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Preparation of Cellulose Adsorbents with Ionic Liquid and Pore Expansion for Chromatographic Applications

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ABSTRACT: Chromatography is a widely used technique in protein separation, and the adsorbents are essential to separate target proteins from raw mixtures. In this study, porous cellulose beads were prepared with a direct dissolution of microcrystalline cellulose (MCC) using an ionic liquid solvent 1-butyl-3-methylimidazolium chloride. Pore expanding agents (cassava starch and cyclohexane) were added in MCC solutions to change the pore structure of the cellulose beads. The results showed that the mean pore size of cellulose beads increased after the application of pore expanding agents, whereas the wet density and the specific surface area were decreased. Residence time distribution studies indicated that the beads prepared with the addition of cyclohexane had the best performance for a series of molecules with different molecular weights. The cellulose beads were coupled with diethylaminoethyl and the adsorption properties with bovine serum albumin as a model protein showed that the beads prepared with cyclohexane had the best protein adsorption capability. The chromatographic results demonstrated that ionic liquids are effective solvents for cellulose dissolution and pore expanding agents can be used to enhance the pore structure of cellulose beads. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40060.

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INTRODUCTION

Chromatography is the most widely used technique in biotechnology industries for separation and analysis of proteins and other biomedical materials.[1] This popular technique offers several advantages in protein purification, such as high capacity, good selectivity, simple methodology, and preservation of protein bioactivity.[2] With the development of biological science, more bioactive proteins or antibodies have been discovered. These new bioactive materials with variety of structures and different physical and chemical properties require the development of bioseparation processes. Two main components in a chromatographic separation process are mobile phases which carry the target molecules,[3,4] and stationary phases which are composed of different adsorbents.[5] Ionic strength and pH are the two important factors of mobile phases in the protein separation,[6] whereas adsorbents can be prepared with a variety of materials and their effects on separation processes may need to be evaluated individually.[7–9]

Cellulose is one of the most abundant natural polymers which has been used for preparing chromatographic adsorbents owing to its excellent hydrophilicity, mechanical strength, and easy modification.[10–12] However, one of the problems using cellulose to prepare adsorbents is the dissolution of cellulose as it is difficult to directly dissolve in water and other common solvents because of the strong hydrogen bonds and partial crystalline structure of most natural celluloses. Traditional dissolution methods involve the usage of toxic chemicals, whereas some new methods used solvents such as lithium chloride and dimethyl sulfoxide/paraformaldehyde, which are complicated when processing is considered and still have problems such as limited dissolution capability and solvent recovery.[13] Ionic liquids are salts in which the ions poorly coordinate with one ion that has a delocalized charge and another one organic, which results in these salts being liquid at relatively low temperatures (usually $<100^{\circ}C$) or at room temperature. They are nonvolatile, nonflammable, chemically and thermally stable, and easy to recycle.[14,15] Rogers and coworkers[16] successfully used ionic liquids to dissolve cellulose for the preparation of cellulose beads. They found that 1-butyl-3-methylimidazolium chloride (BmimCl) can dissolve celluloses with degree of polymerization of 290–1200 and 10% wt % cellulose solutions can be obtained when dissolved at $100^{\circ}C$.[16] Later on, other research was followed to prepare cellulose films and fibers.[17–19] For example, researchers used 1-ethyl-3-methylimidazolium acetate with cosolvent DMSO to dissolve celluloses for electrospun

application and the effects of molecular weight and rheological behavior of the cellulose were studied.[20] Kyllonen et al.[21] used various ionic liquids to study the solubility of wood samples; however, their results showed that intact wood samples were not soluble in cellulose dissolving or swelling ionic liquids unless special treatments were performed.

Moreover, the pore size of cellulose beads prepared can directly affect the mass transfer of proteins in adsorbents and consequently the separation efficiency.[8,22] Proteins might be excluded from the internal pores of adsorbents and bound to the outer layer when the pores are too small, which decreases additional protein adsorption,[23] whereas proteins can access to larger pores more easily but these pores have relatively lower specific surface area and may result in the decrease of adsorption capacity.[24] Pore expanding agents are commonly used in porous media preparation for catalysis applications,[25] and their applications in the preparation of protein adsorbents have also been reported. For instance, calcium carbonate was used in the preparation of superporous agarose beads for protein adsorption applications and the porosity was increased to 6% compared to the beads without calcium carbonate.[26] Two sets of pores, that is flow-through pores created by calcium carbonate and diffusive pores, were found within agarose gel. Cassava starch was also used for similar applications.[27] It is a long-chain polysaccharide with high molecular weight and shows good hydrophilicity, which can be well mixed with cellulose viscose. Moreover, cassava starch can be easily digested by amylase, whereas cellulose can remain unaffected; therefore macropores can be formed in cellulose bead preparation with cassava starch.

For the application of cellulose beads as protein adsorbents, ligands attached to cellulose are necessary to capture certain proteins. Diethylaminoethyl (DEAE) is one of the most widely used ligands and DEAE cellulose is a positively charged resin in ion exchange chromatography to capture negatively charged proteins or nucleic acids. For instance, Lu et al.[28,29] studied the effects of DEAE density on the protein adsorption and found that the adsorption capacities increased with the increase of ligand density, but the pore diffusion coefficient was relatively independent on the DEAE density.

To prepare a porous cellulose-based adsorbent with new cellulose dissolution method, an ionic liquid as the solvent was used to directly dissolve microcrystalline cellulose (MCC) for preparing cellulose beads. Cassava starch and cyclohexane were used separately as pore enlarging agents and the properties such as porosity, mean pore size, and residence time distribution (RTD) of the prepared beads were compared. In addition, the prepared beads were coupled with DEAE and the adsorption behaviors of bovine serum albumin (BSA) were characterized. The effect of pore expanding agents on the protein adsorption was discussed.

EXPERIMENTAL

Materials

MCC with degree of polymerization of 300 was purchased from Hengxing Chemical (Shanghai, China). BmimCl was from Tianxudong Technology (Shenzhen, China). Cassava starch was from Meifeng Food (Shanghai, China). Cyclohexane, acetone, and amylase (6000 U/g) were brought from Sinopharm Chemical Reagent (Shanghai, China). Immunoglobulin of yolk (IgY) was laboratory purified. BSA, lysozyme, and diethylchloroethylamine hydrochloride were purchased from Sigma-Aldrich (USA). These chemicals were all used as received and deionized water was used in the experiments.

Cellulose Bead Preparation

The preparation method of the cellulose beads was similar to that of cellulose composite beads reported earlier.[30] Briefly, MCC was added into BmimCl at 95°C under a stirring rate of 120 rpm and the MCC/BmimCl solution was prepared after 2 h. This solution was then dispersed in an oil phase (vacuum oil with 2% Tween-80) and stirred for 20 min at 90°C. The prepared water-in-oil emulsion was cooled to 40°C under stirring and then ethanol was added to facilitate the bead formation process. The final beads were washed with water and ethanol, screened with standard sieves (74–300 μm), and stored in deionized water. For the beads prepared with cassava starch and cyclohexane as pore expanding agents, cassava starch solution or cyclohexane was mixed with the MCC/BmimCl solution and then water-in-oil emulsion was prepared. Cassava starch was hydrolyzed with amylase and detected with I_2-KI solution to ensure the removal of cassava starch in the beads, whereas cyclohexane was washed away with ethanol and water after the formation of MCC beads.

DEAE Coupling

The coupling of DEAE onto MCC beads was achieved through a method reported earlier.[31] The MCC beads prepared were added into 5.75*M* of NaOH at 15°C and then mixed with diethylchloroethylamine hydrochloride at 85°C for 35 min. The mixture was then treated with 5*M* of HCl and washed with abundant water and 1*M* of NaCl solution. The ionic exchange capacity was determined by titration with 0.1*M* of HCl.

Physical Properties

The methods for the measurements of physical properties of these MCC beads were detailed in our previous studies.[27,31] The wet density of these beads was measured via a mass balance method using a 5-mL gravity bottle.[27] The water content was obtained by dehydration at 120°C to a constant mass. With wet density and water content data and presuming that all pores were full of water, the porosity could be calculated.[32] The mean pore size was calculated according to the cylindrical pore structural model.[31] Specific surface areas were obtained by the adsorption of methylene blue solution with the assumption of mono-molecule-layer adsorption.[33]

Protein Adsorption Equilibrium

BSA was used as a model protein and 0.2, 0.4, 0.6, 1.0, 2.0, and 3.0 g of adsorbents was balanced with Tris-HCl buffer (pH 8.5, 20 m*M*) for 30 min and then drained and added into 15-mL Tris-HCl buffer with 5 mg/mL of BSA. The mixture was kept at 25°C for 8 h in a shaking incubator. The adsorbents were then separated and the supernatant was analyzed via UV spectroscopy (Ultrospec 3300 Pro, Amersham Biosciences, Uppsala). The adsorbed proteins were calculated and the adsorption equilibrium was fitted by Langmuir equation:

$$Q^* = \frac{Q_m \times C^*}{K_d + C^*} \quad (1)$$

where Q^* (mg/mL gel) and C^* (mg/mL) are the equilibrium adsorption capacity and protein concentration in liquid phase, respectively, Q_m (mg/mL gel) is the saturated adsorption capacity, and K_d (mg/mL) is the dissociation constant.

Dynamic Adsorption

A closed loop was set up with a filter and an online UV spectrometer connected. In brief, 1 mg/mL of BSA in Tris-HCl buffer (pH 8.5, 20 m*M*) was flowed in the loop and 0.5 g of drained adsorbents was added in the bulk liquid. The dynamic adsorption process was recorded with online UV spectroscopy and the result was analyzed using the pore diffusion model reported by Chen et al.[34]

Protein Breakthrough

A chromatographic column (diameter 1.0 cm, length 10 cm, Amersham Biosciences) was used for the measurements of protein breakthrough curves. In total, 2 mg/mL of BSA in Tris-HCl buffer (pH 8.5, 20 m*M*) was applied. The dynamic adsorption capacity at 10% breakthrough ($Q_{10\%}$, mg/mL gel) was calculated as described earliler[35]:

$$Q_{10\%} = \frac{C_0 \times \int_0^{V_{\text{solution},10\%}} \left(1 - \frac{C}{C_0}\right) dV_{\text{solution}}}{V_{\text{adsorbents}}} \quad (2)$$

where C (mg/mL) and C_0 (mg/mL) are the protein concentration of the outlet and initial of the liquid phase, respectively, $V_{\text{solution},10\%}$ (mL) is the loading volume corresponding to 10% of breakthrough point. $V_{\text{adsorbents}}$ (mL) is the volume of the adsorbents.

RESULTS AND DISCUSSION

Bead Preparation

The cellulose bead preparation process included MCC dissolution in the ionic liquid, the formation of water-in-oil emulsion, and the precipitation of beads from the mixture system. A number of parameters needed to be optimized in this three-step preparation procedure, such as dissolution temperature, MCC concentration in the ionic liquid, bead size control, and addition of additives in the precipitation process. Similar optimization processes have been reported and discussed earlier[30] and here is a brief result discussion for this study.

It was found in the experiments that the increase of temperature could facilitate the dissolution of MCC in the ionic liquid, but high temperature would likely cause the degradation of MCC molecules. Moreover, high MCC concentration in the ionic liquid would significantly increase the viscosity of the ionic liquid solution, which caused the later treatments problematic. After a series of optimization experiments, the concentration of MCC in the ionic liquid was determined as 7% wt % and the dissolution temperature was set at 95°C. It took approximately 2.0 h for MCC to be fully dissolved in the ionic liquid.

The bead size was found to be controllable and had a strong relationship with the mechanical stirring speed in the water-in-oil emulsion preparation process. The bead size decreased when the stirring speed increased. In all, 700 rpm was chosen as the stirring speed in this study with an ionic liquid/oil phase ratio of 1 : 5 to keep the mean bead size below 200 μm, which is a typical bead size for protein separation applications.[27,36]

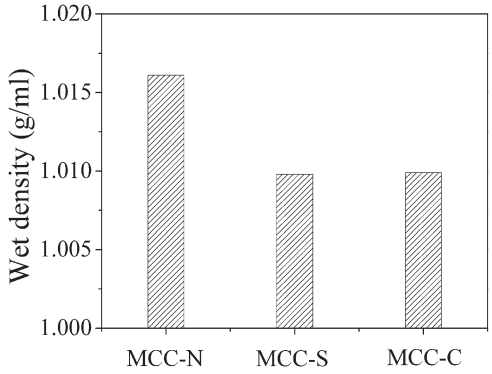

Figure 1. Wet density of cellulose beads prepared with and without pore expanding agents. MCC-N: beads prepared without pore expanding agents; MCC-S: beads prepared with cassava starch; MCC-C: beads prepared with cyclohexane.

The precipitation of cellulose beads from the emulsion system was controlled by cooling the whole system from 95°C with a cooling rate of 5°C/15 min. The results showed that the bead cannot keep spherical shape when the cooling rate was faster than 5°C/15 min. However, when the cooling rate was slower, the mechanical strength of the beads was not good. Ethanol was used as an additive to facilitate the precipitation process and it was found that by adding 100 mL of ethanol into 200 mL of emulsion, the precipitation process can be significantly accelerated and the formed cellulose beads had good mechanical strength and can be used for further treatments and applications.

The addition of cassava starch or cyclohexane did not seem to have an obvious effect on the bead preparation process. Cassava starch was first boiled in water for 10 min to prepare cassava starch solution (5% w/v), and then 4 g of the above cassava starch solution was added into the MCC solution (40 g) to form a liquid mixture. The rest of the beads preparation process was the same as the beads without cassava starch and the starch was hydrolyzed with amylase in the end. In addition, cyclohexane as an expanding agent (20 mL) was added directly into the MCC solution (40 g) to first form a water-in-oil system. Similarly, the rest of the bead preparation process was the same as the beads without cyclohexane and it was later washed away with ethanol and water. The cellulose beads prepared were named as MCC-N, MCC-S, and MCC-C (MCC-N: beads prepared without pore expanding agents; MCC-S: beads prepared with cassava starch; MCC-C: beads prepared with cyclohexane).

Physical Property Characterization

Wet Density. Wet density is a property of particulate materials such as ion exchange resins and is usually used to characterize the real density of materials in their working conditions (normally immerged in water). [37] Figure 1 compares the wet density of MCC-N, MCC-S, and MCC-C. The results show that the wet densities of three samples were in the range of 1.00–1.02 g/mL, which is close to the density of water. These values are much smaller than cellulose composite beads prepared with the addition of tungsten carbide as the densifier, which can reach up to 2.4 g/mL.[31] Moreover, the addition

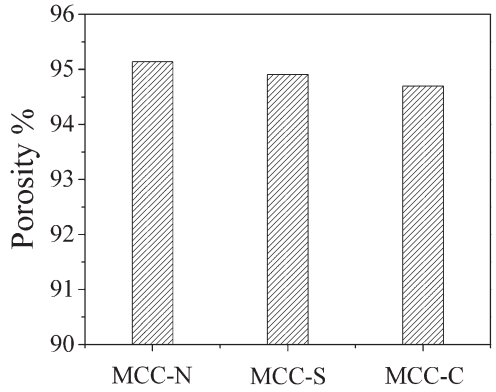

Figure 2. Porosity of cellulose beads prepared with and without pore expanding agents.

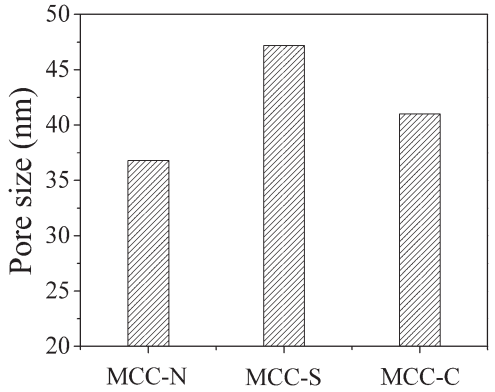

Figure 3. Mean pore size of cellulose beads prepared with and without pore expanding agents.

of pore expanding agents did not have a significant effect on the wet density although detailed comparison of three samples shows that MCC-S and MCC-C had slight lower wet density than that of MCC-N. This is likely because the solid beads had comparable densities as water. When the porosity or pore size of the beads increased, the internal pores would be filled with water owing to the hydrophilic property of the beads. The replacement of cellulose with water did not have an obvious influence on the bead wet density.

Porosity and Pore Size. Figures 2 and 3 compare the porosity and mean pore size of three samples prepared. The results show that all of cellulose beads prepared with/without pore expanding agents had high porosity values (approximately, 95%), which is an important factor for chromatographic applications. Although cassava starch and cyclohexane were used in MCC-S and MCC-C, respectively, to generate or enlarge pores in the beads, the porosities of these beads were comparable. Figure 2 shows that the porosity of MCC-S and MCC-C was even slightly lower than that of MCC-N. These results indicated that the addition of cassava starch or cyclohexane did not increase the porosity of the beads. Alternatively, the addition of these pore expanding agents followed by the removal after the formation of the beads resulted in larger pores in the bead structure. This speculation was confirmed in Figure 3 which shows the mean pore size of these MCC beads. The results show that the mean pore size of MCC-N is 37 nm. When adding cassava starch or cyclohexane in the formulations, which were later removed after the bead formation, the mean pore size increased to 47 and 41 nm, respectively. The size increasing percentages were 27 and 11%.

The specific surface area values of these beads are shown in Figure 4. Owing to the increase of the pore size, the specific surface area of MCC-S and MCC-C beads reduced compared to that of MCC-N. MCC-N had a specific surface area of 104 m^2/mL, which were reduced by approximately 25 and 13% for MCC-S and MCC-C, respectively. Although the specific surface area values decreased after using pore expanding agents, they were still >70 m^2/mL and suitable for coupling with ligands and used for protein separation processes. Moreover, these data were consistent with the pore size increasing trends shown in Figure 3. In addition, Figure 5 shows typical surface morphology of MCC beads prepared with and without pore expanding

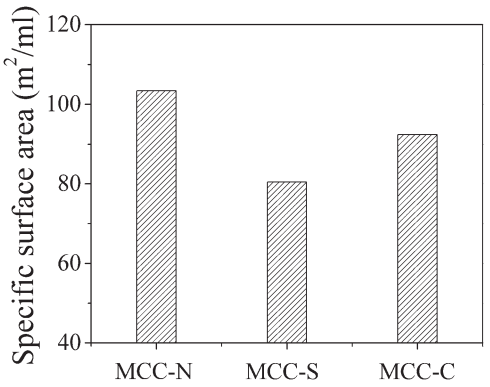

Figure 4. Specific surface area of cellulose beads prepared with and without pore expanding agents.

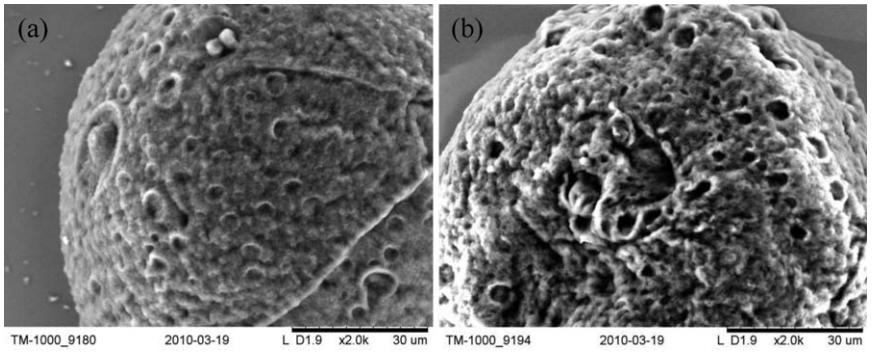

Figure 5. SEM micrographs of (a) MCC-N and (b) MCC-C cellulose beads.

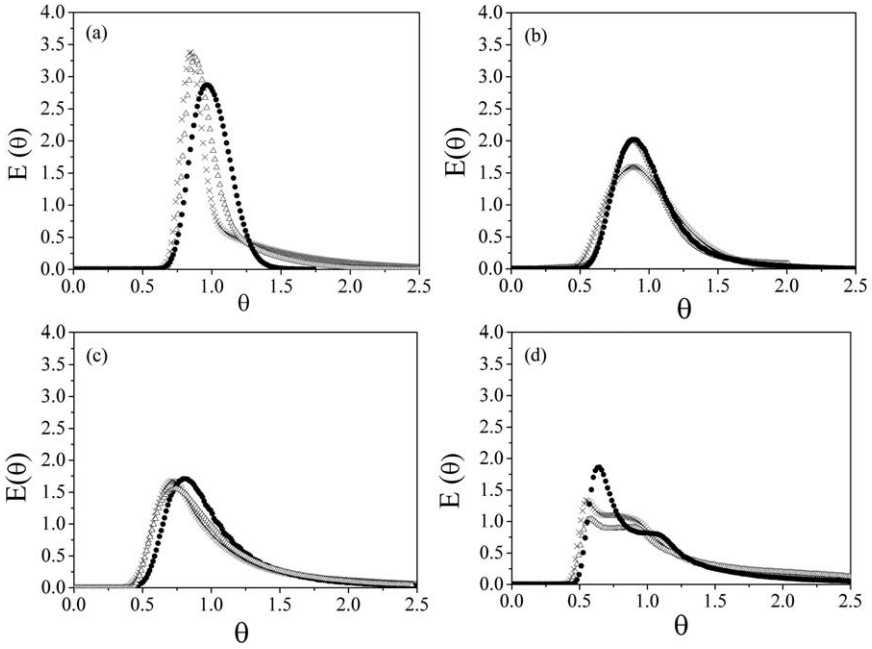

Figure 6. RTD profiles of different solutes in the prepared MCC beads. (a) Acetone, (b) lysozyme, (c) BSA, and (d) IgY. (×):MCC-N; (△): MCC-S; (●):MCC-C. In brief, θ is the dimensionless time and $E(\theta)$ is the dimensionless solute distribution.

agents. The SEM micrographs show that MCC beads without cyclohexane had a more smooth surface with smaller but more homogeneous pore structure on the surface. The beads prepared with cyclohexane had irregular surface pore structure.

Figures 2–5 show that the addition of cassava starch or cyclohexane which was later removed after the bead formation can enlarge the average pore size in cellulose beads. The porosity of the beads kept almost the same with different treatments. The increase of pore size in beads can bring a relatively short path for protein diffusion and increase the protein adsorption efficiency.[38] Mohan and Lyddiatt[39] studied the physical capacity of adsorbents prepared with silica-based beads having pore diameters of 20, 50, and 100 nm, respectively. The results showed that the beads with 20 nm of pore diameter had only 16% of the theoretically expected capacity, whereas the ones with 50 and 100 nm had 70 and 90% of the expected values, and the capacity is strongly related to the protein molecular size. The pore accessibility and transport of proteins are facilitated by large pores but the mechanical strength may also be affected. Moreover, proteins may be excluded from the internal pores when the pores are too small.[23,40] Therefore, it is useful to evaluate the pore enlarging effect on the application in protein separation processes.

Mass Transfer Characteristics. RTD was used to evaluate the mass transfer characteristics in the prepared beads, which shows the solute distribution in columns as a function of time, and the results may imply the pore structure inside the beads and the separation efficiency.[41] Figure 6 shows the RTD peaks of four different solutes with varying molecular weights: acetone, lysozyme (14.9 kDa), BSA (67 kDa), and IgY (180 kDa). The RTD curves are shown in the normalized RTD ($E(\theta)$) as a function of normalized time (θ). The peak intensities of the RTD profiles decreased when the molecular weight of the solutes increased and the peaks turned broader, which was probably owing to the increase of the internal resistance in the beads.

Figure 6(a) shows the RTD profiles of acetone in MCC-N, MCC-S, and MCC-C beads. Acetone as a small molecule can penetrate or diffuse into most of the micropores in the beads. Therefore, the pore size should not have an obvious effect on the RTD profiles. However, the results show that MCC-N and MCC-S had similar RTD profiles, but MCC-C presented a RTD profile with better symmetry. It may be because of the fact that the beads prepared with cyclohexane had well-connected porous structure or the pore structure/size was more uniform and acetone can move freely in MCC-C beads with relatively larger pore size.

Lysozyme has a molecular weight of 14.9 kDa and the RTD profiles shown in Figure 6(b) indicate that it had symmetric peaks in all of the three MCC beads although MCC-C showed a slightly sharper peak with higher intensity. This result indicates that the pore size may not have an obvious effect on low-molecular-weight proteins during separation processes. Moreover, Figure 6(c) shows that the RTD profiles of BSA in three kinds of beads had almost overlaid with each other although tailing phenomenon can be observed. The results indicate that proteins with molecular weight of <67 kDa may be separated with these three beads with similar separation efficiency and pore size was not a critical factor in this case.

When IgY was used as a model solute which had a molecular weight of 180 kDa, the RTD profiles changed significantly. Figure 6(d) shows that the tailing effect is obvious in all of the beads, and MCC-S did not show a sharper and narrower RTD profile than MCC-N although MCC-S had larger pore size. Again, MCC-C showed a sharper profile with the highest peak

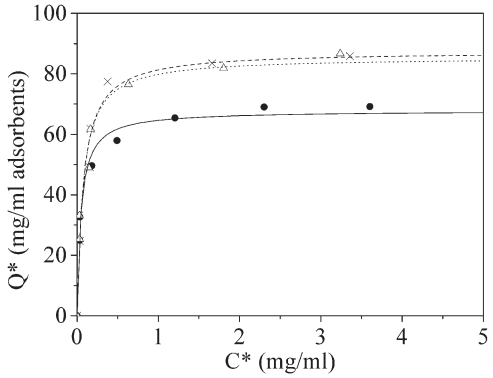

Figure 7. Adsorption isotherm curves of BSA in (×):MCC-N-DEAE, (△): MCC-S-DEAE, and (●): MCC-C-DEAE with corresponding Langmuir equation fitting lines.

intensity. The RTD profiles with severe tailing effects were likely owing to the high molecular size of IgY, which impeded the mass transfer of proteins in the porous beads. The treatments of cyclohexane may result in large and well interconnected pores, which facilitate the mass transfer inside the beads. These results indicate that the pore size is an important factor in the protein separation process; however, other factors such as pore structure are also needed to be considered when preparing porous beads for protein separation.

Adsorbent Characterization. The cellulose beads prepared were coupled with DEAE to obtain ion exchange adsorbents and named as MCC-N-DEAE, MCC-S-DEAE, and MCC-C-DEAE. Their ionic exchange capacities were measured and the results were 249.3, 245.3, and 241.7 μmol/mL. These results were comparable with each other, which indicate that the usage of cassava starch and cyclohexane as pore expanding agents did not have a significant effect on the application of the cellulose beads as ion exchange carriers.

The protein adsorption capacity of these three adsorbents was measured by adsorption isotherms with BSA as a model protein and the experimental results are shown in Figure 7. The data were fitted with the Langmuir equation. Figure 7 shows that the experimental data can be fitted well with the Langmuir equation which further provided the saturated adsorption capacity (Q_m) and the dissociation constant (K_d) of BSA adsorption. Q_m and K_d are summarized in Table I. It can be found that MCC-N-DEAE and MCC-S-DEAE had similar Q_m, whereas MCC-C-DEAE had a lower Q_m value. The pore accessibility and transport of proteins may be facilitated in larger pores, but smaller pores usually have larger specific surface area which results in the increase of adsorption capacity.[24,42] Furthermore, the internal structure and interconnectivity of the pore inside can also have important effects on the protein adsorption capacity. The Q_m values listed in Table I were slightly lower than that of cellulose composite bead adsorbents reported earlier (97.1 mg/mL),[31] but higher than the BSA adsorption with Streamline DEAE (69 mg/mL).[43] Moreover, the dissociation constants in three adsorbents were all lower than 0.1 mg/mL and MCC-C-DEAE had the lowest K_d, which indicates that all of the three adsorbents had good affinity with BSA in the separation process and MCC-C-DEAE may be better for separating solutions with low BSA concentrations.

The adsorption kinetics of BSA in MCC-N-DEAE, MCC-S-DEAE, and MCC-C-DEAE was studied and the results are shown in Figure 8. It was found that MCC-S-DEAE and MCC-C-DEAE had quicker adsorption processes than that of MCC-N-DEAE, and the equilibrium adsorption capacity of MCC-S-DEAE and MCC-C-DEAE was slightly higher than MCC-N-DEAE. Moreover, MCC-C-DEAE may reach to equilibrium faster than both MCC-N-DEAE and MCC-S-DEAE. The experimental results were also fitted by following the pore diffusion model as discussed in the previously published articles. [34,44] The pore diffusivity (D_p) of these three adsorbents was calculated by fitting the experimental data, which can be used to evaluate the mass transfer properties inside the porous adsorbents. Table I summarizes that the pore diffusivity of MCC-S-DEAE is four times higher than that of MCC-N-DEAE, and MCC-C-DEAE had the highest D_p which was approximately eight times higher than that of MCC-N-DEAE. The fitting results indicate that cyclohexane as a pore expanding agent can enhance the BSA kinetic adsorption performance.

BSA breakthrough behaviors with MCC-N-DEAE, MCC-S-DEAE, and MCC-C-DEAE in packed beds were investigated to evaluate the dynamic binding capacity of these adsorbents and the results are shown in Figure 9. The dynamic adsorption capacities at 10% breakthrough ($Q_{10\%}$) were 21.1, 23.5, and 31.9 mg/mL for MCC-N-DEAE, MCC-S-DEAE, and MCC-C-DEAE, respectively. These values were equal to 24.2, 27.5, and 47.1% of the saturated adsorption capacities (Q_m), which means that the pore enlarging process can enhance the dynamic adsorption, especially with cyclohexane as the pore expanding agent. Moreover, the curve of MCC-C-DEAE showed a sharp increasing trend, which indicates that the adsorption rate was higher in MCC-C-DEAE. This result was consistent with the kinetic adsorption results shown in Figure 8. Some related parameters are summarized in Table I.

Table I. Typical Parameters of the Three Prepared Adsorbents

	q (mol/mL)	Q_m (mg/mL)	K_d (mg/mL)	D_p (10^{-10}m^2/s)	$Q_{10\%}$ (mg/mL)
MCC-N-DEAE	249.3	87.5 ± 4.1	0.076 ± 0.015	0.256	21.1
MCC-S-DEAE	245.3	85.5 ± 3.3	0.071 ± 0.012	1.02	23.5
MCC-C-DEAE	241.7	67.8 ± 1.9	0.048 ± 0.008	2.15	31.9

q: Ionic exchange capacity; Q_m: saturated adsorption capacity; K_d: dissociation constant; D_p: pore diffusivity; $Q_{10\%}$: adsorption capacities at 10% breakthrough.

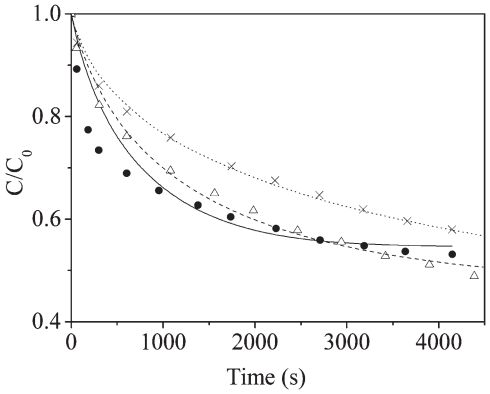

Figure 8. Dynamic adsorption profiles of BSA in (×): MCC-N-DEAE, (△): MCC-S-DEAE, and (●), MCC-C-DEAE with corresponding fitting lines.

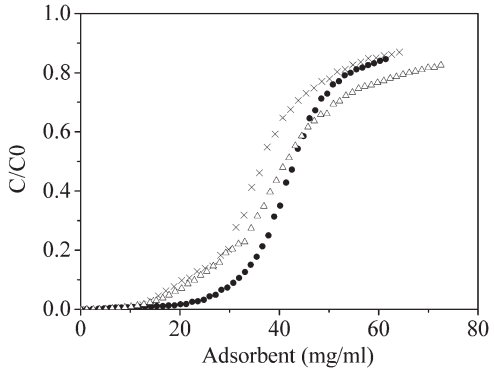

Figure 9. Breakthrough curves of BSA in packed beds with (×): MCC-N-DEAE, (△): MCC-S-DEAE, and (●):MCC-C-DEAE.

CONCLUSIONS

The porous cellulose beads for ion exchange chromatography applications were prepared via a direct dissolution method using ionic liquids. This method provides an easy, short, and environmental friendly procedure for producing porous materials and adsorbents for protein separation applications. Cassava starch and cyclohexane were used as pore expanding agents in some of the bead preparing procedures and different physical properties were analyzed and compared between cellulose beads prepared with/without the pore expanding agents. The results showed that using cassava starch or cyclohexane, the pore size of cellulose beads was increased and the specific surface area was decreased, whereas the porosity of these beads was comparable with the beads prepared without these agents. The enlargement of pores in cellulose beads facilitated the protein mass transfer in the pore, especially for high-molecular-weight proteins. Moreover, DEAE ion exchangers were prepared with these cellulose and the adsorption performances were compared using adsorption isotherm, adsorption kinetics, and protein breakthrough. The results showed that cellulose beads prepared with cyclohexane enlarged pores had the best protein adsorption performance and can be used as a potential chromatographic matrix for protein separation applications.

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