

## CHT™ Ceramic Hydroxyapatite: Use in Expanded Bed Adsorption Mode

### Introduction

Expanded bed adsorption (EBA) is a cost saving procedure in which target molecules are purified from crude unfiltered feedstock without the need for separate clarification, concentration, and initial purification steps.<sup>1</sup> The expansion of the adsorbent bed suspends the adsorbent particles and allows particulates to pass upward through the bed during the application of crude feed to the column. The target molecule is recovered by reversal of the flow to pack the expanded bed and by application of the elution buffer.

CHT (Bio-Rad catalog #157-0080) is an ideal chromatographic support for use in expanded bed systems due to its unique biomolecule binding characteristics,<sup>2</sup> high specific gravity (s.g. = 3), and high mechanical stability. CHT,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , is a sintered form of calcium phosphate that can be used to separate and purify proteins, enzymes, nucleic acids, viruses, and other macromolecules. Its unique mechanism of separation involves both electrostatic interactions and the formation of calcium coordination complexes.

The spherical ceramic nature of CHT overcomes the physical and chemical instability that limits traditional hydroxyapatite use in biotherapeutic/EBA manufacturing processes. CHT is available in two porosities (Type I and Type II) and three particle size ranges (20, 40, and 80  $\mu\text{m}$  mean). For EBA, only the CHT 80  $\mu\text{m}$  bead has the proper particle density and size to achieve a stable fluidized bed and the high throughput required for industrial applications.

This study examines the expansion properties and protein binding characteristics of CHT Type I, 80  $\mu\text{m}$ , in the STREAMLINE 25 column (2.5 x 100 cm).

### Results

CHT Type I, 80  $\mu\text{m}$  (75 ml; 17 cm gravity-settled bed), was added to a STREAMLINE 25 column filled with 10 mM sodium phosphate, pH 6.8. Flow was incrementally increased by 50 cm/hr and held for 30 min (Figure 1). The experiment was repeated using 500 mM sodium phosphate, pH 6.8. Due to the higher viscosity of 500 mM sodium phosphate, pH 6.8 ( $\eta = 1.20$  cp), the degree of expansion ( $H/H_0$ , expanded bed height/settled bed height) was higher than with 10 mM sodium phosphate ( $\eta = 1.01$  cp). Typically, expanded bed systems require degrees of expansion values of 2–3 (Figure 2).<sup>1</sup> This requires a linear velocity range of 100–200 cm/hr for CHT Type I, 80  $\mu\text{m}$ .

The 10% breakthrough binding capacity for purified bovine IgG and bovine serum albumin (BSA) was determined for CHT Type I, 80  $\mu\text{m}$ , at 200 cm/hr in packed bed and expanded bed modes (Table 1). Only small differences were seen between packed and expanded beds, indicating that binding properties are similar. Total protein binding may be increased by increasing the amount of CHT Type I, 80  $\mu\text{m}$ .

### Recommendation

CHT is an ideal support for expanded bed systems. For initial studies, 17 cm of CHT Type I, 80  $\mu\text{m}$  in 10 mM sodium phosphate, pH 6.8, should be fluidized in a STREAMLINE 25 column at 200 cm/hr.

**Table 1. 10% breakthrough capacity for CHT Type I, 80  $\mu\text{m}$ , for bovine IgG (1.1 mg/ml) and BSA (2 mg/ml) in 10 mM sodium phosphate, pH 6.8 at 200 cm/hr.**

Mode	Protein	10% Breakthrough Capacity (mg/ml)*
Packed bed	Bovine IgG	38
Expanded bed	Bovine IgG	37
Packed bed	BSA	14
Expanded bed	BSA	11

\* Packed bed column dimensions, 2.2 x 18.5 cm (70.3 ml).  
Expanded bed height, 2.5 x 40 cm (73.6 ml settled bed volume).  
Column void volume was subtracted from 10% breakthrough volume.

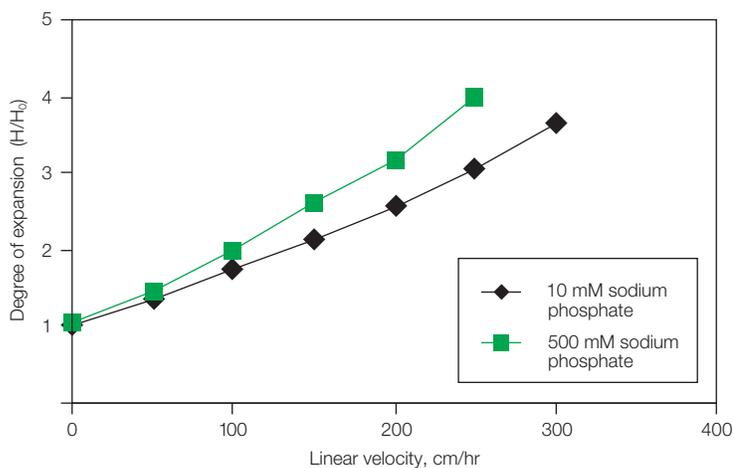


Fig. 1. Relative expansion at different flow velocities of CHT Type I, 80  $\mu\text{m}$ , with 10 mM and 500 mM sodium phosphate, pH 6.8.

### References

- Expanded Bed Adsorption — Principles and Methods, Amersham Pharmacia Biotech 18, 1124–1126
- Ceramic Hydroxyapatite — A New Dimension in Chromatography of Biological Molecules, Bio-Rad Laboratories, bulletin 2156

STREAMLINE is a trademark of Amersham Pharmacia Biotech.

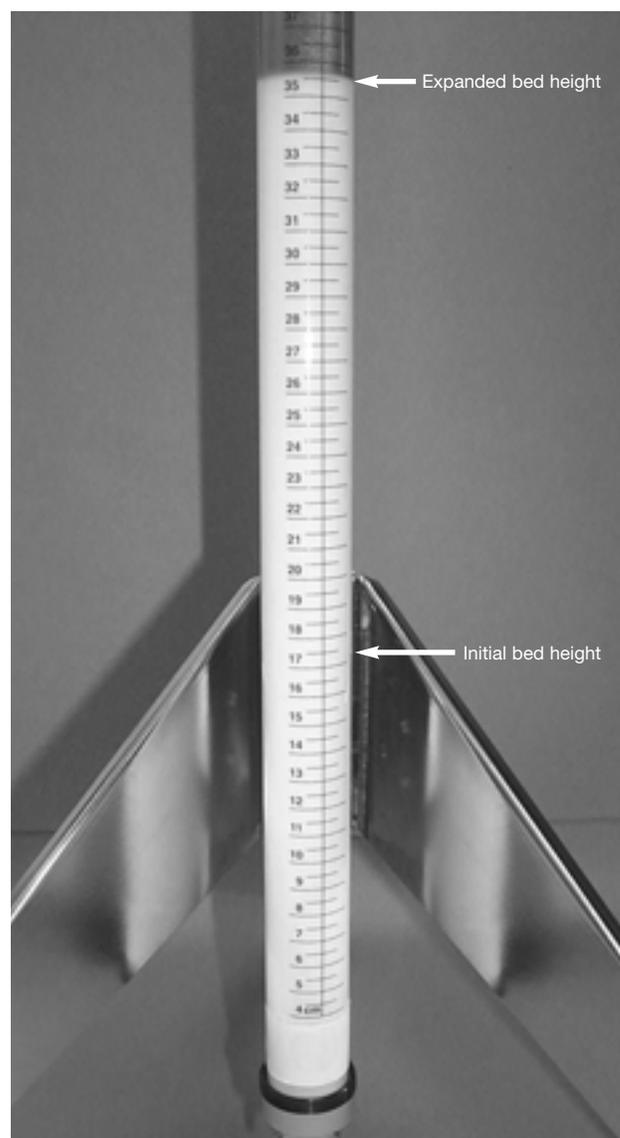


Fig. 2. Expanded CHT Type I, 80  $\mu\text{m}$  (17 cm settled bed height), in 10 mM sodium phosphate, pH 6.8, at 150 cm/hr in a STREAMLINE 25 column.

**BIO-RAD**

**Bio-Rad  
Laboratories, Inc.**

Life Science  
Group

Web site [www.bio-rad.com](http://www.bio-rad.com) USA (800) 4BIORAD Australia 02 9914 2800 Austria (01)-877 89 01 Belgium 09-385 55 11 Brazil 55 21 507 6191  
Canada (905) 712-2771 China (86-21) 63052255 Czech Republic (420) 2-4141 0532 Denmark 45 44 52-1000 Finland 358 (0)9 804 2200  
France 01 47 95 69 65 Germany 089 318 84-177 Hong Kong 852-2789-3300 India (91-124) 6398112/113/114, 6450092/93 Israel 03 951 4127  
Italy 39 02 216091 Japan 03-5811-6270 Korea 82-2-3473-4460 Latin America 305-894-5950 Mexico 52 5 534 2552 to 54  
The Netherlands 0318-540666 New Zealand 64-9-4152280 Norway 47-23-38-41-30 Poland (48) 22-8126 672 Portugal 351-21-472-7700  
Russia 7 095 721 1404 Singapore 65-2729877 South Africa 00 27 11 4428508 Spain 590 5200 Sweden 46 (0)8-55 51 27 00 Switzerland 061 717-9555  
United Kingdom 0800-181134