Preparation and Characterization of Titanium Oxide-Densified Cellulose Beads for Expanded Bed Adsorption

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ABSTRACT: Expanded bed adsorption (EBA) has been widely used in industrial downstream bioprocessing. Solid matrix is the principal pillar supporting the successful application of the EBA. A new type of TiO_2-densified cellulose beads as matrix for EBA is exploited through the method of water-in-oil suspension thermal regeneration. The typical physical properties of beads are wet density 1.21 g/cm^3, specific surface area 38.7 m^2/mL, porosity 83.7%, and water content 69.5%. The results indicate that the custom-assembled matrix can be derivated to function as an anion exchanger for EBA, by possessing a significant mechanical strength, stable hydrodynamics, and comparable protein breakthrough capacities with the commercial Streamline diethylaminoethyl (DEAE) adsorbent. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 90: 2848–2854, 2003

Key words: expanded bed adsorption; composite matrix; cellulose; TiO_2; proteins

INTRODUCTION

Cellulose is the most abundant naturally occurring polymer in the world. Its replaceable, renewable, and biodegradable properties have make it one of the most widely studied polymers.[1,2] In the 1970s, Peška et al.[3] developed a method for manufacturing cellulose beads based on the thermal sol–gel transition process of cellulose xanthate viscose. From then on, porous cellulose bead has been widely used as column packing material for liquid chromatography and as matrix for further derivatization for ion exchange or affinity chromatography.[4, 5]

Expanded bed adsorption (EBA) is a novel bioseparation technique, which integrates clarification, concentration, and initial purification into a single step. It enables proteins to be recovered directly from unclarified cultivations of microorganisms or homogenates of disrupted cells, without the need for prior removal of suspended solids.[6] It is obvious, therefore, that the use of this technique would be both technically and economically valuable. Solid matrix is the principal "hardware" pillar supporting the successful application of EBA. The basic criteria of the matrices for EBA are formulated as being a sufficient density and a distribution of particle size. Some other physicochemical properties of matrices, such as shape, porosity, hydrophilicity, and modifiability, are also important for efficient protein adsorption.[7] In 1994, Gilchrist et al.[8] prepared an anion exchanger with TiO_2-densified cellulose particles as matrix for EBA. The adsorbent was found to have high protein binding capacities. However, their matrix was provided with irregular shape that possibly leads to unstable expansion behavior, and with large particle size up to 600 μm indicating long mass transfer distance thus slow adsorption kinetics.

The purpose of this work is to prepare a spherical TiO_2-densified cellulose composite matrix for EBA. After activated by epichlorohydrin and coupling with diethylamine, the matrix is derived to function as an anion exchanger. The effects of the weight ratio of TiO_2 to viscose and the content of cellulose in xanthate viscose on the physical properties of matrices are studied in details. After that, the expansion behavior and protein adsorption performance of the aimed adsorbent in an expanded bed are also investigated. As a control test, a commercially available adsorbent Streamline diethylaminoethyl (DEAE), which was exploited by Amersham Biosciences and has been widely used on pilot and industry scales,[9] is chosen to compare with our absorbent.

EXPERIMENTAL

Materials and chemicals

Degreasing cotton with a degree of polymerization 1620 was ordered from Huamei Biotech Ltd. (Shanghai, China). Rutile TiO_2 with a density of 4.2 g/cm^3 and a size of 300 nm was provided by Huachang Polymer Ltd. (Shanghai, China). Streamline DEAE and Streamline base matrix were purchased from Am-

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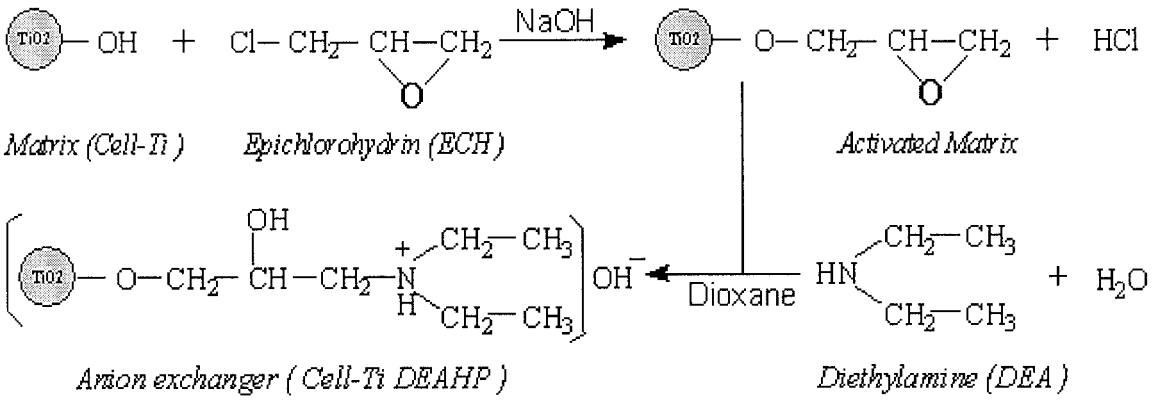

Figure 1 Preparation route of Cell-Ti DEAHP.

ersham Biosciences (Uppsala, Sweden). Bovine Serum Albumin (BSA) (A-7030) was obtained from Sigma (Milwaukee, WI, USA). Pump oil was of technical grade and supplied by Shanghai Chemical Reagent Co. All other reagents were of analytical reagent grade and purchased from local suppliers.

Preparation of composite beads

The cellulose xanthate viscose was prepared by reacting 45 g alkali-treated and aged degreasing cotton with 20 mL CS_2 and then dissolving into 6% (w/w) NaOH solution. A given mass TiO_2 powder was mixed thoroughly with 100 g viscose. Then the mixture was dispersed in a solution of 200 mL chlorobenzene in 400 mL pump oil in a 1-L flask with agitation at 350 rpm for 0.5 h at 25°C. The suspension was heated to 95°C for 1 h under continuous stirring, then cooled down and filtered. The resulting particles were washed successfully with benzene and methanol. The decomposition of cellulose xanthate was completed by stirring in a solution of 50 mL acetic acid and 200 mL ethanol. After washing with water and sieving with standard test sieves in water, about 30 mL beads (designated Cell-Ti) were obtained.

Preparation of cellulose-based adsorbent

The matrix Cell-Ti was activated by epichlorohydrin, and then attached to diethylamine to produce an anion exchanger (Cell-Ti DEAHP) with diethylaminohydroxypropyl weak base groups (as shown in Figure 1). Typically, the activation of matrix was completed by stirring 30 mL Cell-Ti in 60 mL 3*M* NaOH solution containing 12 mL epichlorohydrin at 25°C for 8 h. After extracting with acetone in a Soxhlet apparatus for 4 h to remove low polymers, the beads were mixed with 90 mL dioxane and 90 mL diethylamine. The mixture was stirred and heated at 55°C for 12 h. The reaction product was washed with 500 mL water and stored in 20% (v/v) ethanol.

Expanded bed operation

A homemade column (1000×20 mm I.D.) was used for expanded bed experiments. About 2 mL glass beads (0.3 mm diameter) were added to improve flow distribution at the column inlet. A movable top adapter was employed to adjust the position of liquid outlet just above the bed surface. Proper column vertical alignment was confirmed in all experiments. A peristaltic pump (Lan'ge Ltd., Baoding, China) was used for fluid supply. All operations were performed at 25°C with a settled bed height of 150 ± 2 mm.

Protein adsorption

BSA was used as a model adsorbate for protein adsorption. In all adsorption tests, the aqueous phase was 50 m*M* Tris HCl buffer (pH 7.5). The rates of protein uptake were measured in a batch stirred vessel. To explain briefly: 1 mL preequilibrated adsorbent was added into 40 mL homogeneous solution (3 mg/mL BSA in buffer) in a 100 mL beaker. The flow phase was continuously circulated by a pump and BSA concentration was monitored by an ultraviolet (UV) detector (Amersham Biosciences). The adsorbents were also challenged in the presence of suspended biomass, for the adsorption rate in a simulated fermentation broth (20 dry g/L baker's yeast, dosed with 3 mg/mL BSA in buffer). A XK16/20 column (Amersham Biosciences) was employed for the packed bed adsorption with a settled bed height of 100 ± 1 mm, while the expanded bed mentioned above was adopted for EBA. The concentrations of protein at both column outlets were monitored with the UV detector at 280 nm. All experiments were conducted at 25°C.

ANALYSIS AND CHARACTERIZATION

Physical properties

The physical properties of prepared matrices were characterized as follows. The definite size distribution

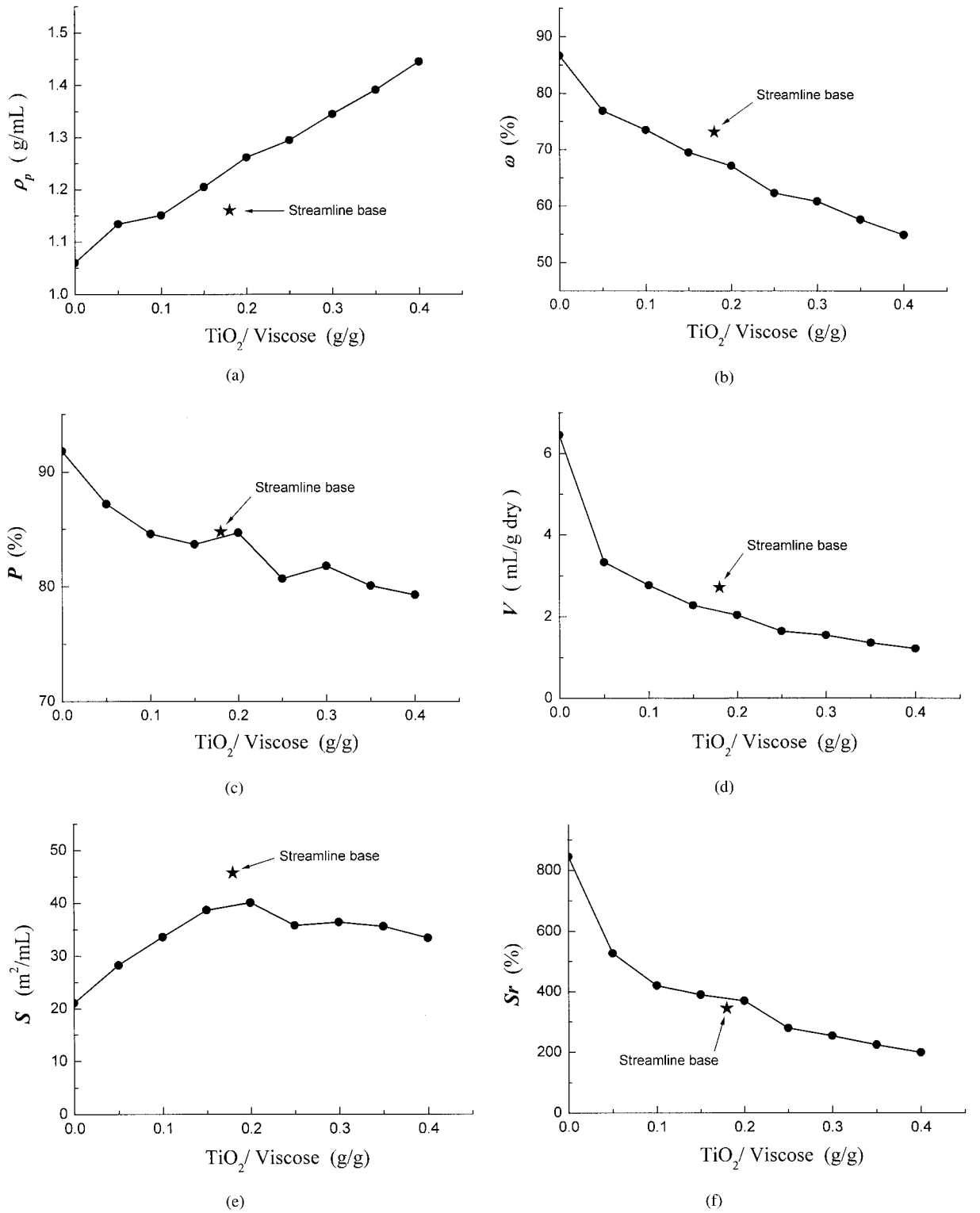

Figure 2 Effects of TiO_2 content on physical properties.

of Cell-Ti beads was determined with a LS-230 laser particle size analyzer (Coulter Co., Miami, FL, USA). A 37XAZ inverted biologic microscope (Shanghai Optical Instrument Plant, Shanghai, China) was used to observe the shape of particles. Shrinking behavior was characterized as the shrunk volume percentage, $Sr = (V_w - V_d)/V_d \times 100\%$, in which V_w and V_d represented the volumes of the wet and the dried particles, respectively. Specific surface area S is defined as the accessible area of solid/mL wet beads, including ex-

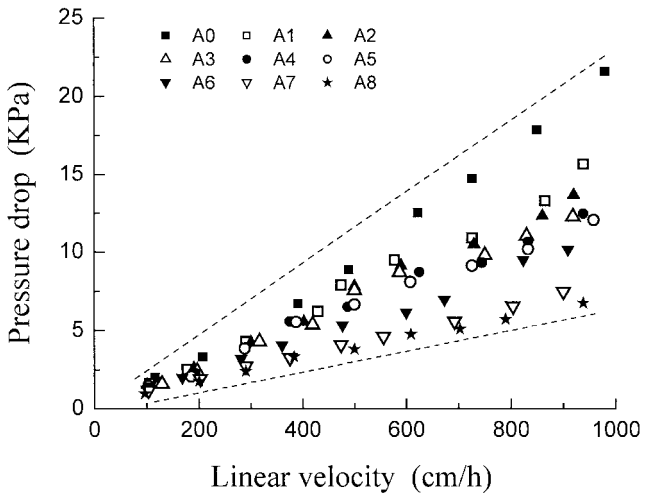

Figure 3 Flow characteristics of matrices.

ternal and internal surface area, and determined by the method of adsorption of methylene blue in liquid phase.[10] The flow tests in a column (1000×20 mm I.D.) with a packed height of 500 ± 2 mm were carried out to evaluate the mechanical strength of the prepared beads. Results from the flow tests were obtained as a relation between pressure drop and linear velocity, which corresponds to the hydraulic property of beads. The wet density ρ_p of sucked beads was determined by water displacement method in a 10 mL gravity bottle, while water content ω was obtained by dehydration at 120°C to a constant mass. Presuming that all of pores in particles were full of water, porosity P expressing pore volume per mL wet particles and pore volume V expressing pore volume per gram dried particles can be roughly calculated as $P = \rho_p \cdot \omega/\rho_{water}$ and $V = \omega/(1-\omega)$.[11]

Expansion characteristics

The degree of expansion E is measured as H/H_0, where H is the expanded bed height and H_0 the sedimented bed height. Therefore, E values at a variety of flow velocities in different mobile phases were used to exhibit the expansion characteristics of adsorbents.

Protein adsorption performance

The concentration of protein in the simulated broth was determined by the Bradford method[12] with a centrifugally separated supernatant, while the others were monitored spectrophotometrically at 280 nm by a UV detector. Breakthrough capacities (Q_b) in packed bed and expanded bed were calculated as the amount of protein bound to the adsorbent when a breakthrough of 10% of the initial protein concentration occurred. Simultaneously, the available protein binding capacity (Q_m) was calculated from the mass balance at 100% breakthrough of the initial concentration. Both Q_b and Q_m were given as mg adsorbed protein/mL sedimented adsorbent.

RESULTS AND DISCUSSION

Effect of TiO_2 content

The amount of TiO_2 and cellulose in mixed viscose influences the physical properties of the prepared composite beads. First, the effects of the weight ratio of TiO_2 to viscose on the physical properties including wet density ρ_P, water content ω, porosity P, pore volume V, specific surface area S, and shrinking degree Sr, were investigated. The results are shown in Figure 2(a)–(f), respectively, in which the cellulose content in viscose is wt 7.5%. Figure 2(a) indicates that wet density increases almost linearly with the increase of the TiO_2 content, confirming that superfine TiO_2 had been successfully entrapped within the cellulose matrix to impart an increased density to particles. From Figure 2(b)–(f), it is also found that the increase of the TiO_2 content results in the corresponding decreases in water content, porosity, pore volume, and shrinking degree. It is noticeable that specific surface area has an obvious ascent when TiO_2 is added to produce beads, and stands at a relatively high level even at high TiO_2 dosages (up to a weight ratio of TiO_2 to viscose 40/100). This will benefit the separation of biomolecules, especially for those small molecular peptides, with supplying a multitude of adsorption sites. In general, a TiO_2-densified cellulose matrix with comparable properties to the commercial Streamline base matrix can be achieved at a weight ratio of TiO_2 to viscose 15/100.

TABLE I
Effects of Cellulose Content on Physical Properties

Cellulose in viscose (wt %)	ρP (mg/mL)	ω (%)	P (%)	V (mL/g dry)	S (m^2/mL)	Sr (%)
6.0	1.18	76.3	90.0	3.22	24.3	450
7.5	1.21	69.5	83.7	2.28	38.7	390
9.0	1.22	63.7	77.7	1.75	31.4	325

The weight ratio of TiO_2 to viscose 15/100.

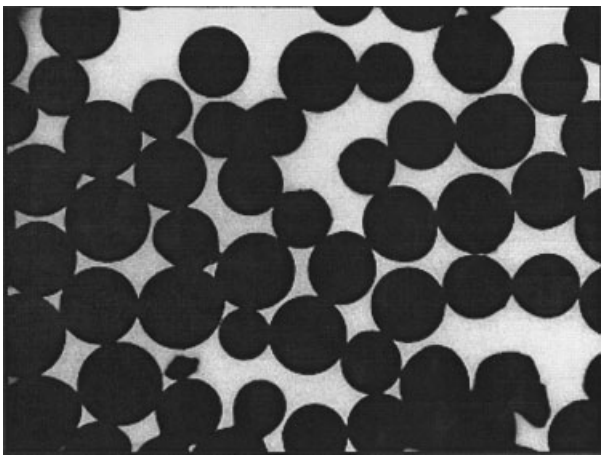

Figure 4 Morphology of Cell-Ti.

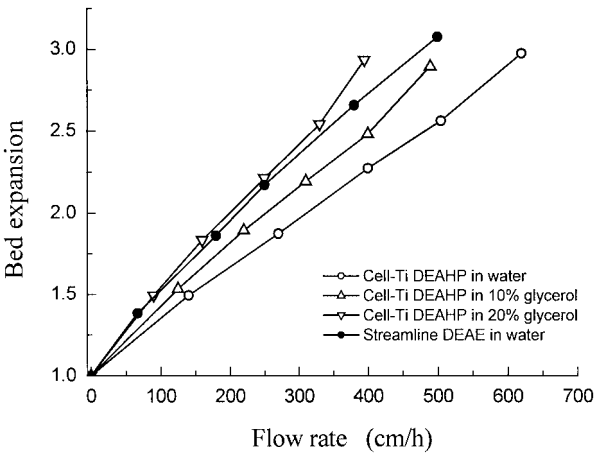

Figure 6 Expanded bed characteristics of adsorbents.

The flow characteristics of chromatographic matrix are remarkable for a bead-formed polymer without crosslinking, as is demonstrated by the dependence of the operating pressure on the linear flow rate. The results are shown in Figure 3, in which items A0 to A8 respectively express matrices prepared at the weight ratio of TiO_2 to viscose from 0/100 to 40/100, at an interval of 5/100. The cellulose content in viscose is also wt 7.5%. From the figure, a lower pressure at the same flow rate, suggesting increased mechanical stability, is observed after entrapping more TiO_2 into particles. This may probably be attributed to the functions that TiO_2 plays not only as a densifier but also as a supporting skeleton.

Effect of cellulose content

The effects of the cellulose content in initial viscose were further examined. The results are listed in Table I, showing that the increase of cellulose content (from 6 to 9%) leads to the slight rise in wet density, but also the evident falls in water content, porosity, pore volume, and shrinking degree. This means that not only the chemical modification but also the pore structure of composite beads can be adjusted by altering the cellulose content in initial viscose.

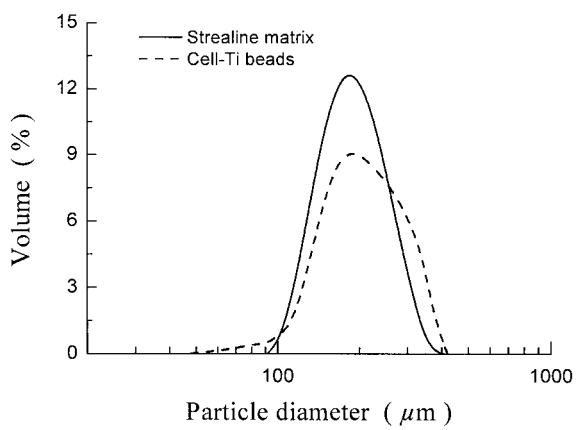

Figure 5 Particle size distribution of matrices.

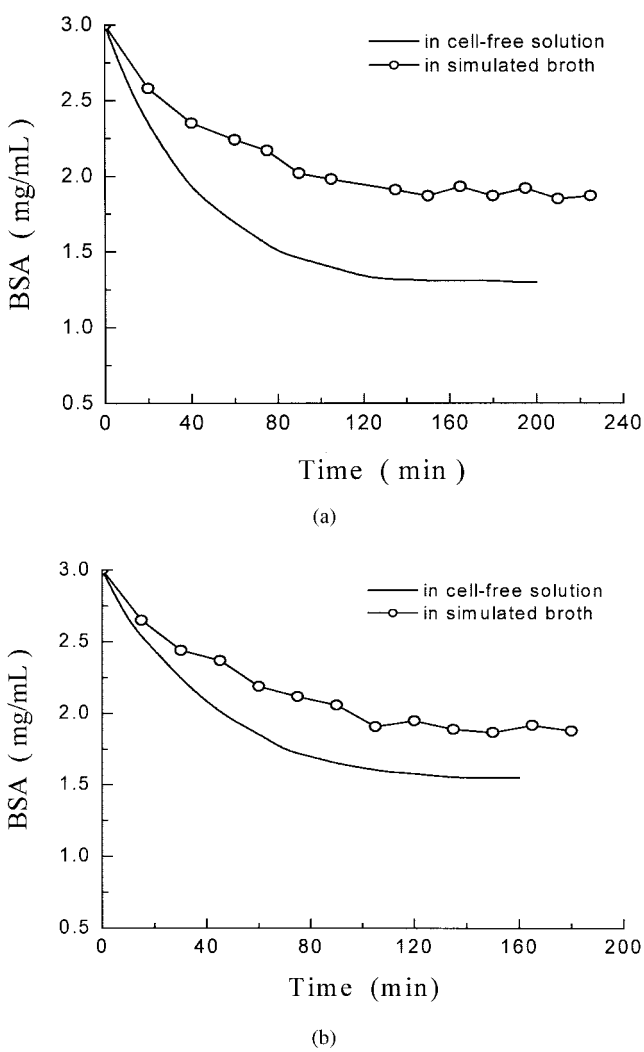

Figure 7 BSA dynamic binding profile in cell-free and simulated systems: (a) Streamline DEAE and (b) Cell-Ti DEAHP.

TABLE II
Comparison of Dynamic Binding Capacities (*DBC*)

Adsorbent	DBC_1 (mg/mL)	DBC_2 (mg/mL)	DBC_2/DBC_1 (%)
Streamline DEAE	67.9	45.2	66.5
Cell-Ti DEAHP	58.1	43.8	75.3

DBC_1: in cell-free system; DBC_2: in simulated system.

TABLE III
Comparison of Breakthrough Capacities

Mode	U (cm/h)	Q_b (mg/mL)	Q_m (mg/mL)	Q_b/Q_m (%)
PB	60	52.6	55.6	94.6
PB	190	49.3	54.7	90.1
PB	410	46.2	52.6	87.8
EB	410	42.1	48.9	86.1

PB: packed bed; EB: expanded bed.

Sphericity and size distribution

Figure 4 displays the morphology of matrix particles. As shown in Figure 4, no tendency for cracking of the particles is observed, and the most of particles are of regular sphericity except for a little adhesion among particles. The size distribution of particles is also shown in Figure 5. Compared with the case of the commercial Streamline matrix, Cell-Ti possesses a relatively wider distribution. Palsson et al.[13] has reported that a wider particle size distribution of adsorbent could contribute to the stability of expanded bed. In the following study for hydrodynamic properties of Cell-Ti matrix in expanded bed, this viewpoint will be restated.[14]

Expanded bed characteristics

After activated by epichlorohydrin and coupling with diethylamine, the matrix Cell-Ti was derivated to function as an anion exchanger Cell-Ti DEAHP. Expanded bed characteristics shown in Figure 6 indicate that the bed expansions of Cell-Ti DEAHP are considerably lower than those of Streamline DEAE at identical flow rates, either in water or in glycerol solutions. This means that the aimed adsorbent expands more slowly with increasing flow rate than the commercial Streamline adsorbent. Thus, the former is more suitable for higher flow rates. Typically, an appropriate expansion was arrived by an E value of 2.5 for Cell-Ti DEAHP at close to 500 cm/h in water.

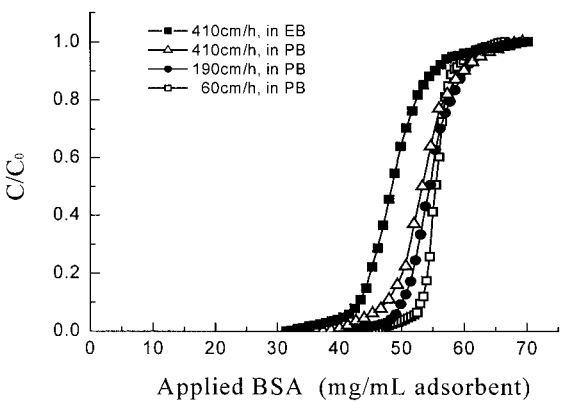

Figure 8 Breakthrough curves for 1 mg/mL BSA.

Protein dynamic adsorptions

The dynamic capacities for BSA in the homogeneous/cell-free system [Fig. 7(b)] indicate that Cell-Ti DEAHP performs at a lower capacity than Streamline DEAE [Fig. 7(a)]. However, in the presence of suspended solids, it is observed that dynamic capacities are reduced. It should be noted that the presence of suspended solids has the lesser effect on the binding capacity of Cell-Ti DEAHP (data summarized in Table II). In spite of the lower binding capacity in the cell-free system, Cell-Ti DEAHP displays quicker adsorption kinetics in both systems. This may be due to its macroporous and lowly crosslinked structure.

Protein breakthrough capacities

The influence of flow velocity on breakthrough behavior for Cell-Ti DEAHP in packed bed is shown in Figure 8. As expected, the increase of flow rate resulted in the decreases in both breakthrough capacity (Q_b) and available protein binding capacity (Q_m), arising from the more and more gentle breakthrough behavior. However, Q_b in expanded bed was more than 90% of that in packed bed at a flow velocity of 410 cm/h, indicating a low axial dispersion in the system (see Table III). From an practical viewpoint, the exploited adsorbent can therefore be applied to bioseparation and purification.

CONCLUSIONS

The fabrication of a TiO_2-densified cellulose composite has yielded macroporous beads that possess physical attributes and hydrodynamic characteristics required for use in an expanded bed. After activation and derivation, the composite serves as an anion exchanger and has comparable protein binding capacities with the commercial adsorbent. The results encourage the further development of a custom-built matrix designed for specific recovery applications exhibiting problems of varied biomass and broth rheologies.

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