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Journal of Chromatography A, 1165 (2007) 78–85
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Use of an alternative scale-down approach to predict and extend hydroxyapatite column lifetimes

Justin T. McCue *, Douglas Cecchini, Kenneth Hawkins, Eric Dolinski

Biogen Idec Corporation, Bioprocess Development, 14 Cambridge Center, MA 02142, USA

Received 30 May 2007; received in revised form 20 July 2007; accepted 23 July 2007
Available online 31 July 2007

Abstract

Ceramic hydroxyapatite (CHT) chromatography offers unique selectivity for protein purification. However, columns composed of CHT, a crystalline form of calcium phosphate, often suffer from short column lifetimes, particularly under acidic operating conditions. In this paper, CHT was used under slightly acidic conditions (pH 6) for the production scale purification of a recombinant protein. Under these conditions, the packing quality of production scale CHT columns (45 cm diameter) degraded after 5–10 cycles of operation. This was not reproduced using a conventional scale-down chromatography model, in which a constant column bed height was maintained across scales. Thus, an alternative scale-down model was developed to better predict the lifetime of large scale CHT columns. The alternative approach, which utilized a constant column diameter-to-height aspect ratio, was able to predict column failure that approximated that of the manufacturing scale column. The alternative scale-down approach was then used to test alternate buffer formulations that significantly improved the CHT column lifetime. Screening studies, which assessed the effects of mobile phase pH and composition on the dissolution (weight loss) of CHT, were used to identify the alternative mobile phase formulations. Results from the study showed that slight changes to the existing mobile phase compositions significantly increased the column lifetime, from approximately 10 cycles to approximately 65 cycles of use, without altering the purification of the recombinant protein. The alternative scale-down model, together with relatively rapid mobile phase screening studies, provides a practical approach for predicting and optimizing the useful lifetime of CHT columns for large scale applications.

Keywords: Hydroxyapatite; Preparative chromatography; Scale-up; Column lifetime

1. Introduction

Hydroxyapatite (HAP) consists of a crystalline form of calcium phosphate ($Ca_5(PO_4)_3OH_2$). When used in chromatography applications, HAP possesses unique protein separation properties, which are a result of the heterogeneous functional groups present on the crystal surface. Hydroxyapatite contains both positively charged calcium ions and negatively charged phosphate sites, which give rise to complex protein-surface interactions [1–6]. Ceramic hydroxyapatite (CHT), a sintered form of the crystalline material, is a spherical, macroporous form of HAP. Ceramic hydroxyapatite provides improved stability and flow properties, which allows for its use in laboratory and process scale applications [1,5,6].

Ceramic hydroxyapatite chromatography has been used as a purification step in the preparative scale manufacturing of recombinant proteins [6,7]. However, use of CHT in large scale purification processes can often be limited due to adsorbent dissolution and discoloration, which results in reduced column lifetimes. Severe discoloration of CHT can occur due to the binding of trace metals that are present in the chromatography buffers [7]. This can potentially alter the chromatographic properties of CHT [8] and may further contribute to the degradation of the packed bed. Previous work [9–12] has shown the dissolution mechanism of HAP to be complex, depending on the mobile phase pH, mobile phase composition and the HAP surface properties. Hydroxyapatite is known to dissolve in acidic solutions (pH <7), with the rate of dissolution increasing at lower pH [9]. Thomann et al. [9] showed the dissolution rates depended not only on pH, but also on the solution composition and the initial state of the HAP. The presence of calcium or phosphate in the solution was shown to inhibit the dissolution

* Corresponding author. Tel.: +1 617 679 3500; fax: +1 617 679 3408.
E-mail address: Justin.mccue@biogenidec.com (J.T. McCue).

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doi:10.1016/j.chroma.2007.07.053

kinetics in weakly acidic conditions (pH 4.5–6.5). The authors demonstrated the rate controlling process for HAP dissolution was the diffusion of calcium and/or phosphate ions through the solid-liquid interfacial surface layer [11,12].

Despite the large amount of studies, there is a need to better understand the impact of mobile phase composition and pH on CHT stability in the form of a packed chromatography bed, especially in the presence of weakly acidic solutions. Weakly acidic solutions (pH 5.5–6.5) are often employed to maximize the CHT protein binding capacity and selectivity [1]. However, under such conditions the column lifetime can be relatively short [7] because of CHT degradation. Thus, it is desirable to understand the effects of the mobile phase properties, such as the operating pH and buffer composition, and how they impact CHT column lifetime in large scale manufacturing applications.

CHT chromatography was used to purify a recombinant protein in a large scale manufacturing process using a CHT column with a diameter of 45 cm. At the recommendation of the vendor (Bio-Rad Laboratories Inc., Hercules, CA), a small guard column, also packed with CHT (Fig. 1), was implemented to trap trace metals and prevent discoloration of the main CHT column. To achieve the required selectivity, the CHT chromatography step was operated under slightly acidic (pH 6.1) conditions. In addition, the tendency of relatively low concentrations of phosphate to decrease the binding capacity of CHT [5] and alter the selectivity for the recombinant protein limited the use of phosphate buffers (which stabilize CHT) during the chromatography process. Thus, the production scale CHT column suffered from a short lifetime. Severe peak fronting was observed during the determination of the height equivalent of a theoretical plate (HETP) following only 5–10 cycles of operation (Fig. 2) and the main column had to be replaced frequently. The rapid column degradation observed at the production scale was not observed in prior development studies, which used columns that were scaled down by diameter only. Thus, an alternative scale-down model for CHT chromatography was developed to better model the lifetime of CHT columns used for large scale chromatography. The alternative approach maintained the same column diameter-to-height aspect ratio that was used for the production scale column (Fig. 1). This alternative scale-down model reproduced the early column failure first observed in the production scale column and was also used to compare the effects of two modified mobile phase compositions on CHT column lifetime. The modified mobile phase conditions were selected based on screening studies, which assessed the effects of mobile phase pH and composition on the dissolution (weight loss) of CHT. The alternative scale-down model, together with relatively rapid mobile phase screening studies, provides a practical approach for predicting and optimizing the useful lifetime of CHT columns in large scale applications.

2. Experimental

2.1. Materials

2.1.1. Adsorbents

The CHT adsorbent (MacroPrep, ceramic hydroxapatite, type I) was manufactured by Bio-Rad Laboratories Inc. Both 40 μm (Cat. #157-0045) and 80 μm (Cat. #157-0045) particle sizes were used in the studies.

2.1.2. Solvents and chemicals

Chemicals used to produce various mobile phase buffer solutions in the studies included MES (Cat. #4014-40, J.T. Baker,), MOPS (Cat. #4004, J.T. Baker), sodium chloride (Cat. #3627-07, J.T. Baker,), sodium hydroxide (Cat. #3718-01, J.T. Baker), hydrochloric acid (Cat. #9535, J.T. Baker), potassium phosphate (monobasic) (Cat. #7778-77-0, Mallinckrodt), potassium phosphate (dibasic) (Cat. #P288, Fisher) and calcium chloride (Cat. #10035-04-8, Fisher).

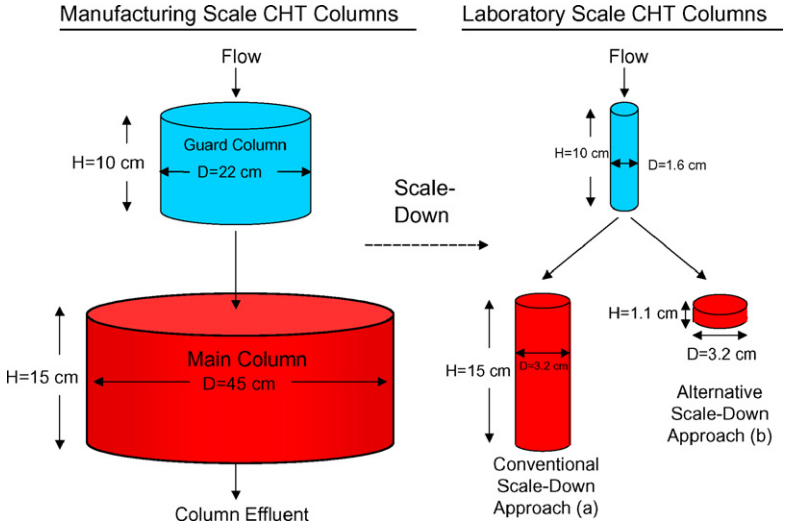

Fig. 1. Conventional (a) and alternative (b) scale-down approaches used to model the manufacturing scale CHT chromatography column. Laboratory scale cycling studies using the alternative scale-down approach (constant aspect ratio) were used to evaluate the column lifetime of the CHT columns (Table 3, described in Section 2.3.2).