

## Best Practices for Process Scale Column Packing of CHT™ Ceramic Hydroxyapatite Media

### Introduction

CHT ceramic hydroxyapatite media require specific considerations during process-scale chromatography packing due to high specific gravity, rapid settling rate, and sensitivity to mechanical shear. CHT ceramic hydroxyapatite has a free settling velocity of 35–125 cm/hr for 40 µm particles and 125–275 cm/hr for 80 µm particles. If subjected to mechanical shear, CHT media particles can fracture, creating fines. This article describes straightforward best practices for successful and reproducible CHT media column packing at process scale.

### Preliminary Considerations

- For 40 µm CHT, equip the column with frits that have a pore size of ≤10 µm. Although the median particle size is 40 µm, a small population down to 16 µm exists. These smaller particles can clog frits with larger pore sizes and cause increased backpressure
- Ensure that the column is level in all directions
- Know the functional column height, defined as the usable height of the column tube with the adaptor in place
- Calculate appropriate slurry percentage and slurry volume based on functional column height; consult the tables in section 7 of the CHT Ceramic Hydroxyapatite Instruction Manual (Bio-Rad bulletin LIT611)

### Buffers and Solutions

The buffers and solutions recommended for hydrating, packing, equilibrating, and testing CHT media are presented in Table 1. The following general guidelines should be used when choosing a buffer for CHT media packing:

- For hydrating new, unused CHT media, a minimum concentration of 15 mM phosphate buffer is required. Additionally, the ionic strength of the buffer should be at least equivalent to 150 mM NaCl, and the pH should be 6.8 or higher
- The sample injection solution should contain the same concentration of phosphate as the equilibration buffer



Fig. 1. Use a polypropylene paddle to manually mix the CHT media.

### Slurrying

- For manual mixing, use a plastic paddle such as the polypropylene paddle\* shown in Figure 1. Mix with a J stroke or side-to-side motion. Do not mix in a circular manner
- For automated mixing, use a low-shear hydrofoil impeller\*\* as shown in Figure 2
- Minimize the time that CHT media are mixed. Even with a plastic paddle or low-shear hydrofoil impeller, bead fracture is still possible
- Use the thinnest slurry possible (≤26% v/v or ≤16% w/v) to minimize particle-to-particle collisions during transfer

Table 1. Buffers and solutions recommended for hydrating, packing, equilibrating, and testing CHT media.

	Hydrating Buffer	Equilibration Buffer	Sample Injection
20 mM phosphate buffer, 150 mM sodium chloride, pH 7.2–7.4 (phosphate buffered saline)	•	•	
Sodium or potassium phosphate buffer, 200–400 mM, pH 6.8–10	•		
0.15 N to 1.0 N NaOH	•		
20 mM phosphate buffer, 0.6–1.2 M sodium chloride, pH 7.2–7.4			•
20 mM phosphate buffer, 1–2% acetone, pH 7.2–7.4			•

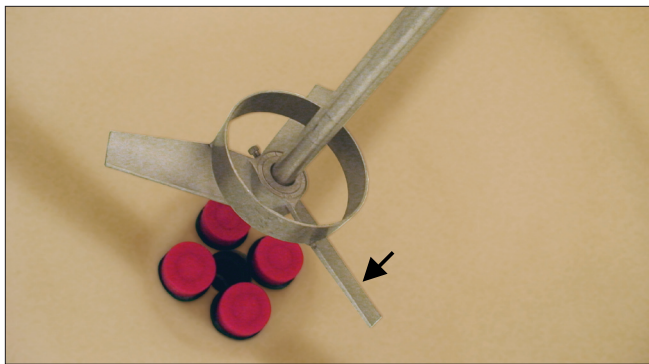


Fig. 2. Use a low-shear hydrofoil impeller for automated mixing of CHT media.

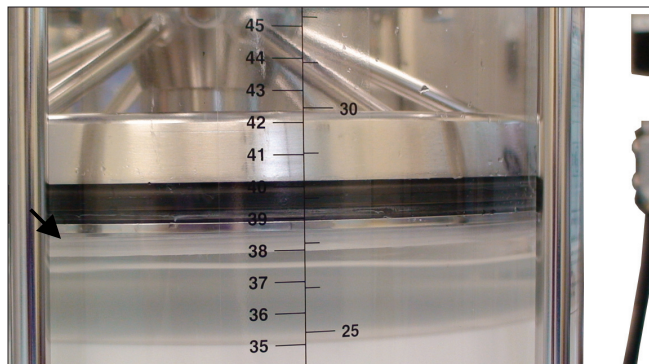


Fig. 3. A 2–3 cm buffer layer forms on top of the media bed.

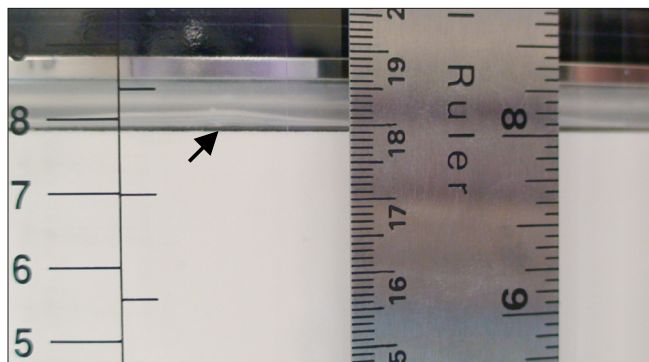


Fig. 4. Headspace of 1 mm between the top of the media bed and the adaptor: Leaving a headspace (indicated by arrow) between the top of the media and the adaptor is critically important to avoid crushing the CHT media beads.

- For open column systems, form slurry directly in the column tube by adding the calculated amount of buffer and then slowly adding the dry CHT media while mixing with a plastic paddle
- For slurry tanks, add calculated amount of buffer to tank, then add CHT media while the impeller mixes slowly. Ensure impeller speed is just rapid enough to maintain a uniform slurry
- Slurry transfers should be done with a diaphragm pump or, for smaller volumes, by pouring from a carboy or pitcher

### Packing

To pack CHT media in Bio-Rad® InPlace™ columns and in open column systems:

- CHT media settle rapidly (35–125 cm/hr for 40 μm CHT beads; 125–275 cm/hr for 80 μm CHT beads), so it is important to work quickly
- Within 5 min, a 2–3 cm buffer layer will develop over the settling media (Figure 3). Lower the top adaptor into the liquid and activate the seal
- Begin flow packing or axial packing as soon as possible to consolidate the bed. A flow rate of 100–300 cm/hr is recommended
- CHT ceramic hydroxyapatite does not rebound and is not compressible. Only one consolidation step is needed
- The single most important guideline for successful CHT media packing is to always leave a headspace of 1–5 mm between the top of the bed and the adaptor to avoid crushing the beads (Figure 4)
- When the bed is consolidated, the column is ready for equilibration and testing
- Once the column is successfully packed, do not perform buffer upflow. All column operations should be performed in downflow mode

\* Remco Products Corporation.

\*\*Lightnin Model A310 (SPX Corporation).

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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