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Preparation and characterization of crosslinked porous cellulose beads

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Abstract

Porous cellulose beads have been prepared from cellulose diacetate under the conditions of the pore-forming agents and latter-crosslinked with epichlorohydrin. The reaction conditions and the amount of the pore-forming agents have been optimized in order to obtain beads with various macropore sizes. The crosslinking density of these beads was calculated from their swelling properties by a modified Flory equation. The pore volume, pore structure, porosity and the swelling behavior in water of these beads have been studied.

Keywords: Porous cellulose beads; Cellulose diacetate; Carrier; Latter-crosslinked

1. Introduction

There have been many reports on the production of useful products by immobilized microorganisms, cells and enzymes. In these reports, several kinds of immobilization carriers including ceramics (Jee, Yano, Nishio, & Nagai, 1987; Melin & Shieh, 1992; Passarinho, Vieira, Cabral, Novais, & Kennedy, 1989), ion-exchange resins (Bar, Gainer, & Kirwan, 1986; Yoshioka & Shimamura, 1986), polyurethane (Endo, Nagamune, Kato, Nishimura, & Kobayashi, 1988; Sanroman, Pintato, & Lema, 1994), chitosan (Shinonaga, Kawamura, Shimbo, & Yamane, 1996; Shinonaga, Kawamura, & Yamane, 1992), PVA (Doria-Serrano, Ruiz-Trevino, Rios-Arciga, Hernandez-Esparza, & Santiago, 2001; Wan, Luo, & Zhu, 2004) and cellulose (Fuji, Yasuda, & Sakakibara, 1994; Ogawa et al., 1992) were used. Many polymers are rather hydrophobic and thus their applications in aqueous media are limited. Hydrophilic polymers should have a better swellability in water (Muzzarelli, Stanic, Gobbi, Tosi, & Muzzarelli, 2004; Wan, Huang, Wang, & Zhu, 2004a). Therefore, cellulose, a hydrophilic and low cost polymer, would be a good candidate as the carriers for immobilization uses.

Regenerated cellulose and cellulose acetate as carriers after membrane can be obtained through phase inversion. They present a hydrophilic surface and abundant reactive hydroxyl groups, as well as a low, non-specific adsorption. However, the mechanical strength of these membranes is poor and is not suitable for column system (Zou, Luo, & Zhou, 2001). To overcome these shortcomings, several methods have recently been proposed (Guo, Shang, Yu, & Zhou, 1994; Kubota, Nakagawa, & Eguchi, 1996; Malakian, Golebiowska, & Bellefeuille, 1993). In addition, there were many methods to prepare spherical cellulose sorbent, but they have some fatal disadvantages such as low porosity, poor strength, high cost, low applicability, difficulty of production scaleup and requirement of waste treatment for environmental protection (Zhu, Shi, & He, 1996). Therefore, it is still a challenge to obtain crosslinked porous cellulose beads (PCB) with well-defined macropore structures and acceptable mechanical properties.

In this work, we have developed a new and economical way to prepare macroporous spherical cellulose beads from cellulose diacetate, and investigated the characterization of the beads which were crosslinked through epichlorohydrin to the hydroxyl groups on it. The macropore structure, the swelling behavior and the functionalized groups make the beads potentially useful as polymeric carrier or support in biotechnology and biochemistry field.

2. Experimental section

2.1. Materials

Cellulose diacetate (CDA, $M_n = 98{,}300$, the content of acetyl is 55.4%) was purchased from Shanghai Cellulose

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Corporation (China). Epichlorohydrin (EP, 99%), dimethyl sulfoxide (DMSO) and other chemicals were all obtained from Tianjing Chemical Reagent Company (China) and used without further purification.

2.2. Preparation of porous cellulose beads (PCB)

Porous cellulose beads (PCB) were prepared as follows. 3.0 g cellulose diacetate was dissolved in 27 ml dimethyl sulfoxide (DMSO), then 0.3 g sodium bicarbonate (powder) and 1.2 g anhydrous sodium sulfate (0.2 mm particle mean diameter) are added, and blended adequately with a mechanical stirrer. After that, the mixture was added dropwise into an acid coagulation bath (500 ml of 0.015 M HCl) from a nozzle (different size of the spherical beads could be gained along with the change of the diameter of the nozzle). When the bleb emitted from the coagulation was over, the coagulated cellulose acetate beads were collected by filtration using a sieve. In order to form abundant pores in interior of the beads, they were immerged in large quantities of hot water to dissolve and remove sodium sulfate after being filtrated from the bath. Also, in order to create symmetrical and macropore in surface layer of the beads and regenerated cellulose from cellulose diacetate, the beads were immersed into 100 ml sodium hydroxide solution (2 M) and stirred gently at 40 °C for 1.5 h, and at the same time quantificational epichlorohydrin was added into the reactor for a latter-crosslinking. Finally, the crosslinked porous cellulose beads were rinsed thoroughly with distilled water till pH 7.0. Five sets of bead samples were obtained, and they were designated as PCB-2, PCB-4, PCB-6, PCB-8 and PCB-10, respectively, where the number following the PCB indicates the volume (×10 ml) of crosslinker (EP) used in the crosslinked reaction. Dimethyl sulfoxide was recycled when the water was vaporized from the acid coagulation bath.

2.3. Optimum amount of the pore-forming agents

The total amount and the ratio of the pore-forming agents are the important factors which decide the uniformity, aperture distribution and elasticity of the porous beads. The apparent characters of the porous cellulose beads were studied when different total amount and different ratio of the sodium bicarbonate with anhydrous sodium sulfate were added.

2.4. Infrared spectroscopy

The FT-IR spectra of the porous cellulose diacetate beads, porous cellulose beads before and after crosslinked were recorded by using a Nicolet NEXUS 670 FT-IR spectrophotometer, and the sample and KBr were pressed to form a tablet.

2.5. Characterization of the porous cellulose beads

2.5.1. Bulk density

To estimate the bulk density of dried porous cellulose beads, they were first dried until a constant weight at 20 °C, and then filled with dry beads to the mark of a 10 ml volumetric flask (W_1) and was weighed (W_2). The bulk density of porous cellulose beads was calculated according to

$$d_o = (W_2 - W_1)/10 \tag{1}$$

where W_2 is the total weight of the porous cellulose beads and the flask, and W_1 is the weight of the flask only.

2.5.2. True density

To estimate the true density of dried porous cellulose beads, 0.35 g of porous cellulose beads was first dried until a constant weight (W_0) was reached and then placed into a 10 ml volumetric flask of known weight at 20 °C. Into the flask was added 8 ml of cyclohexane and the mixture was kept at 20 °C for 24 h. The flask was then filled with cyclohexane to the mark and was weighed (W). The true density of PCB was calculated according to

$$d = W_0/[10 - (W - W_0)/d_c] \tag{2}$$

where W is the total weight of the porous cellulose beads and the solvent, and W_0 the weight of the dry porous cellulose beads, d_c the density of the solvent ($d_{\mathrm{cyclohexane}} = 0.778$ g/ml).

2.5.3. Degree of swelling

The swelling rate of the porous cellulose beads in distilled water was determined by monitoring the weight gain of the porous cellulose beads in water. The porous cellulose beads were dried and then placed into tubes with a sintered glass bottom. The tubes were immersed in distilled water for 48 h at 20 °C. The excess of water was removed by centrifugation at 1500 rpm during 1 min. The water content (W_Q) at equilibrium was calculated according to

$$W_Q(\%) = [(W - W_o)/W] \times 100\% \tag{3}$$

and the degree of swelling (S_w) according to

$$S_w = (W - W_o)/W_o \tag{4}$$

where W and W_o denote the weight of porous cellulose beads with absorbed water and that of the dry porous cellulose beads, respectively.

2.5.4. Pore volume and porosity

The pore volume of the porous cellulose beads was studied by monitoring the weight gain of the beads (Greig & Sherrington, 1978). The dried beads were placed into tubes with a porous glass bottom. The tubes were kept inside a flask filled with cyclohexane for 48 h at 20 °C. The excess cyclohexane was removed by centrifugation at 1500 rpm for 1 min. The volume of cyclohexane absorbed in the beads was used to estimate the porosity of the beads. The porosity of the beads was indicated by

$$\varnothing = V_p/V_o \tag{5}$$

where V_p is the pore volume in the beads and V_o is the true volume of beads.

$$V_o = W_o/d \tag{6}$$

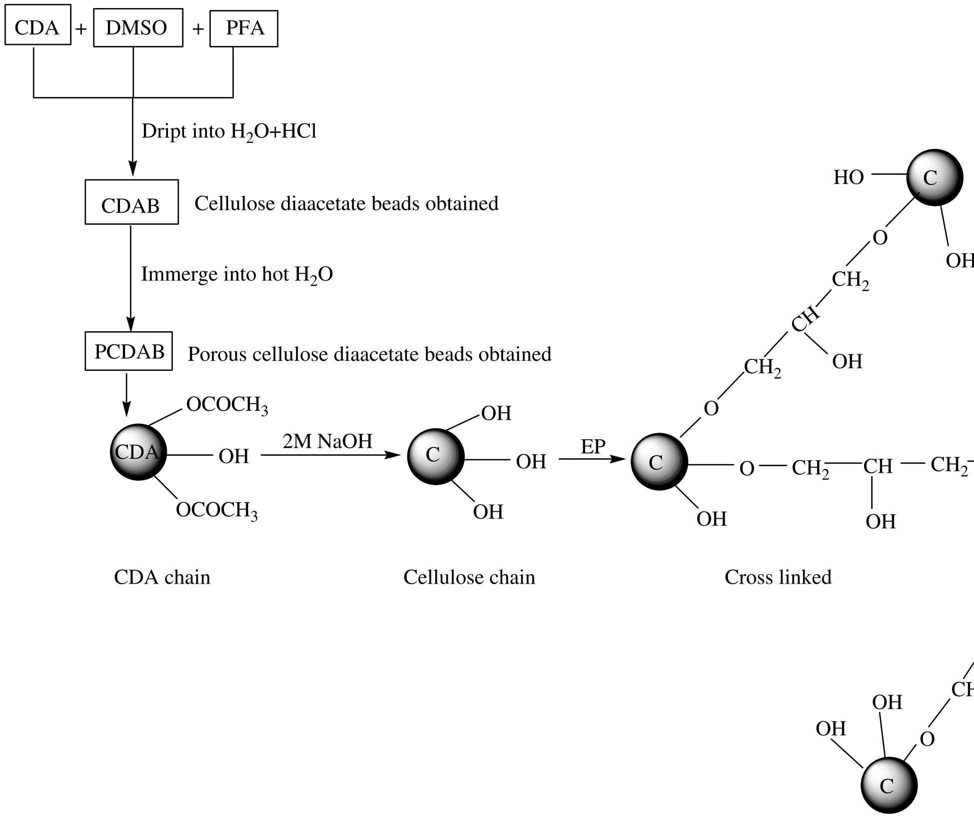

Fig. 1. Strategy for preparation of latter-crosslinked porous cellulose beads: (PFA) pore-forming agents; (CDA) cellulose diacetate chain; (C) cellulose chain.

where W_o is the weight of dry beads, and d (the true density of dried beads) was determined according to Eq. (2).

The morphologies of the macropores in the beads were observed by the use of a scanning electron microscope (Philips, XL-20). The beads in small amounts were frozen in liquid nitrogen and freeze-dried for 8 h and no collapsing of the macropores or of the network was observed.

3. Results and discussion

3.1. Preparation of porous cellulose beads (PCB)

The synthetic scheme for the preparation of the porous cellulose beads is shown in Fig. 1. EP was used as the crosslinker. If each EP molecule reacted with two hydroxyl groups on the cellulose chains, it could be expected that each EP molecule would yield one hydroxyl group while consuming two hydroxyl groups of PCB. However, it is known that there may be some side reactions, i.e. some EP molecules may only react with one hydroxyl group or react with unreacted pendant epoxy groups. Since it is difficult to quantify such side reactions, it is reasonable to assume that amount of functional groups from such side reaction is also directly related to the amount of EP used in the crosslinking reaction. Therefore, the optimum amount of EP added was estimated from the apparent characters of the porous cellulose beads. The latter-crosslinked porous cellulose beads in the spherical form were found to be swellable in water and have the desired mechanical strength.

3.2. Optimum amount of the pore-forming agents

The apparent characters of the porous beads were partly based on the amount of the pore-forming agents. Study shows the optimum amount of the total pore-forming agents was 1.5 g, and the ratio of anhydrous sodium sulfate with the sodium bicarbonate was 4:1 (W/W) when 2.4 g cellulose diacetate was used (Table 1).

Table 1
Optimum amount and ratio of the pore-forming agents

Total amount (g)	1.0			1.5			2.0		
Ratio (W/W)[a]	3:1	4:1	5:1	3:1	4:1	5:1	3:1	4:1	5:1
PCB uniformity	Bad	Good	Bad	Good	Better	Good	Bad	Bad	Bad
Aperture distribution	Wide	Narrow	Wide	Narrow	Very narrow	Narrow	Wide	Narrow	Wide
Elasticity	Good	Better	Bad	Better	Best	Better	Bad	Good	Good

[a] The weight ratio of anhydrous sodium sulfate to sodium bicarbonate.

3.3. IR spectrum

The infrared spectra for porous cellulose diacetate beads, porous cellulose beads and latter-crosslinked porous cellulose beads are represented in Fig. 2(a)–(c), respectively. From the IR spectra presented in Fig. 2(a), the absorption peaks of the stretching vibration of the νC=O from porous cellulose diacetate beads should be at 1740 cm^{-1}. But this absorption peak disappeared absolutely in the IR spectra of the latter-crosslinked porous cellulose beads (b) and porous cellulose beads (c). This indicated that the porous cellulose diacetate beads have completely hydrolyzed to the porous cellulose beads. The changes of the band of the hydroxyl groups at 3400 cm^{-1} of the three beads also show the occurrence of the hydrolyze reaction of the porous cellulose diacetate beads (large amount hydroxyl groups have been produced for the hydrolyzed reactions (c)) and the latter-crosslinked of the porous cellulose beads (some hydroxyl groups have been consumed for the latter-crosslinking reactions (b)).

3.4. Porous structure

The morphologies of the latter-crosslinked porous cellulose beads were observed with SEM. Table 2 shows the pore volume, pore size and porosity of the PCB beads. In comparison with PCB-10, the pore volume and porosity of PCB-2 are more than five times larger. The large differences in the pore volume and porosity can also be seen in Fig. 3, which shows the scanning electron micrographs of the porous structure for selected PCB beads with varying crosslinking density. The size and shape of the pores of three different beads are strikingly different. It is clear that PCB-8 has regular pores with smaller pore sizes than that of PCB-4 and PCB-6, while the porous structure of PCB-6 is less uniform than the other two beads. Since the total amount and ratio of the pore-forming agents are uniform in the three PCB, these differences may be attributed to the difference in the density of crosslinking of the different PCB (i.e. the difference amount of the crosslinker added).

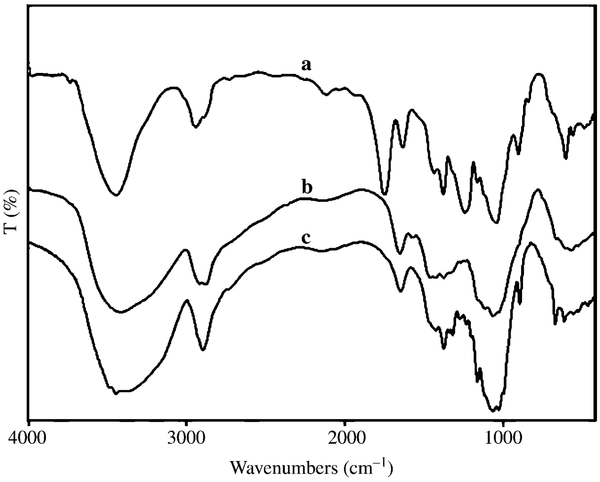

Fig. 2. FT-IR spectra of the porous beads: (a) porous cellulose diacetate bead; (b) latter-crosslinked porous cellulose bead; (c) porous cellulose bead.

Table 2
Pore parameters of the latter-crosslinked porous cellulose beads

PCB	Pore volume (V_p) (ml/g)	Porosity (Ø)	Pore size (μm)
PCB-2	2.46	10.39	20–60
PCB-4	1.72	7.26	20–50
PCB-6	1.25	5.28	20–40
PCB-8	0.80	3.38	10–25
PCB-10	0.48	2.03	10–20

The pore size of the beads was estimated from their SEM images.

3.5. Swelling behavior and crosslinking density

Crosslinked polymeric networks can be conveniently characterized by the crosslinking density ρ (Bray & Merrill, 1973a), which is inversely related to the average molecular weight percrosslinking unit M_c, according to

$$\rho = 1/vM_c \tag{7}$$

where v is the specific volume of the polymer. From the true density of the dry beads, the volume fraction of PCB in the swollen beads ($v_{2,s}$), and the volume fraction of cellulose diacetate in solution before crosslinking ($v_{2,r}$), can also be calculated (Kawai, 1958)

$$V_p = W_p/\rho_p \qquad v_{2,r} = V_p/V_r \qquad v_{2,s} = V_p/V_s \tag{8}$$

where W_p is the weight of polymer added, ρ_p is the real density of PCB, V_p is volume of polymer, V_r is volume of polymer and DMSO added, V_s is volume of swollen bead.

Following closely Flory's derivation, a modification was made taking into account that the crosslinkers were introduced between polymer chains of number-average molecular weight M_n. Calculate the crosslinking density ($1/M_c$) when

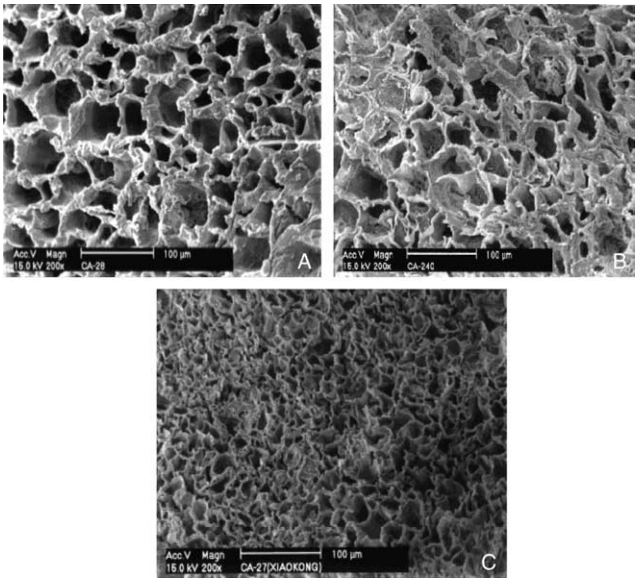

Fig. 3. Scanning electron micrographs of latter-crosslinked porous cellulose beads: (A) PCB-4; (B) PCB-6; (C) PCB-8.

Table 3
Swelling behavior and crosslinking density of porous cellulose beads

PCB	Bulk density (g/ml)	Real density (g/ml)	W_Q (%)	S_w	$v_{2,s}$	$v_{2,r}$	M_c	$\rho \times 10^{-3}$
PCB-2	0.308	1.39	92.9	14.2	0.052	0.074	1121	1.25
PCB-4	0.308	1.41	90.6	10.8	0.069	0.073	584	2.40
PCB-6	0.311	1.40	88.8	8.8	0.083	0.074	379	3.70
PCB-8	0.314	1.42	86.3	7.0	0.101	0.073	297	4.72
PCB-10	0.316	1.42	84.8	5.6	0.112	0.073	162	8.65

All data in the table are the average of the triplicate of experiments.

crosslinkers were introduced between polymer chains while the polymer was dissolved (Bray & Merrill, 1973b).

$$\frac{1}{M_c} = \frac{2}{M_n} - \frac{\frac{v}{V}\left[\ln(1-v_{2,s}) + (v_{2,s}) + \chi(v_{2,s})^2\right]}{(v_{2,r})\left[\left(\frac{v_{2,s}}{v_{2,r}}\right)^{1/3} - \frac{1}{2}\left(\frac{v_{2,s}}{v_{2,r}}\right)\right]} \qquad (9)$$

where v is the specific volume of polymer in the amorphous state (0.714 ml/g) and V is the molar volume of the water (18 ml/mol). The Flory polymer–solvent interaction parameter χ takes a value of 0.188 (Kawai, 1958), while M_n of CDA is known. Since the crosslinked beads are similar to a gel, the equation of above can be applied in this case. The calculations for such beads were made with the help of all the equations above, and the results are given in Table 3. Unlike a linear homopolymers of cellulose, many of the OH groups of the beads have reacted during crosslinking. Therefore, the parameters used in the calculations are rather approximate, and the results obtained here are likely also approximate.

Although S_w and M_c differed greatly for the five PCB samples, the W_Q value did not change much. It is natural that S_w will decrease when M_c decreases (i.e. ρ increases) since the cellulose chains were held together more tightly. In addition, we have also noted that both S_w and M_c decreased sharply at the beginning followed by a more gradual decrease when the amount of crosslinker was more than 0.6 ml. It is clear that ρ should have a direct influence on the swelling behavior of the PCB. The number of free hydroxyl groups in PCB is more important in determining the swelling properties of these beads, since the amount of bound water in the beads is closely related or even proportional to the number of hydroxyl groups. Although PCB-10 has the highest crosslinked density in the series of PCB the W_Q value (84.8%) is also high enough. Therefore, we can conclude that these Porous cellose beads all have a fairly high loading of hydroxyl groups.

4. Conclusions

Porous cellulose beads have been prepared from cellulose diacetate with the pore-forming agents (i.e. sodium bicarbonate and anhydrous sodium sulfate) and latter-crosslinked with epichlorohydrin. Under optimized reaction conditions which were 1.5 g pore-forming agents with the ratio of sodium bicarbonate and anhydrous sodium sulfate is 1:4 added in 3.0 g cellulose diacetate solution, these beads possess better macropore structures and high porosity. The spherical carriers are easily swellable in water and their pore size and volume and the porosity depend strongly on their crosslinking density when the optimum amount and ratio of the pore-forming agents is constant. In general, a low crosslinking density results in the spherical carriers with relatively large pore volume, high porosity, large pore diameter and high degree of swelling, i.e. when the crosslinker (EP) is 0.2 ml, the above parameters is 2.46 ml/g, 10.39, 20–60 μm and 14.2, respectively. In addition, the prepared process is simple, economical and environmental friendly because the only solvent (DMSO) can be recycled. The porous cellulose beads, after being modified (if needed), would be a valuable carriers or supports in biotechnology and biochemistry field.

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