Packing and Slurry-in-Place Procedures for CHT™ Ceramic Hydroxyapatite Using a Bio-Rad® InPlace™ Process-Scale Chromatography Column

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Introduction

Ceramic hydroxyapatite requires special consideration during process-scale chromatography packing primarily due to its high specific gravity and rapid settling rate. Ceramic hydroxyapatite media have a bulk density of 0.63 g/ml and a free settling velocity of 35–125 cm/hr for 40 µm and 125–275 cm/hr for 80 µm. Additionally, ceramic hydroxyapatite media are sensitive to mechanical shear, which can fracture the particles and produce fines.

Bio-Rad InPlace columns offer a unique solution for packing media because they are designed with low-shear slurry valves, which minimize damage to media such as ceramic hydroxyapatite (Figure 1). Another feature of the Bio-Rad InPlace column slurry valves is their use for transferring media into and out of the column without removing the top flow adaptor. The valves are on the side of the column wall and do not interfere with flow dynamics.

The design of the Bio-Rad InPlace column allows a slurry-in-place operation so that the column can be repacked without removing the media or the top flow adaptor. With this procedure, the column can be repacked and ready for processing in less than 1 hr.

Unpacking of ceramic hydroxyapatite media can be challenging due to the high specific gravity of the particles, which form a dense packed bed. With a special unpacking protocol, ceramic hydroxyapatite can be unpacked from a Bio-Rad InPlace column in less than 1 hr and using less than 2 CV of buffer.

In this study, six independent column packing cycles, including reslurrying, packing, and testing, were performed in less than 8 hr.

Materials and Equipment

New, unopened containers of CHT Type I support, 40 µm, were used (4 x 5 kg, total = 20 kg). PBS (150 mM NaCl, 20 mM sodium phosphate, pH 7.0) was used for hydrating, packing, repacking, and equilibrating the CHT support. A Bio-Rad InPlace column with an acrylic tube (inner diameter, 446 mm; height, 600 mm), a Bio-Rad® process chromatography skid, and a Bio-Rad® media transfer device were used for the procedures.

Methods

Preparation of Slurry and Packing the Column

The slurry was prepared by mixing 20 kg of dry CHT Type I support with 50 L of PBS directly in the slurry tank. An additional 10 L of buffer was reserved for use later in the packing process to make a final slurry concentration of 40% (v/v) as described in the CHT support instruction manual (bulletin 611). The suspension was mixed using a low-shear hydrofoil impeller to ensure homogeneity without damage to the particles.

A Bio-Rad InPlace column (inner diameter, 446 mm) was prepared for packing by raising the upper piston assembly to its maximum height, leaving a functional column height of approximately 54 cm. The slurry valves were opened via pneumatic pressure controlled by the Bio-Rad media transfer device (bulletin 5661).

The slurry was transferred from the reservoir to the column using the Bio-Rad media transfer device diaphragm pump. Once the slurry tank was emptied, the walls and lines were rinsed with the reserved 10 L of packing buffer, resulting in complete transfer of the remaining media.

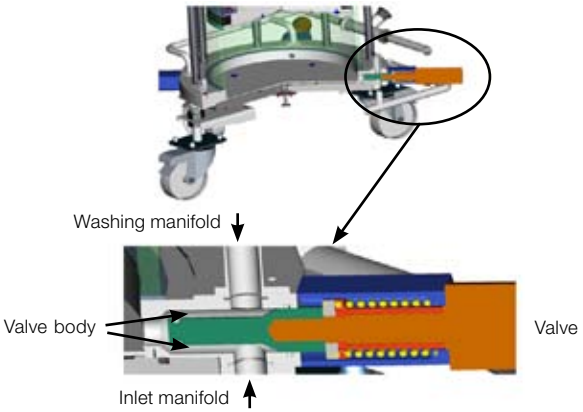

Fig. 1. Slurry valve on Bio-Rad InPlace column. The separated inlet and washing manifolds make it easy and convenient to fill and unload the chromatography media in the contained system and to sanitize the manifold. The valve body allows buffer to pass behind the valve when it is closed.

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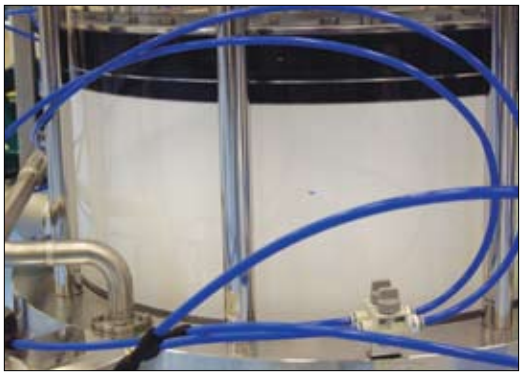

Fig. 2. Fully packed 45 cm Bio-Rad InPlace column. CHT Type I support, 40 µm, was hydrated and packed in a 40% (v/v) suspension in PBS.

The slurry valves were closed using the Bio-Rad media transfer device controls. Upflow with packing buffer was initiated briefly at a low flow rate (50–75 cm/hr) to remove any trapped air bubbles from the slurry. After 3–5 minutes, the upflow was stopped, the bottom valve was closed, and the ceramic hydroxyapatite was allowed to settle to establish a buffer layer approximately 3 cm high, above the bed.

The top piston assembly was lowered until the liquid level rose above the seal, and the seal was inflated with air to 4 bar. The piston was lowered at 100 cm/hr until air was purged from the top frit assembly and piston.

Next, the bottom valve was opened, and downflow was initiated at 100 cm/hr. The piston was simultaneously lowered at 200–250 cm/hr for a combined total flow rate of 300–350 cm/hr. This operation should take approximately 10 min.

The piston motor was stopped when the piston was 1–2 mm above the top of the packed bed, which was determined via visual inspection. The final bed height was 21 cm, with a bed volume of 33.4 L (Figure 2). For stainless steel columns, calculations should be determined to allow 1 cm between the piston and the top of the packed bed.

The column was equilibrated with 3 CV of buffer applied as downflow at 100 cm/hr. Following equilibration, the column packing was evaluated by injecting a test solution (1.2 M NaCl, 20 mM, NaH_2PO_4, pH 7) equivalent to 2% of the column volume. Peak asymmetry (A_s) factors and number of theoretical plates per meter were calculated using BioLogic™ HR chromatography software.

Column Repacking Using Slurry-in-Place Procedure

It is possible to repack the column without removing the media or the top flow adaptor. The slurry-in-place procedure allows the column to be repacked and ready for processing in less than 1 hr with minimal buffer consumption.

Downflow was initiated at 200 cm/hr while the top piston was simultaneously raised (Figure 3A). When sufficient space was obtained between the top flow adaptor and the top of the

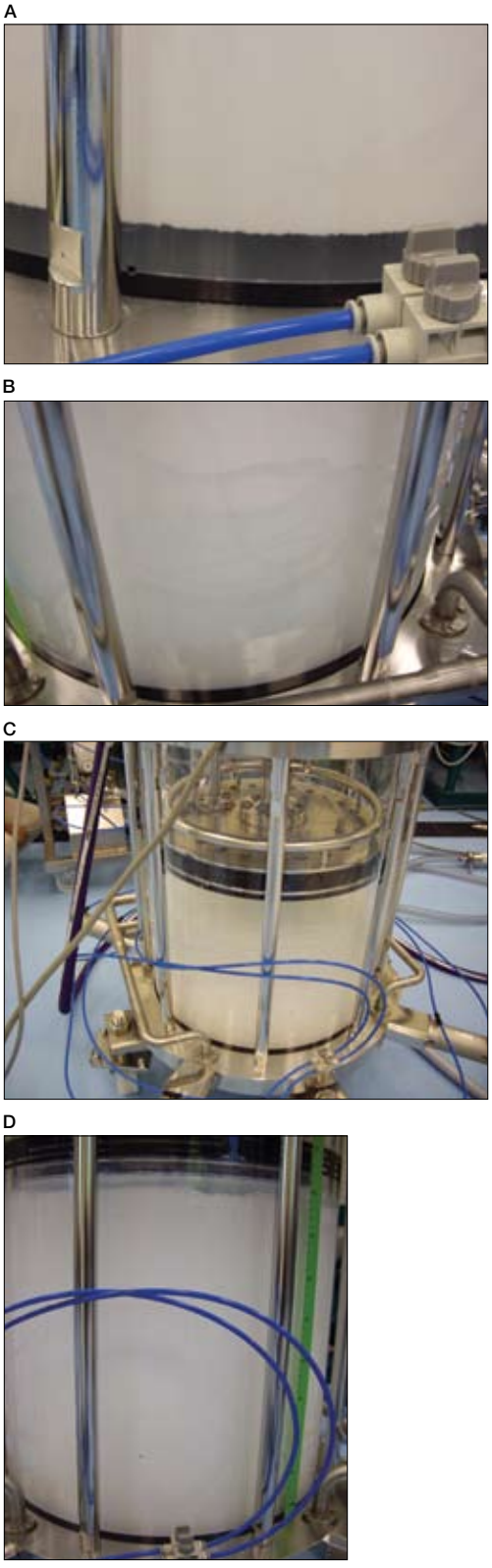

Fig. 3. Bio-Rad InPlace column repacking. A, initiation of upflow at 100 cm/hr while the top piston was raised; **B**, end of upflow and bed resuspension in PBS at 40% (v/v); **C**, fully resuspended bed; **D**, sparging with air was performed for >10 min to bring the suspension to homogeneity while preventing shearing of CHT.

bed, the seal was deflated, and the piston was elevated to its uppermost position at maximum speed. The buffer flow to the column was switched to upflow. As upflow continued, the bed was resuspended (Figure 3B). When the slurry percentage was approximately 40% (v/v), upflow was stopped (Figure 3C). A compressed air hose was attached to the bottom inlet, and air was sparged at a pressure no greater than 3 psi. This is a gentle yet effective way to create a homogeneous slurry. Sparging continued for at least 10 min to ensure thorough mixing (Figure 3D).

The column was then repacked according to the procedure outlined previously. The CHT support was resuspended and repacked a total of six times using this method. Between the third and fourth packing, the CHT support was removed entirely from the column and transferred into the slurry tank.

Unpacking and Cleaning the Column

Similarly to the slurry-in-place procedure, unpacking was performed by initiating upflow at 100 cm/hr while the top piston was raised. Once the bed was resuspended, sparging with air was used to keep the particles in suspension.

The slurry valves were opened using the Bio-Rad media transfer device, and the CHT support was transferred out of the column and into storage containers using a combination of air pressure and pumping with the Bio-Rad media transfer device pump.

Once the majority of the support was transferred out of the column, the sides of the column were washed by running packing buffer through the upper cleaning-in-place (CIP) ring above the top piston (Figure 4). The residual support and accompanying buffer were removed through the slurry valves until the support was no longer visible on the bottom frit. Complete unpacking of the support was accomplished with approximately 1.5 CV of buffer.

Results and Discussion

The results of asymmetry factors and plates/meter (N/m) calculations after each packing procedure are presented in Table 1, with accompanying chromatograms in Figure 5. Symmetrical peaks and the high theoretical plates value indicate a high separation efficiency of CHT support packed in a Bio-Rad InPlace column.

Our results indicate that ceramic hydroxyapatite can be very efficiently packed in a Bio-Rad InPlace column, with no loss of resin. A slurry-in-place procedure was developed to allow repacking without removing the column top or the support from the column. The method is highly reproducible, and the column can be repacked and ready for use in less than 1 hr. Unpacking the Bio-Rad InPlace column can be achieved with less than 2 CV of buffer.

Fig. 4. Unpacking the column. Bottom frit after removal of media and termination of final sparging.

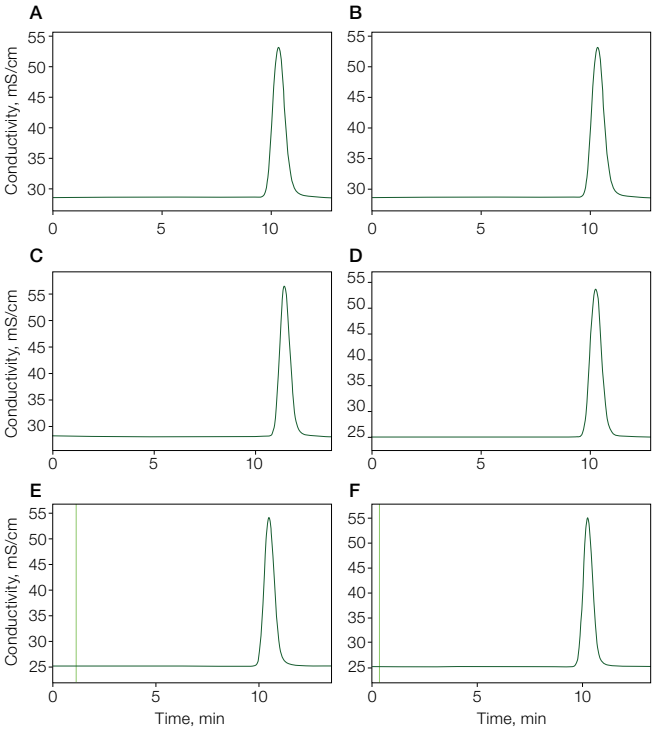

Fig. 5. Asymmetry tests at different packing stages. A, first packing; **B**, second packing, following slurry-in-place procedure; **C**, third packing, following second slurry-in-place procedure; **D**, fourth packing; CHT media was completely removed from column using the Bio-Rad media transfer device and repacked according to the initial procedure; **E**, fifth packing, following slurry-in-place procedure; **F**, sixth packing, following slurry-in-place procedure.

Table 1. Column characteristics after each packing procedure.

Packing Number	Asymmetry Factor	Number of Theoretical Plates/Meter
1	1.22	10,201
2	1.16	7,849*
3	1.15	11,692
4	1.09	8,968*
5	1.14	10,392
6	1.12	11,570
Average	1.15	10,112
Standard Deviation	0.04 (3%)	1,493 (15%)

* Lower plate count was attributed to slurry percentage being denser than 40%.

Information in this tech note was current as of the date of writing (2008) and not necessarily the date this version (rev A, 2008) was published.

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