Process-Scale Packing Procedures for CHT™ Ceramic Hydroxyapatite in Open Column Systems: Best Practices

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Introduction

Ceramic hydroxyapatite media have been shown to be an effective multimodal chromatographic support for the purification of a variety of biomolecules including monoclonal and polyclonal antibodies, isoproteins, antibody fragments, and DNA. Ceramic hydroxyapatite requires special considerations during process-scale chromatography column packing due to its high specific gravity and rapid settling rate. Ceramic hydroxyapatite media have a free-settling density of 35–125 cm/hr for 40 µm particles and 125–275 cm/hr for 80 µm particles. Additionally, ceramic hydroxyapatite media are sensitive to mechanical shear, which can fracture the particles and create fines.

Open column systems such as the GE BioProcess Glass (BPG) column are commonly used for pilot-scale protein purification because of their simple design and ease of use. To transfer media into an open column, remove the top piston from the column tube.

In this report, a procedure for packing CHT ceramic hydroxyapatite media (Bio-Rad Laboratories, Inc.) in a BPG open column system is described, and column evaluation results after five independent packing studies are shown.

Materials and Equipment

A 30 cm BPG column with a polyamide filter with a pore size of 10 µm was used for these experiments. The tube height of this column is 50 cm, and the functional column height is 38 cm. Phosphate buffered saline (20 mM NaH_2PO_4, 150 mM NaCl), pH 8.0, was used as the packing and equilibration buffer.

Procedure

Column Preparation

- With the top piston removed, wet the bottom frit with 20% ethanol, then drain
- Fill the column with 5–7 cm of packing buffer and drain until approximately 1 cm of packing buffer remains in the bottom of the column (Figure 1)
- Close the bottom process valve
- Make sure that the column is level by checking with a bubble level in several positions (Figure 2)

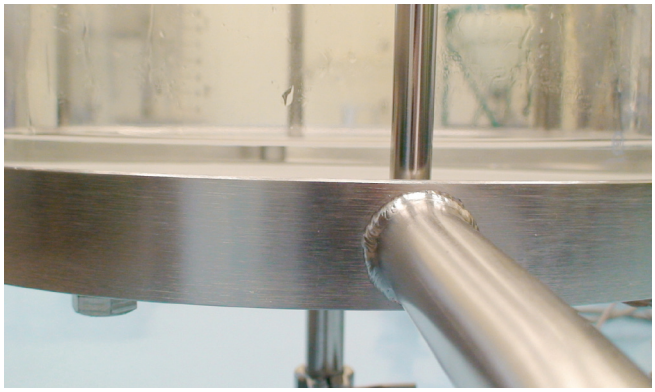

Fig. 1. Prepare the column with packing buffer.

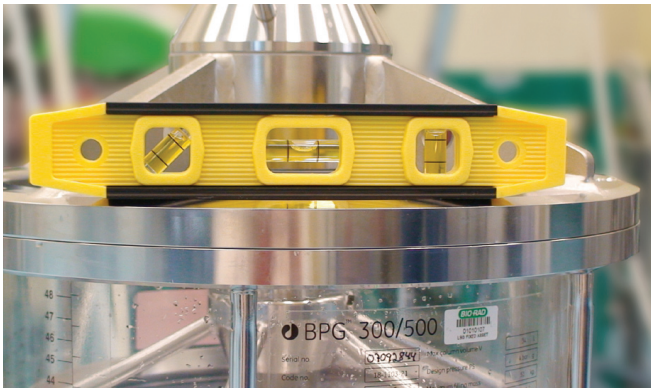

Fig. 2. Use a bubble level to check that the open column system is level.

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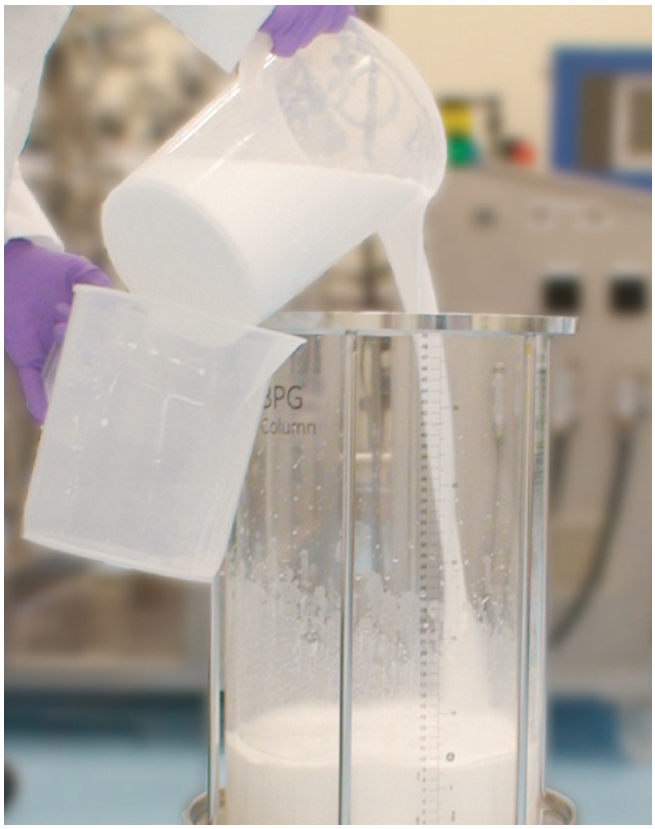

Fig. 3. Transfer prepared slurry to open column system manually, as shown, or with a pump.

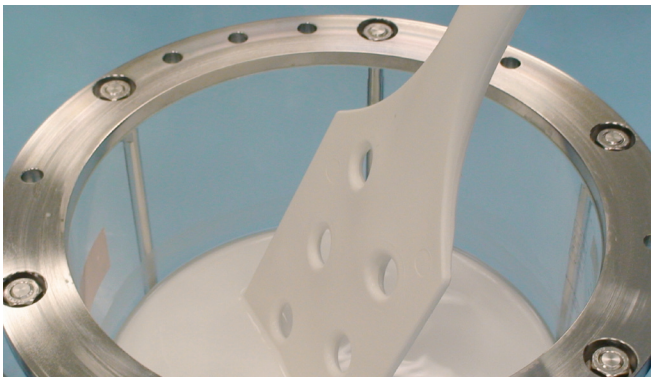

Fig. 4. Use a plastic paddle to manually mix the slurry.

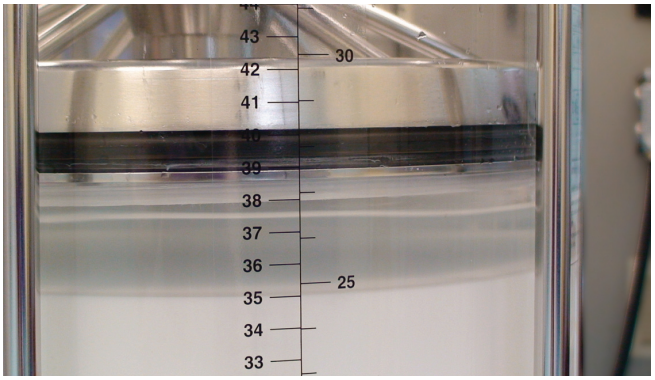

Fig. 5. Lower the top piston into the supernatant layer.

Slurry Preparation

- Packing and equilibration buffers for ceramic hydroxyapatite should always contain at least 20 mM phosphate. Slurries with an at most 50% packed volume to slurry volume ratio are recommended
- To pack with a prepared slurry, use the CHT ceramic hydroxyapatite instruction manual to calculate the appropriate slurry percentage and volume. Carefully transfer the slurry by pouring (Figure 3) or by using a diaphragm pump
- The amount of slurry that can be used in an open process column is dependent on the functional column height, which is the height of the column that can be used with the top piston in place. For instance, with a 50 cm tube height, only 38 cm can be used with the top piston in place. Therefore, the maximum bed height that can be packed with a 50% slurry is 19 cm
- Use a plastic paddle for manual mixing (Figure 4). Use a J stroke or back-and-forth motion; do not use a circular stirring motion. For automated mixing, the use of a low-shear impeller is recommended
- For new media, find the appropriate volume of packing buffer to use in the CHT ceramic hydroxyapatite instruction manual. Fill the column with the calculated volume of buffer and add powdered CHT media slowly, while stirring with a plastic paddle

Packing Procedure

- Replace the column top assembly, and secure it with the bolts. After a buffer layer of 1–2 cm forms on top of the packed media, lower the top piston into the supernatant layer using the height adjuster handle, and tighten the seal (Figure 5)
- Purge the air from the top frit and piston assembly using a short period of upflow or by keeping the bottom process valve closed and lowering the top. When the top piston assembly is purged of air, initiate downflow at a flow rate that is at least 50% faster than the processing flow rate. If a peristaltic pump is used, the flow rate may need to be adjusted as the bed is packed due to increased backpressure
- After the bed has consolidated (Figure 6), stop the flow and close the bottom process valve. There should be no rebound of the bed, because ceramic hydroxyapatite is incompressible. Lower the piston adapter using the height adjuster handle. During this phase, the top process valve must be open for the excess buffer to be purged through it. This procedure reduces or eliminates fluctuations in the flow that is applied to the bed. Lowering the top assembly by hand can be difficult as it requires a significant amount of force. The seal can be loosened slightly to allow for easier movement of the top assembly if care is given to preserve the integrity of the seal

- Continue lowering the top piston until a headspace of approximately 1–5 mm can be seen between the packed bed and the top assembly (Figure 7). Do not perform any additional consolidation, and do not lower the top assembly into the bed as damage to the media may result
- Once the column is packed, further tighten the seal and initiate buffer flow for equilibration and column evaluation

Column Evaluation

After equilibration with at least 1 column volume (CV) of buffer and when a stable baseline is achieved, introduce, by injection, a sample that is 1–3% of the CV. For a conductivity trace, use 1 M NaCl in the equilibration buffer (for example, PBS + 1 M NaCl); for a UV trace, acetone may be mixed with the equilibration buffer (for example, PBS + 1% acetone). Connect the injection loop as close to the column inlet as possible to minimize dilution and band broadening. Perform asymmetry and HETP testing at a flow rate that is representative of your process.

Column Unpacking Procedure

Initiate buffer downflow at 300 cm/hr, and raise the piston slowly by hand to create a headspace of 5–7 cm. Release the seal, and bring the piston upward rapidly. Simultaneously, decrease the flow to 100 cm/hr and reverse the buffer flow direction (upflow). The bed will be lifted and disrupted by steady flow in the upward direction (Figure 8). When the liquid level approximates a 50% slurry (or thinner), halt the upward flow. Remove the top piston assembly. Gently mix the media into slurry using a plastic paddle, and transfer the slurry to a storage container by pouring or by using a diaphragm pump.

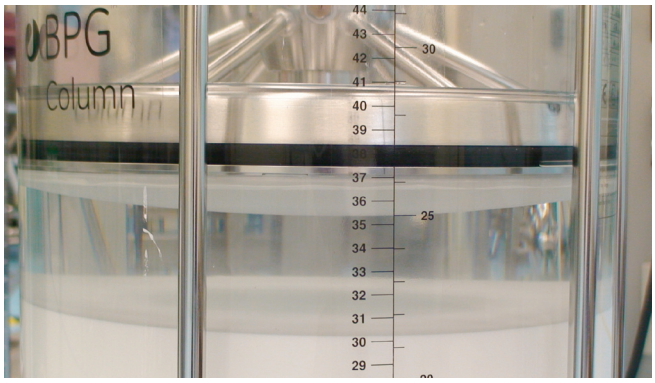

Fig. 6. The bed is consolidating during downflow.

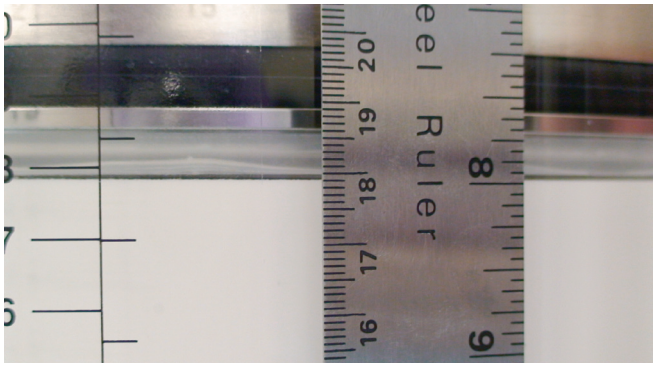

Fig. 7. Leave 1–5 mm of headspace between the packed bed and top piston assembly.

Fig. 8. The bed collapses during unpacking.

Replicate Packing Experiment

	Independent Packing Studies				
Column Evaluation Tests	**1**	**2**	**3**	**4**	**5**
Approximate linear flow (cm/hr)	100	100	100	100	100
HETP*	0.0109	0.0101	0.0098	0.0107	0.0096
rHETP*	2.7	2.5	2.4	2.7	2.4
Asymmetry factor*	1.21	1.38	1.24	1.40	1.20
Plates/meter*	9171.79	9866.01	10221.66	9354.55	10382.46

* These values were calculated using the Bio-Rad® process chromatography skid software.

Information in this tech note was current as of the date of writing (2008) and not necessarily the date this version (Rev A, 2009) was published.

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Bulletin 5831 Rev A US/EG *08-1009 0309 Sig 0308*