT Ips² packing station can also be used for fully automated packing and unpacking.

Slurry preparation

Slurry Preparation: CHT being supplied in dry form, it must be wetted with packing buffer for preparation of the slurry. Consider 0.63g of dry media per liter of packed bed. If the syringe mode is used for slurry transfer, prepare at least 5% of media overage. PBS buffer will be used as the packing buffer. Do not use water or any solution with pH less than 6.5. Prepare a slurry concentration of 40% (v/v) max.

Fig.1. VERDOT Ips² InPlace column

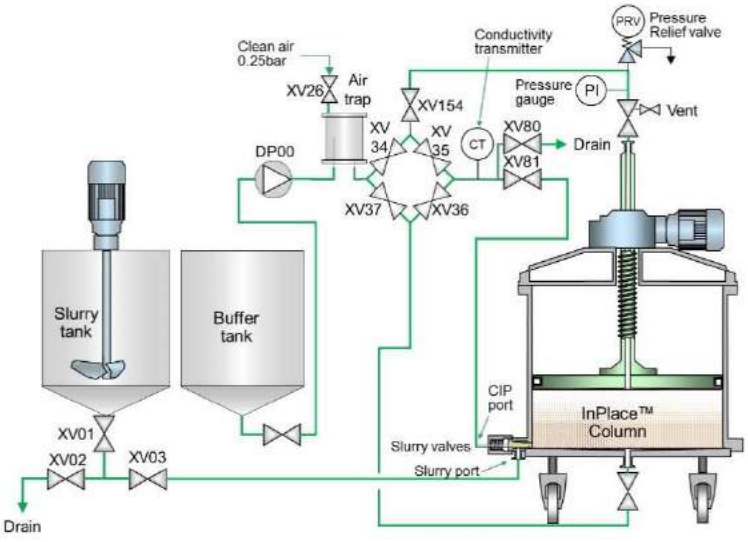
Media transfer in syringe mode

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

Column priming

The slurry valves being closed, their CIP ports and slurry ports are connected. Using DP00, the slurry valve manifold and transfer hoses are primed by injecting packing buffer through XV81, flowing through XV03 and XV02.

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



(1) Bio-Rad tech note 5739

The slurry valves are opened and the column is filled with PBS to a height of 15cm. The slurry valves are cycled (opened and closed) to release any potential air that could be caught in the lines. The column is degassed by using the tilting actuator and by introducing water into the column until the inflatable seal is fully submerged. The inflatable seal is inflated and the column tilting actuator is disengaged, placing the column back into the level position. The top isolation valve is opened to the drain position (XV154-35-80). Using the control console, the top adapter is lowered at a speed of 200cm/h for 5cm in order to prime the top process line. Once primed, the top isolation valve is immediately closed. Conversely, the bottom process line is primed by opening the bottom process isolation valve to the drain position (XV36-80) and lowering the top adapter at a speed of 200cm/h until it is positioned approximately 5cm above the bottom frit. Once primed, the bottom isolation valve is immediately closed.

Media transfer via syringe method

Both the bottom slurry tank valve (XV01-03) and the column slurry valves are opened. Media transfer from slurry tank to column is performed using the syringe method. The top adapter, initially positioned at a height of 5 cm above the bottom frit (with the seal inflated) is raised at a speed of 200cm/hr initializing slurry transfer via suction. The stroke of the piston is chosen to transfer enough slurry for the target bed height:

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

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

Because the slurry valves and transfer lines contain residual slurry, approximatively 20L of water is pumped through the slurry valve cleaning ports (XV81-03-01) to recover 100% of the unused slurry back to the slurry tank.

Re-slurrying within the column after media transfer

If CHT80µm is used, the settling might be too much for being packed while being transferred in the column, as shown on figure 3. Right after the slurry transfer, proceed to a resuspension with air sparging. The inflatable seal is deflated and the top adapter is moved to 8 cm above the liquid level. The inflatable seal is re-inflated and the top process valve is connected to drain (XV154-35-80). Compressed air, injected through XV26 is sparged through the bottom frit at 0.25 Bar for 20 minutes. After resuspension, the air is shut off and the slurry settles until a 4cm clear supernatant layer is formed. With the inflatable seal deflated, the top adapter is lowered until the inflatable seal is submerged into buffer. The column is degassed with the tilting actuator. Once air bubbles are removed, the inflatable seal is inflated at 4bar and the tilting actuator disengaged.

Figure 3 - Slurry transfer in syringe mode

For priming again the top connection, the top isolation valve is opened to the drain position (XV154-35-80). Using the control console, the top adapter is lowered at a speed of 200cm/h for 3-5cm. Immediately proceed to axial compression packing.

Packing method -Dynamic Axial compression

Packing is 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 port is closed upstream the pressure gauge (close XV154) and the bottom process isolation valve is opened to the drain (XV36-80)

This configuration allows the bed to build from the bottom to the top while avoiding bed drying. The top adapter is lowered at 200 cm/hr for CHT40µm and 300cm/h for CHT80µm until the bed is consolidated. The top adapter speed is then reduced to 60 cm/hr until the top filter stands 3-5mm above the packed bed. The top adapter should not touch the bed.

Figure 4 - Axial compression

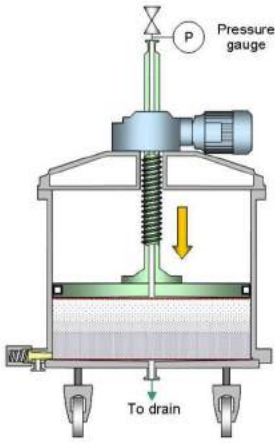
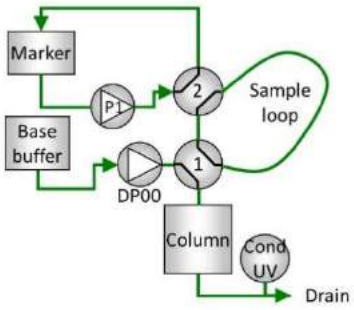


Figure 5 – Marker injection

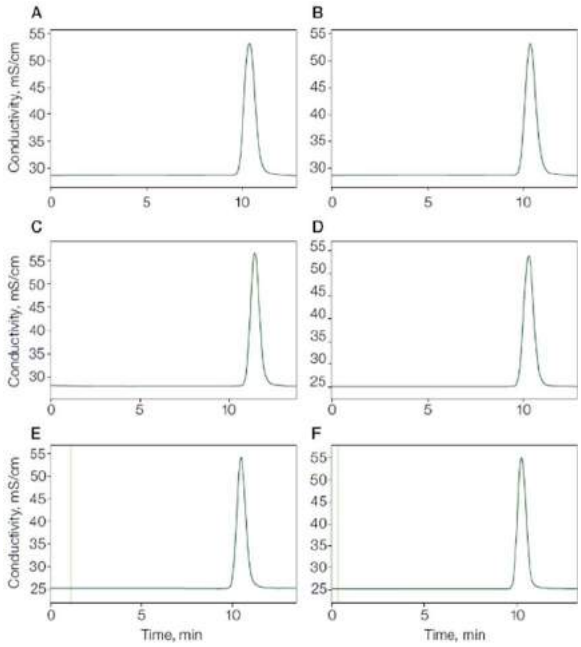


Column equilibration and validation (HETP, As)

The installation described in figure 5 is ideal for HETP test as it allows injection of a sharp marker peak while keeping a constant flow. 1 and 2 are 4-ways valves. The sample loop is a flexible hose with a volume corresponding to 1-1.5% CV. Please refer to our tech note DP-MKT-338 HETP test for more details on column qualification.

Packing buffer is used as a baseline and PBS with 1M NaCl is used as marker. The column is equilibrated with 3CV of packing buffer in downflow while pump P1 primes the loop with marker (valve 2 configured as valve 1 on picture). The loop is isolated by switching valve 2 as on picture. After column equilibration, valve 1 is switched as valve 2 on picture so as to push the sample in the column and the flow continues until the peak, measured with conductivity probe, elutes from the column.

For example, the following results have been obtained on a campaign of 6 packings (Bio-Rad tech note 5739) in an InPlace D446mm column with 20cm bed. Lower plate counts (*) were attributed to slurry ratio over 40%.



Packing #	Asymmetry	N/m
#A	1.22	10201
#B	1.16	7849*
#C	1.15	11692
#D	1.09	8968*
#E	1.14	10392
#F	1.12	11570
Average	1.15	10112

Re-slurrying within the column

The following can be applied before repacking the column without transfer of the media or before unpacking. The unpacking buffer should at least contain 10mM phosphate buffer with a pH above 6.5. For instance Sodium phosphate dibasic.

A 5cm headspace is created above the packed bed by injecting water at 220cm/h downflow while simultaneously raising the top adapter at 200cm/h to maintain a net positive down flow. Once the headspace is created, flow direction is changed to up flow and the top adapter is raised with the control console. Up flow continues until the top adapter reaches a height corresponding to 2 times the bed height (for obtaining a 50% slurry).

At this stage, if the media bed is still intact, the flow from the skid is reversed (down flow) and the direction is changed several times for 2 minutes between up flow and down flow to raise and lower the media bed until it collapses. Once the media bed is collapsed, the inflatable seal is deflated and the top adapter is moved 8 cm above the liquid level. The inflatable seal is re-inflated and the top process valve is connected to drain (XV36-80). Compressed air, injected through the cover of the bubble trap (XV26-37) sparges through the bottom frit at 0.25 Bar for at least 20 minutes with new media, and at least 1 hour in columns larger than 1m or with used media.

Unpacking method

Maintaining the air sparging, the top process isolation valve (XV154) is closed to pressurize the column at 0.25bar and the column tilting actuator is activated. The slurry valve located at the lowest point of the tilted column is solely opened to the slurry tank through a flexible hose (via XV03-01 or connected to the top of the tank). This allows transferring of the media from the column to the slurry tank in a few minutes.

Air sparging is maintained during the slurry transfer to keep the media suspended, maintain slight pressurization and help the media flow toward the slurry valve.

For removing the traces of media, close the slurry valve, disengage the tilting actuator and open the top process isolation valve. Equivalent to 1cm of water is injected in up flow (XV36-80), followed with air sparging for 30 seconds. The top process isolation valve is then closed to pressurize the column at 0.25bar and the column tilting actuator is engaged. The slurry valve located at the low point of the tilted column is opened to transfer any remaining media. This rinsing may need to be repeated one or two times more, eventually flowing to the drain (XV03-02) if media tracks is neglectible.

Usually less than 2.5CV of unpacking buffer is 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 visible trace of media.

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

Conclusion

The VERDOT Ips² InPlace column is ideally suited for processes involving chromatography media, such as CHT. The low shear slurry valves ensures the integrity of the media during transfer. 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 two hours using minimal equipment (via air sparging) and minimal buffer usage.