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Preparation and characterization of porous polysucrose microspheres

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Abstract

A set of novel porous polysucrose microspheres were prepared by the inverse suspension polymerization using soluble polysucrose, epichlorohydrin (EP) as crosslinker and dimethyl ether of polyethylene glycol (DMPE) as porogen. The fourier transform infrared spectrometer (FTIR), optical microscope (OM), scanning electronic microscope (SEM) and laser diffraction method were utilized to characterize the structure and morphology of the porous microspheres. The results indicated that these beads had spherical shape with the mean particle size of around 340 μm, narrow distribution, and porous structure. The equilibrium water contents of these porous microspheres ranged from 92.1% to 96.6% with the increasing contents of porogen. The porosities ranged from 82.3% to 90.3% with the increasing hydroxyl contents from 19.3 to 21.8 mmol/g, and bovine serum albumin (BSA) was used as adsorbate model to examine the adsorption behavior of the porous microspheres. The saturated adsorption capacities of these microspheres ranged from 42.6 to 98.5 mg/g.

Keywords: Polysucrose; Porous microspheres; Inverse suspension polymerization; Adsorption

1. Introduction

Recently, the preparation and application of polysaccharide microspheres have been paid attention due to the good performance of these microspheres such as low toxicity, good biocompatibility and biodegradability. The microspheres including cellulose, dextran and chitosan have been widely used in absorbents (Gustavsson, Axelsson, & Larson, 1998; Kim, Kim, Yang, & Cho, 2004), affinity bioseparators (Fu et al., 2006; Per-Erik, Anders, & Per-Olof, 1999), drug and enzyme carriers (Mi, Shyu, Chen, & Schoung, 1999; Remunan-Lopez, Lorenzo-Lamosa, Vila-Jato, & Alonso, 1998). These microspheres have been prepared directly by emulsion polymerization (Caglayan, Unsal, Camli, Tuncel, & Tuncel, 2006; Kang, Kan, Du, & Liu, 2006), dispersion polymerization (Camli, Tuncel, Senel, & Tuncel, 2002) and suspension polymerization (Lu, Liu, & Liu, 2003). Usually, these hydrophilic microspheres were ''gel-type'' with low surface area and low porosity, which limited their applications. Porous microspheres with large specific surface area and high porosity would be favorite to be used in the separation field. The porous structures of polysaccharide microspheres could be able to obtain via double emulsification procedure (Gustavsson & Larsson, 1996), wet phase inversion method (Fwu-Long, Shin-Shing, Chin-Ta, & Juin-Yih, 2002), or adding porogen such as inorganic particles (Shi, Zhou, & Sun, 2005) in the preparation of microspheres.

Sucrose, a disaccharide, is liable to be crosslinked because it has eight chemically active hydroxyl groups. In this paper, our aim is to synthesize porous hydrophilic polysucrose microspheres by the inverse phase suspension polymerization. The chemical structures, morphology, mean particle size and polydispersity index of the microspheres were characterized. The porosity, equilibrium water content and hydroxyl content of porous microspheres were investigated as well. Bovine serum albumin (BSA) was used as adsorbate model to examine the adsorption behavior of the porous microspheres.

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2. Experimental

2.1. Materials

Soluble polysucrose was purchased from Polymer Science & Commerce Co. Ltd. (Tianjin). DMPE ($\overline{M_n} = 7798, \mathrm{PDI} = 2.78$) was purchased from Tianjin chemical engineering stock company (Jiangsu Province). Epichlorohydrin (EP) was obtained from Tianjin No. 1 Chemical Reagent Plant (Tianjin, China). Chlorobenzene was purchased from Beijing Chemical Agents Company (China). Sodium hydroxide (NaOH) (50 wt% aqueous solution) was prepared in laboratory. Acetic anhydride and pyridine were purchased from Tianjin Kewei Company (Tianjin, China). All the chemical regents were analytical pure grade and used without further purification.

2.2. Preparation of porous polysucrose microspheres

To a 250 mL three-necked flask were added subsequently soluble polysucrose (5.0 g), epichlorohydrin (2.54 mL), DMPE (2.0 g) in distilled water (35 mL) at room temperature. Then chlorobenzene (160 mL) and span80 (3 g) were added to the mixture under stirring. The pH value of the mixture was adjusted to 14 by aqueous NaOH solution (50 wt%). The microspheres were formed during the crosslinking reaction carried out at 70 °C for 90 min with appropriate mechanical stirring. The temperature was then raised to 90 °C and the reaction continued for 4 h. The microspheres were washed by ethanol and water several times to eliminate the chlorobenzene and DMPE.

Five microsphere samples were synthesized using the method above, labeled as PPS-1, PPS-2, PPS-3, PPS-4, PPS-5, respectively, where the numbers following the PPS indicated the mass of porogen (g) used in the reaction.

2.3. Characterization of porous polysucrose microspheres

2.3.1. Fourier transform infrared spectra

The fourier transform infrared (FTIR) spectra were obtained using Bio-Rad FTS 135 FTIR instrument (Bio-Rad, USA), the dry samples were powdered and mixed with KBr and then pressed into pellets under reduced pressure.

2.3.2. The morphology of porous microspheres

The Optical microscope (Olympus BX51, Japan) and scanning electron microscope (PHILPS XL-30 apparatus) were used to determine the size and morphology of porous polysucrose microspheres. The samples were sputter coated with a thin layer of gold to enhance the surface contrast and reduce surface charging prior to SEM examination.

2.3.3. The particle size and distribution

The particle sizes and the size distribution of PS microspheres were determined using Mastersizer S particle size analyzer (Malvern Instrument, UK).

2.3.4. Pore volume and porosity of microspheres

It is important to investigate the porosity, which reflects the pore volume and the ability of adsorption of microspheres. The excess surface-adhered water on the microspheres was removed by blotting. Then the microspheres were dried to constant weight at 25 °C in vacuum oven for 24 h. The average pore volume V_p and porosity P_r were calculated according to Eqs. (1) and (2) (Zhang, Zhou, Yang, & Chen, 1998):

$$V_p\ (\mathrm{mL/g}) = (V - W_d/d)/W_d \quad (1)$$

$$P_r\ (\%) = V_p/(V_p + 1/d) \quad (2)$$

where V was the volume of the wet microspheres, W_d was the dried weight of microspheres, d was the density of porous microsphere framework.

2.3.5. Hydroxyl content (Dong, 2004, pp. 94–95)

Acid number was determined through the following method: 0.1 g of dried porous polysucrose microspheres were introduced into ethanol (20 mL). The solution of NaOH (1.0 mol/L) was used to titrate the excess of acetic acid using a phenolphthalein solution as the indicator. Blank titration was performed in the same way to avoid systematic errors. The acid number was calculated according to:

$$\text{Acid number}\ (\mathrm{mg/g}) = \frac{40 \times (V - V_0) \times c}{m} \quad (3)$$

where V and V_0 were the volume of NaOH solution for the experimental and blank titration, respectively, c was the concentration of NaOH solution (mol/L) and m was the mass of sample (g).

Non-aqueous titration was employed to determine the hydroxyl content in porous polysucrose microspheres: to a pyridine solution of acetic anhydride (20 mL, 25%, v/v) was added 0.30 g of porous polysucrose microspheres at room temperature, and then raised to 100 °C to acetylate for 1 h. Afterwards, to the reaction system was added 5 mL of distilled water and the system was allowed to stand for another 30 min. The aqueous solution of NaOH (1.0 mol/L) was used to titrate the excess of acetic acid using phenolphthalein solution as indicator. A blank titration was performed in the same way to avoid systematic errors. The hydroxyl content was calculated according to Eqs. (4) and (5):

$$\text{Hydroxyl number}\ (\mathrm{mg/g}) = \frac{40 \times (V_1 - V_2) \times c}{m} + \text{Acid number} \quad (4)$$

$$\text{Hydroxyl content}\ (\mathrm{mmol/g}) = \frac{\text{Hydroxyl number}}{N} \quad (5)$$

where V_2 and V_1 are the volume of NaOH solution for the experimental and blank titration, respectively, c is the concentration of NaOH solution (mol/L), m is the mass of sample (g), and N was the mole mass of NaOH (g/mol).

2.3.6. Equilibrium water content

The swelling behavior of the porous polysucrose microspheres was determined by monitoring the equilibrium

water content. The sample microspheres were immersed into the distilled water at 25 °C for 24 h. The excess surface-adhered water was removed by blotting and then dried in vacuum oven at 25 °C for 24 h. The equilibrium water content X (%) was calculated according to Eq. (6) (Hou, Yang, Tang, et al., 2006):

$$X\ (\%) = \frac{m_2 - (m_1 + m_3)}{m_2 - m_1} \quad (6)$$

where m_1 is the weight of empty container, m_2 was the total weight of wet sample microspheres and container, and m_3 is the constant weight of dry sample microspheres.

2.4. The determination of protein adsorption

Bovine serum albumin (BSA) was used as model protein to test the adsorption behavior of porous polysucrose microspheres. All the adsorption experiments were conducted at 25 °C in 0.01 mol/L Tris–HCl buffer solution. Typically, 0.05 g of porous microspheres was added into 5.0 mL of BSA solution (0.5 mg/L) for 24 h in a shaking incubator (pH 7.6, NaCl 0.2 mol/L). A series of the adsorption experimentals were carried out with different concentrations (ranging from 0.5 to 3.0 mg/mL) under the same conditions. The BSA standard curve was determined firstly. After centrifugation, the optical density at 280 nm of the supernatant solutions was recorded, and the amount of adsorbed protein was calculated by mass balance according to Eq. (7) (Xue & Sun, 2002):

$$q\ (\mathrm{mg/g}) = (C_0 - C_1) \times V/W \quad (7)$$

where C_0 is the concentration of BSA in the liquid phase before adsorption (mg/mL), C_1 is the concentration of BSA in the liquid phase after adsorption (mg/mL), V is the volume of solvent (mL) and W is the mass of microspheres (g).

3. Results and discussion

3.1. Preparation of porous microspheres

The reaction of soluble polysucrose and EP obeyed the Williamson Synthesis and nucleophilic substitution (Morrison & Boyd, 1980, pp. 379–384) under alkaline solution and at high temperature in this experiment. The synthetic scheme of porous polysucrose microspheres is shown in Fig. 1. DMPE was added as porogen and the suspended droplets of soluble polysucrose and EP in chlorobenzene were stabilized by Span80. Then the reaction took placed with the adding of alkaline solution and increasing temperature. The phase-separation occured in the suspended droplets during the process of crosslinking and solidifying. DMPE did not take part in the crosslinking reaction of soluble polysucrose and EP in alkaline solution. At last, the microspheres consisting of solid polysucrose and DMPE were obtained and the porous structure was resulted through elimination of DMPE by washing steps.

3.2. FTIR spectrum

The FTIR spectra of sucrose, soluble polysucrose and porous polysucrose microspheres were shown in Fig. 2. The sharp peak at 3560 cm^{-1} of primary hydroxyl groups only existed in sucrose and disappeared in soluble polysucrose and porous polysucrose microspheres. This may be that all the primary hydroxyl groups were consumed in the polymerization. The peak at 3394 cm^{-1} of porous polysucrose microspheres attributed to the combined hydroxyl groups (Bellamy, 1975) showed a decrease in its intensity in comparison to that of soluble polysucrose

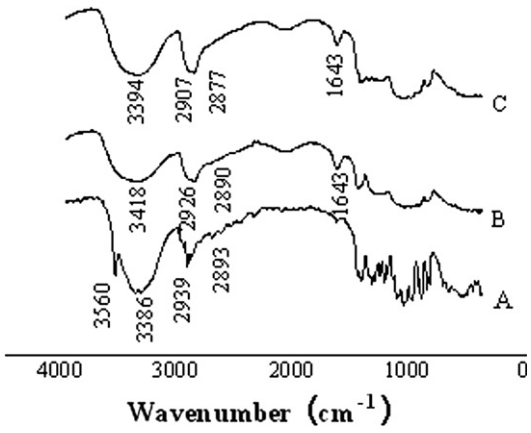

Fig. 2. FTIR spectra of soluble polysucrose, soluble polysucrose and porous polysucrose microspheres. A. sucrose; B. soluble polysucrose; C. porous polysucrose microspheres.

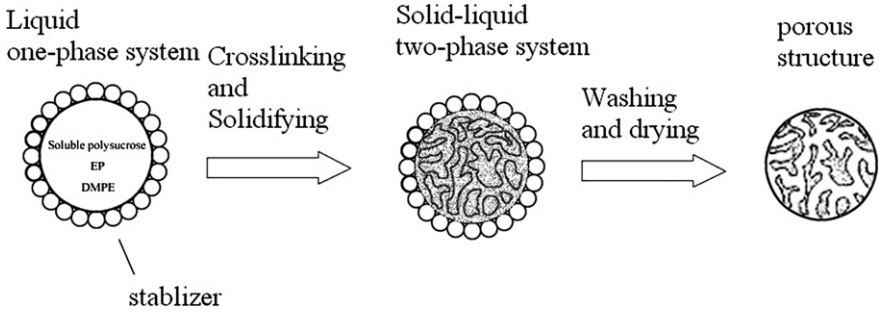

Fig. 1. Synthesis scheme for the preparation of porous polysucrose microspheres.

and sucrose. This indicated that some hydroxyl groups were consumed during the reaction. The intensities of bands at 2907 cm^{-1} and 2877 cm^{-1} of methylene groups (Nakanshi Koji & Solomon, 1977) in the spectrum of porous polysucrose microspheres increased in comparison to that of soluble polysucrose and sucrose, which suggested that more methylene groups were introduced into porous microspheres by EP.

3.3. Morphology of microspheres

Fig. 3 showed the morphology of wet porous polysucrose microspheres in the Optical Microscope photographs. It was clear that the beads had porous interior structure. Fig. 4 showed the SEM images of dried porous polysucrose microspheres. It revealed that the surface of the microsphere was rough with round cavities and the interior of the bead was porous. Compared with the wet beads, the mean particle size decreased and porous structure shrunk after drying. This may be caused by that the evaporation of water in hydrophilic microspheres after drying leading to the shrinkage of interior porous structure, while the skeleton of microspheres was relatively rigid, and the porous structure of microspheres retained to a certain extent after drying.

3.4. The particle size and distribution

The particle size and distribution of wet porous polysucrose microspheres were listed in Table 1. It showed that the particle size of porous beads was about 340 μm and PDI was closed to 1, which indicated that good monodispersity of microspheres could be obtained. The effects of porogen amount on the particle size and distribution of porous polysucrose microspheres were shown in Fig. 5. The results showed that the variety of porogen amounts did not remarkably affect the particle size and distribution under this preparation condition. The particle size and dis-

Table 1
Mean particle size and polydispersity index (PDI) of porous polysucrose microspheres with different porogen amounts

Microspheres	Porogen amount (%)	D_n (μm)	D_w (μm)	PDI
PPS-1	16.7	338.6	345.7	1.017
PPS-2	28.6	347.4	352.7	1.015
PPS-3	37.5	334.3	340.6	1.019
PPS-4	44.4	352.0	356.1	1.012
PPS-5	50.0	336.1	341.6	1.016

Preparation conditions: W/O ratio (v/v) = 1:5; temperature = 70 ± 2 °C; stirring speed = 240 ± 10 rpm; porogen amount = sporogen/porogen and soluble polysucrose.
D_n represents the number average diameter which is calculated according to Eq. (9), while D_w represents the weight average diameters which is calculated according to Eq. (10):

$$D_n = \frac{\sum_{i=1}^{n} d_i}{n} \quad (9)$$

$$D_w = \frac{\sum_{i=1}^{n} d_i^4}{\sum_{i=1}^{n} d_i^3} \quad (10)$$

$$\mathrm{PDI} = \frac{D_w}{D_n} \quad (11)$$

where the d_i means the diameter of the microspheres, n represents the number of the microspheres (Lu & Zhang, 2003).

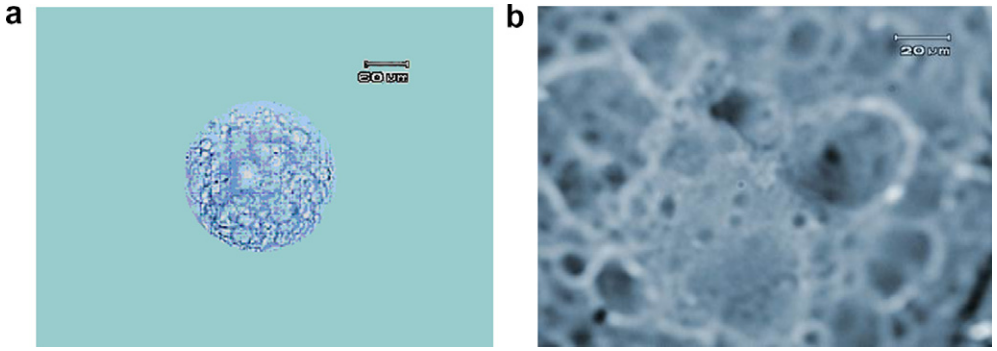

Fig. 3. OM photographs of wet PPS-3 (a) 100×; (b) 300×.

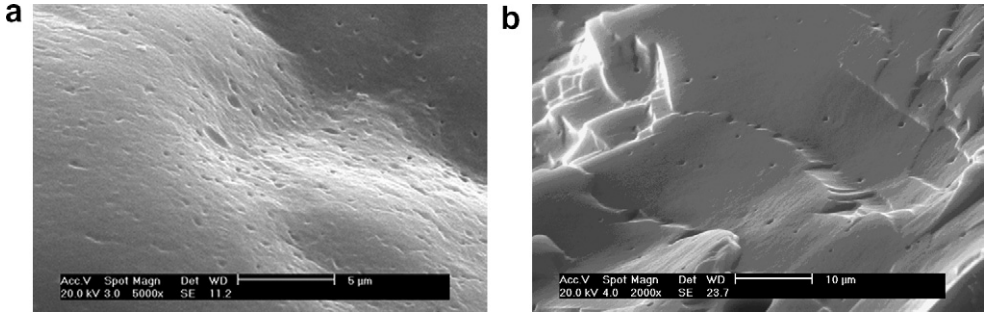

Fig. 4. SEM images of dried PPS-3 (a) 5000×; (b) 2000×.

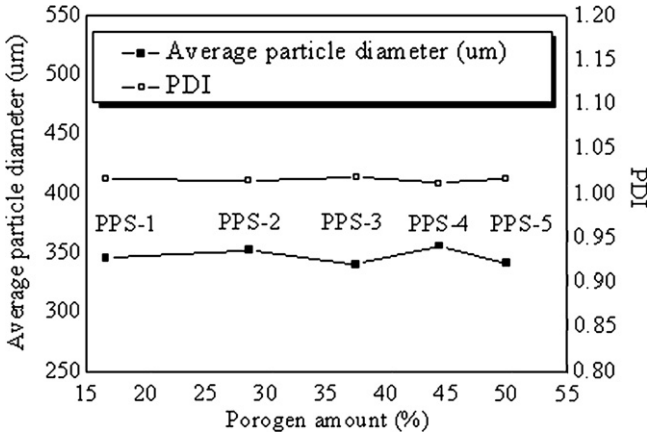

Fig. 5. Effects of porogen amount on mean particle size and polydispersity index (PDI) of porous polysucrose microspheres.

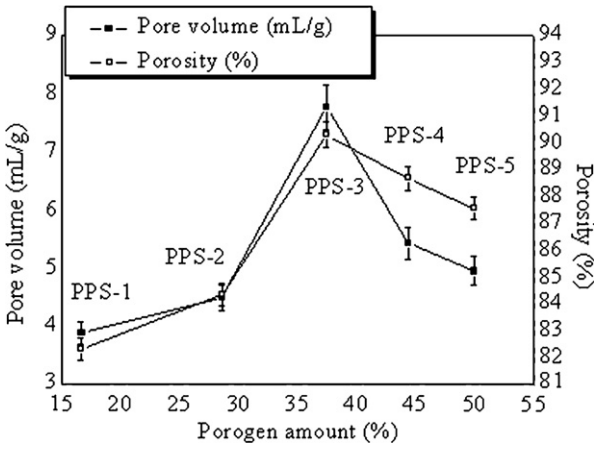

Fig. 6. Effects of porogen amount on porosity and pore volume of porous polysucrose microspheres.

tribution of wet microspheres might be likely influenced by the stirring rates and dispersant amounts according to the principle of suspension polymerization.

3.5. Porosity of polysucrose microspheres

The average pore volume and porosity of five dried porous polysucrose microspheres were shown in Table 2. It could be seen from Table 2 that when the porogen amount reached 37.5%, the average pore volume and porosity reached a peak value as shown in Fig. 6. This might be due to the fact that: (1) when the less porogen amount would yield less porosity, the porosity would increase with the increasing of porogen amounts; (2) when the added porogen amount increased, more porogen molecules would be embedded in the crosslinked network, such the average pore volume and porosity decreased. The porous structure of PPS-3 was available in order to prevent porous structure from large shrinkage in different solvents under the process of adsorption.

3.6. Hydroxyl content

To determine the loading of hydroxyl groups, the polysucrose microspheres were acetylated by the reaction with an excess of acetic anhydride. The excess acetic anhydride was then converted to acetic acid and titrated by standardized NaOH solution. Some of the results were listed in Table 3. The loading of hydroxyl groups of microspheres increased from 19.30 to 21.80 mmol/g with increase of porogen amounts, as shown in Fig. 7. During the polymerization of polysucrose and EP, each EP molecule could consume two hydroxyl groups of polysucrose and generate new one, which lead to decrease of hydroxyl groups; while the exist of porogen molecule decreased the opportunity of reaction between the soluble polysucrose and EP in the droplets, leading to accessibility of active hydroxyl groups on the microspheres. So the determined hydroxyl content of porous microspheres was higher than that of the gel polysucrose microspheres.

Table 2
The V_p and P_r of porous polysucrose microspheres with different porogen amounts

Microspheres	Porogen amount (%)	Pore volume (mL/g)	Porosity (%)
PPS-1	16.7	3.88 ± 0.12	82.3 ± 0.4
PPS-2	28.6	4.49 ± 0.15	84.4 ± 0.6
PPS-3	37.5	7.75 ± 0.16	90.3 ± 0.3
PPS-4	44.4	5.42 ± 0.13	88.7 ± 0.5
PPS-5	50.0	4.94 ± 0.18	87.6 ± 0.5

Preparation conditions: W/O ratio (v/v) = 1:5; temperature = 70 ± 2 °C; stirring speed = 240 ± 10 rpm; porogen amount = porogen/porogen and soluble polysucrose.

3.7. Equilibrium water content

Fig. 8 showed the dependence of equilibrium water contents of microspheres on the porogen amounts added in preparation of microspheres. The results indicated that equilibrium water contents of microspheres increased with the increasing of porogen amount. As mentioned in Section 3.6 the hydrophilic hydroxyl contents in the microspheres increased with increasing of added porogen amounts; the average pore volumes and porosities of the microspheres in wet state showed the increase with added porogen

Table 3
Hydroxyl content and equilibrium water content of porous polysucrose microspheres with different porogen amounts

Microspheres	Porogen amount (%)	Hydroxyl content (mmol/g)	Equilibrium water content (%)
PPS-1	16.7	19.3 ± 0.18	92.1 ± 0.24
PPS-2	28.6	20.0 ± 0.15	93.1 ± 0.18
PPS-3	37.5	21.5 ± 0.16	95.9 ± 0.13
PPS-4	44.4	21.6 ± 0.12	96.2 ± 0.19
PPS-5	50.0	21.8 ± 0.18	96.6 ± 0.17
GPS[a]	0.0	17.9 ± 0.12	86.4 ± 0.13

Preparation conditions: W/O ratio (v/v) = 1:5; temperature = 70 ± 2 °C; stirring speed = 240 ± 10 rpm; porogen amount = porogen/porogen and soluble polysucrose.

[a] GPS was the abbreviation of gel polysucrose microspheres, which were synthesized and characterized in the previous study (Hou, Yang, Tang, et al., 2006).

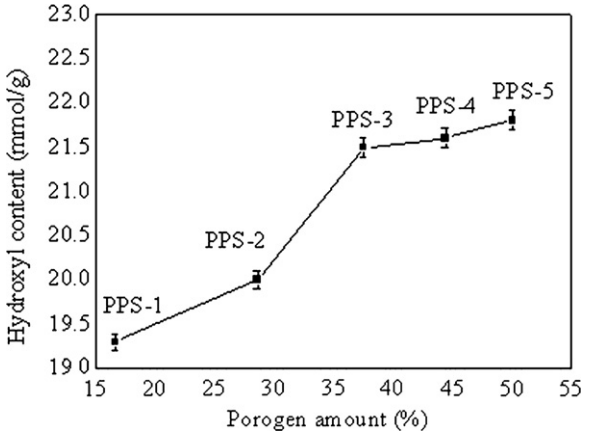

Fig. 7. Effects of porogen amount on hydroxyl content of porous polysucrose microspheres.

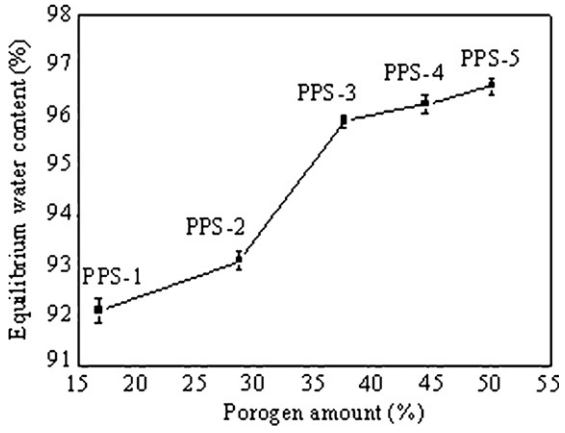

Fig. 8. Effects of porogen amount on equilibrium water content of porous polysucrose microspheres.

amounts, therefore the equilibrium water contents of microspheres increased. It was concluded that the hydroxyl content affected the equilibrium water content more significantly than the porosity and average pore volume.

3.8. Protein adsorption

The adsorption behavior of the porous polysucrose microspheres for BSA can be described by the Langmuir equation:

$$q = \frac{q_m c}{K_d + c} \tag{8}$$

where c (mg/mL) is the equilibrium concentration of BSA in bulk solution, q (mg/g) is the adsorption quantity of microspheres, q_m is the saturation capacity, and K_d is the dissociation constant. The parameters in the Langmuir equation were estimated by fitting the equation to the experimental results using the least-square regression (Zhou, Xue, Bai, & Sun, 2002).

The influence of added porogen amounts in preparation of beads on the adsorption capacity of porous polysucrose microspheres was displayed in Fig. 9. Compared with the curve of gel GPS with the same particle size (Hou, Yang,

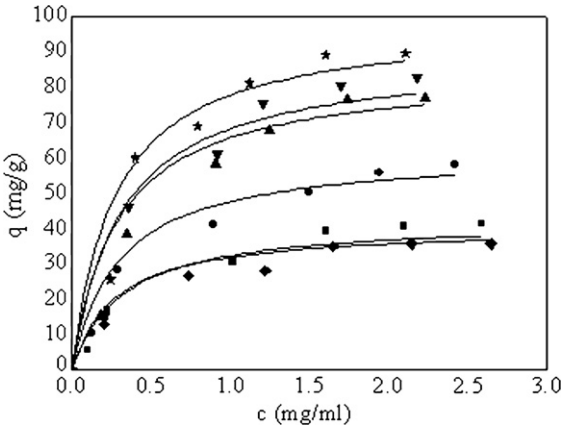

Fig. 9. Isotherms of BSA adsorption to polysucrose microspheres (♦) porogen amount 0.00, hydroxyl content 17.90 mmol/g, q_m (mg/g) = 40.44, K_d (mg/mL) = 0.27; (■) porogen amount 16.7%, hydroxyl content 19.30 mmol/g, q_m (mg/g) = 42.56, K_d (mg/mL) = 0.32; (●) porogen amount 28.6%, hydroxyl content 20.00 mmol/g, q_m (mg/g) = 61.84, K_d (mg/mL) = 0.30; (▲) porogen amount 37.5%, hydroxyl content 21.50 mmol/g, q_m (mg/g) = 84.65, K_d (mg/mL) = 0.29; (▼) porogen amount 44.4%, hydroxyl content 21.60 mmol/g, q_m (mg/g) = 89.24, K_d (mg/mL) = 0.31; (★) porogen amount 50.0%, hydroxyl content 21.80 mmol/g, q_m (mg/g) = 98.54, K_d (mg/mL) = 0.27.

Huang, Wang, & Yao, 2006), the porous microsphere PPS-1 had higher hydroxyl content (19.3 mmol/g) and higher saturation capacity (42.6 mg/g) due to the porogen (16.7%).

The adsorption capacities of microspheres behaved different trends as porogen amounts increased as shown in Fig. 10. The curve of saturation capacity to porogen amount was similar to that of the average pore volume and porosity to porogen amount. When the average pore volume and porosity increased with increase of porogen amount, the larger pore volumes of the microspheres was in favor of protein adsorption. The adsorption capacities increased from 42.6 to 98.5 mg/g as the hydroxyl contents of wet beads increased from 19.3 to 21.8 mmol/g in Fig. 11. This indicated that the hydroxyl group of microspheres was an important factor for protein adsorption due to their hydrophility and the for-

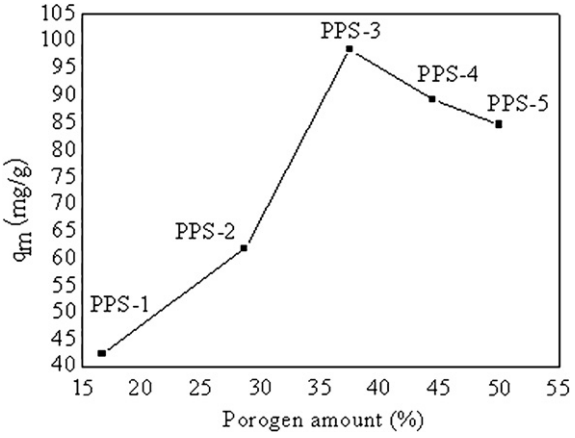

Fig. 10. Effect of porogen amount on saturated adsorption capacity of porous polysucrose microspheres.

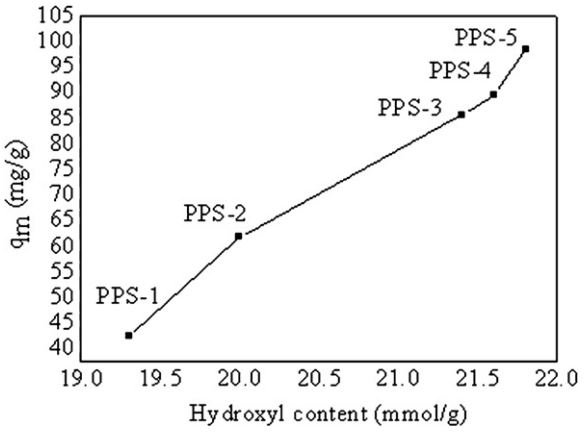

Fig. 11. Effect of hydroxyl content on saturated adsorption capacity of porous polysucrose microspheres.

mation hydrogen bonds between microsphere and protein. The results demonstrated that the hydroxyl contents and porosities of porous microspheres both played important role in adsorption of the porous beads for protein.

4. Conclusions

In this study, a set of novel porous polysucrose microspheres were prepared by the inverse suspension polymerization using soluble polysucrose, epichlorohydrin (EP) as crosslinker and dimethyl ether of polyethylene glycol as porogen. The yielded beads have spherical shapes and porous structures, and approximately 340 μm of the mean particle size with narrow size distribution. The porous microspheres synthesized under optimal condition (crosslinker content 37.5%, porogen content 37.5%) possessed 21.5 mmol/g hydroxyl content, 90.3% porosity and 95.9% equilibrium water content. The saturated adsorption capacities of the porous microspheres for bovine serum albumin as model ranged from 42.6 to 98.5 mg/g. Compared with gel polysucrose microspheres, the porous polysucrose microspheres had higher hydroxyl contents, porosities and saturation capacity for BSA.

Acknowledgements

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References

Bellamy, L. J. (1975). *The infrared spectra of complex molecules*. London: Chapman and Hall.

Caglayan, B., Unsal, E., Camli, S. T., Tuncel, M., & Tuncel, A. (2006). Monodisperse porous poly(vinyl acetate-co-divinylbenzene) particles by single-stage seeded polymerization: A packing material for reversed phase HPLC. *Journal of Separation Science, 29*, 936–944.

Camli, T., Tuncel, M., Senel, S., & Tuncel, A. (2002). Uniform, and macroporous latex particles: Preparation, electron microscopic characterization, and nonspecific protein adsorption properties. *Journal of Applied Polymer Science, 84*(2), 414–429.

Dong, Yanming (2004). *The analysis manual of polymers*. Beijing: Chinese Petroleum and Chemistry Press.

Fu, G. Q., Li, H. Y., Yu, H. F., Liu, L., Yuan, Z., & He, B. L. (2006). Synthesis and lipoprotein sorption properties of porous chitosan beads grafted with poly(acrylic acid). *Reactive and Functional Polymers, 66*, 239–246.

Fwu-Long, Mi, Shin-Shing, Shyu, Chin-Ta, Chen, & Juin-Yih, Lai (2002). Adsorption of indomethacin onto chemically modified chitosan beads. *Polymer, 43*, 757–765.

Gustavsson, P. E., Axelsson, A., & Larson, P. O. (1998). Direct measurements of convective fluid velocities in superporous agarose beads. *Journal of Chromatography A, 795*, 199–210.

Gustavsson, P. E., & Larsson, P. O. (1996). Superporous agarose, a new material for chromatography. *Journal of Chromatography A, 734*, 231–240.

Hou, X., Yang, J., Huang, D. G., Wang, X. L., & Yao, K. D. (2006). Preparation and protein adsorption of hydrogel polysucrose microspheres. *Journal of Applied Polymer Science, 102*, 5934–5940.

Hou, X., Yang, J., Tang, J. C., Chen, X. M., Wang, X. K., & Yao, K. D. (2006). Preparation and characterization of crosslinked polysucrose microspheres. *Reactive & Functional Polymers, 66*, 1711–1717.

Kang, K., Kan, C. Y., Du, Y., & Liu, D. S. (2006). Influences of the polymerization ingredients on the porous morphology of soap-free poly(methyl methacrylate/ethyl acrylate/methacrylic acid) seeded latex particles. *Journal of Applied Polymer Science, 99*, 1934–1939.

Kim, T. Y., Kim, S. J., Yang, J. H., & Cho, S. Y. (2004). Environmentally friendly separation of heavy-metal ions onto porous chitosan beads. *Journal of Industrial and Engineering Chemistry, 10*, 201–207.

Lu, Z. H., Liu, G. J., & Liu, F. T. (2003). Water-dispersible porous polyisoprene-block-poly(acrylic acid) microspheres. *Journal of Applied Polymer Science, 90*, 2785–2793.

Lu, R., & Zhang, H. T. (2003). Synthesis of the crosslinked functional polystyrene microspheres in micro-size monodisperse by dispersion polymerization. *Journal of Functional Polymer, 16*, 54–58.

Mi, F. L., Shyu, S. S., Chen, C. T., & Schoung, J. Y. (1999). Porous chitosan microspheres for controlling the antigen release of Newcastle disease vaccine: Preparation of antigen-adsorbed microsphere and in vitro release. *Biomaterials, 20*, 1603–1612.

Morrison, R. T., & Boyd, R. N. (1980). *Organic chemistry*. Beijing: Science Press.

Nakanshi, K., & Solomon, P. H. (1977). *Infrared Absorption Spectroscopy*. San Francisco: Holden-Day Inc.

Per-Erik, Gustavsson, Anders, Axelsson, & Per-Olof, Larsson (1999). Superporous agarose beads as a hydrophobic interaction chromatography support. *Journal of Chromatography A, 830*(2), 275–284.

Remunan-Lopez, C., Lorenzo-Lamosa, M. L., Vila-Jato, J. L., & Alonso, M. J. (1998). Development of new chitosan-cellulose multicore microparticles for controlled drug delivery. *European Journal of Pharmacy Biopharmacy, 459*, 49–56.

Shi, Q. H., Zhou, X., & Sun, Y. (2005). A novel superporous agarose medium for high-speed protein chromatography. *Biotechnology and Bioengineering, 92*, 643–651.

Xue, B., & Sun, Y. (2002). Fabrication and characterization of a rigid magnetic matrix for protein adsorption. *Journal of Chromatography A, 947*, 185–193.

Zhang, L. N., Zhou, J. P., Yang, G., & Chen, J. H. (1998). Preparative fractionation of polysaccharides by columns packed with regenerated cellulose gels. *Journal of Chromatography A, 816*, 131–136.

Zhou, X., Xue, B., Bai, S., & Sun, Y. (2002). Macroporous polymeric ion exchanger of high capacity for protein adsorption. *Biochemical Engineering Journal, 11*, 13–17.