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Fabrication and characterization of superporous cellulose bead for high-speed protein chromatography

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Abstract

Novel superporous cellulose (SC) matrix has been fabricated by water-in-oil emulsification–thermal regeneration using granules of calcium carbonate as porogenic agents. As a control, microporous cellulose (MC) bead was fabricated in the absence of calcium carbonate. Simultaneously, double cross-linking was applied to enhance the mechanical strength of the particles. The photographs by scanning electron microscopy of the SC bead illustrated that there were more "craters" of several microns scattering on the surface of the beads. It led to a higher water content and effective porosity of the SC medium. The two beads were then modified with diethylaminoethyl (DEAE) group to prepare anion exchangers. The dynamic uptake results of bovine serum albumin (BSA) exhibited that the pore diffusivity of BSA in the DEAE-SC bead was two to three times larger than that in the DEAE-MC bead. In addition, the column packed with the DEAE-SC showed lower backpressure, higher column efficiency and dynamic binding capacity than the column packed with the DEAE-MC at a flow rate range of 150–900 cm/h. Moreover, the column efficiency of the DEAE-SC column was independent of flow velocity up to a flow rate of 1200 cm/h. All the results exhibited the superior characteristics of the SC bead as a potential medium for high-speed protein chromatography.

Keywords: Cellulose bead; Superpores; Anion exchanger; Protein chromatography; Column efficiency; Dynamic binding capacity

1. Introduction

Diverse adsorption mechanism and mild separation condition make preparative liquid chromatography (LC) become a widespread method for the recovery and purification of biological substances such as proteins in the downstream processing of biotechnology [1,2]. It is well known that the LC technology mainly relies on the structure of its stationary phase. However, most of commercial chromatographic media provide nano-pores with a size range of 10–200 nm for diffusing proteins [3], so the intraparticle mass transfer of proteins exhibits a serious hindered diffusion fashion and the performance of chromatography processes is decreased at higher flow rate. Generally, the height equivalent to a theoretical plate (HETP) is used to measure the chromatographic efficiency. The HETP is generally described by the Van Deemter equation [4].

$$\mathrm{HETP} = \frac{A}{u} + B + Cu \tag{1}$$

where u is the superficial velocity; A, B and C are parameters depending on chromatographic system and operation conditions. Based on the equation, lower intraparticle diffusivity expressed in term C leads to an aggravated column efficiency and band broadening at fluid velocities commonly used in conventional preparative chromatography. Hence, in view of the fragility of proteins and biological degradation of proteases in feedstock [5], intraparticle mass transfer should be enhanced to obtain a fast and high-resolution separation as well as a high binding capacity.

Currently, there have been various kinds of stationary phases designed for the separation of proteins. In the beginning of 1990s, a new packing material, referred as perfusion adsorbent, was introduced to mend the stagnant mass transfer resistance [6,7]. In the perfusion packing materials, the flow-through pores with a size of 600–800 nm are formed to connect diffusive pores

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with a pore size of 80–150 nm and bring finite intraparticle convection into the bead. It is well known that a rather small pore flow can significantly augment intraparticle mass transfer [8]. Therefore, perfusion particles provide a more elegant way to enhance mass transfer inside the beads whilst a large surface area is still remained as a result of the presence of diffusive pores. So, the column packed with the matrices can be operated at high speed while maintaining high column efficiency and dynamic binding capacity. Up to date, a few superporous adsorbents, such as polymeric biporous resins [9–11] and superporous agarose beads [12–15], have been fabricated.

Polysaccharides such as agarose, cellulose and dextran, are the most commonly used chromatographic matrices for the purification of biomolecules. These materials provide versatility in chemical structure, biocompatibility to biological molecules, availability in price and ease in modification [16]. However, low mechanical strength restricts their applications at high superficial velocities [17]. Gustavsson et al. prepared superporous agarose beads by double emulsification, in which flow-through pores were formed by organic solvents [14,15]. As expected from perfusive adsorbents, column packed with superporous agarose beads could be operated at high flow rates while retaining high capacity and efficiency. A rigid superporous cellulose material prepared by Lali et al. could be used to purify plasmid DNA in a single chromatographic step [18]. Recently, Shi et al. [12] fabricated superporous agarose beads using granules of calcium carbonate as a porogens [19,20]. The column packed with the superporous agarose gel could be operated at a superficial velocity over 1000 cm/h with little decrease in the column resolution. Thus, by introducing superpores by adding porogenic agents during the preparation, the drawback of soft matrices was partly overcome.

This work is a further contribution to the development of superporous chromatographic matrices with the porogenic method using solid granules to form superpores. Here, we have fabricated a novel rigid superporous cellulose bead by water-in-oil emulsification–thermal regeneration. As a control, microporous cellulose bead was fabricated in the absence of calcium carbonate. Mechanical strength of the matrices was further enhanced by a double cross-linking procedure. By modifying the matrices with diethylaminoethyl groups, anion exchangers were prepared. The superporous structure of the cellulose beads was characterized by scanning electronic microscopy, effective porosity for protein and water content. Then, the flow hydrodynamics and chromatographic performance of the anion exchangers were investigated in detail to debate the feasibility and potential of the superporous cellulose bead for high-speed protein chromatography.

2. Materials and methods

2.1. Materials

Degreasing cotton was purchased from League Health Materials (Jiaozuo, China). Calcium carbonate granule with an average particle size of 2.34 μm and a density of 1.84 g/ml was received from Lihe (Tianjin, China). Tris(hydroxymethyl) aminomethane (Tris), diethylaminoethyl chloride (DEAE-Cl) and bovine serum albumin (BSA) with a purity of 96% were obtained from Sigma (St. Louis, MO, USA). Transformer oil was provided by the Transformer Station of Tianjin University (Tianjin, China). Other reagents were of analytical grade from local sources.

2.2. Preparation of cellulose beads

The superporous cellulose bead was fabricated by a water-in-oil emulsification–thermal regeneration method. Briefly, 5 g of degreasing cotton was submerged in 50 ml of 19% (w/w) hydroxide sodium (NaOH) solution for 2 h. The cotton was shredded and kept for aging in an ambient temperature for 48 h. It was then reacted with 2.5 ml of carbon disulfide in a shaking incubator (shaking rate, 150 stokes/min, spm) at 25 °C for 10 h to produce cellulose xanthate. The viscose solution with a given concentration was prepared by dissolving cellulose xanthate into 30 ml of 6% (w/w) NaOH solution, and then 7 g of calcium carbonate granules were added to prepare 7.6% (v/v) calcium carbonate-viscose suspension. Such a well-mixed suspension was dispersed in a reactor containing 600 ml of transformer oil, in which 0.1 g of potassium oleate and 0.4 g of Span 60 were dissolved. The suspension was stirred at 500 rpm. After 1 h, the suspension in the vessel was heated to 90 °C and kept for 2.5 h at the same stirring speed. Thereafter, the mixture was cooled to room temperature and white spherical particles were collected by centrifugation. The beads were washed with deionized water to remove the residual transformer oil and subsequently screened with standard sieves of 100 and 200 meshes by wet sieving. In the present article, the bead obtained as stated above was nominated as superporous cellulose (SC) bead. Moreover, microporous cellulose (MC) bead was fabricated by the same procedure without adding calcium carbonate granules to the viscose solution in the above procedure.

2.3. Cross-linking and carbonate removal

Prior to the removal of calcium carbonate, the SC particles were successively double cross-linked with glycol diglycidyl ether and epichlorohydrin (ECH) as described by Pernemalm et al. [21]. In brief, 5 g of drained cellulose beads was mixed with 5 ml of glycol diglysidyl ether in a flask. The suspension was shaken in an incubator at 170 spm for 1 h, and then 10 ml of 3.0 mol/L NaOH was added. After 2.5-h reaction at 40 °C, the cellulose particles were filtered off the solution and rinsed with an excess of deionized water to remove residual cross-linking agent in the beads. Then, the beads were further cross-linked by reacting with epichlorohydrin. The procedure for ECH cross-linking was the same as the step described above, just replacing glycol diglycidyl ether with epichlorohydrin. The MC particles were cross-linked by the same procedures.

The removal of calcium carbonate granules from the SC beads was carried out by reacting with a dilute acidic solution. One volume of the cross-linked beads was mixed with eight volumes of hydrochloric acid (1 mmol/L) in a flask and the suspension was shaken on an incubator at 150 spm until no bubbles were