Preparation and Protein Adsorption of Hydrogel Polysucrose Microspheres

Xin Hou, Jing Yang, Dongge Huang, Xiaoli Wang, Kangde Yao

College of Materials and Engineering, Tianjin University, Tianjin 300072, People's Republic of China

Received 25 April 2006; accepted 10 July 2006
DOI 10.1002/app.25147
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: A series of novel hydrogel polysucrose microspheres with the mean size ranging from 200 to 500 μm were prepared via two-step method. First, soluble polysucrose was synthesized by solution polymerization between sucrose and epichlorohydrin; second, a reversed phase suspension crosslinking reaction was performed to prepare polysucrose microspheres. The SEM images indicated that these spherical beads had smooth surface and hydrogel interior structure. FTIR was used to characterize the chemical structure of the beads. The hydrated and dry densities, equilibrium water content, and hydroxyl content of polysucrose microspheres were also investigated. The characteristic of high hydroxyl content (15.48–19.04 mmol/g) make these microspheres suitable for protein adsorption. Meanwhile, bovine serum albumin was used to examine the adsorption capacity of the microspheres. These microspheres had a capacity as high as 49.28 mg/g. The adsorption kinetics and recycling of the beads were also investigated. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 5934–5940, 2006

Key words: polysucrose; microspheres; reversed suspension polymerization; epichlorohydrin; protein adsorption

INTRODUCTION

Sucrose, a disaccharide, is ubiquitously present in nature with world production over 124 million tons annually.[1] Natural sucrose has quite high purity and does not require further purification. So many derivatives, such as sucralose (Splenda), sucrose polyester (Olestra), and polysucrose (Ficoll400), etc., have been commercially available.[2] Sucrose-based polymers have been extensively studied since 1950s.[3] Their properties such as low toxicity, good biodegradability and biocompatibility make them suitable for wide applications in biomedical, pharmaceutical, and related fields.[4–6] Sucrose has eight chemically active hydroxyl groups (three primary and five secondary), which contain labile atoms that can be easily crosslinked. Furthermore, crosslinking reaction could take place in water due to its excellent solubility.

Hydrogel polysucrose (polysucrose) networks (sucrose, hydrogels, or sucrogels) are generally obtained by introducing vinyl groups to sucrose and sequent polymerization. This has been accomplished by enzymatic way,[7,8] chemical way,[9] and chemoenzymatic way.[10] Superporous sucrose hydrogels have also been prepared by utilizing the similar way.[11] Generally these polymers are gels without fixed shapes, thus hinder their applications. Hydrogel beads based on agarose and dextran have been developed and extensively used as supporting materials for protein purification by affinity, ion exchange, and hydrophobic interaction chromatography.[12] So we hope to obtain a kind of soft beads using sucrose as raw material for multiple applications including protein adsorption.

The present article focused on preparation and protein adsorption of novel hydrogel polysucrose microspheres using epichlorohydrin (EP) as crosslinker by a two-step method. Solution polymerization was employed for the first step, while reversed phase suspension polymerization was utilized for the second step. The reason for that was: (a) to avoid high viscosity during reaction; (b) considering that EP is somewhat oil-soluble; (c) emulsion system would be destroyed during long-time polymerization in alkali medium.

EXPERIMENTAL

Materials

Sucrose (edible) was purchased from Lande Food (Tianjin, China). Epichlorohydrin (analar) was purchased from Tianjin No.1 Chemical Reagents Factory (Tianjin, China). Chlorobenzene (analar) was purchased from Beijing Chemical Reagents (Beijing, China). Span80 (analar) was purchased from Tianjin No.3 Chemicals Reagents Factory (Tianjin, China). Sodium hydroxide (NaOH, analar), acetic anhydride (analar), and pyridine

Correspondence to: X. Hou (houxin@tju.edu.cn).
Contract grant sponsor: National Nature Science Foundation of China ; contract grant number: 50403017.
Contract grant sponsor: Nature Science Foundation of Tianjin ; contract grant number: 05yfgpgx06600.

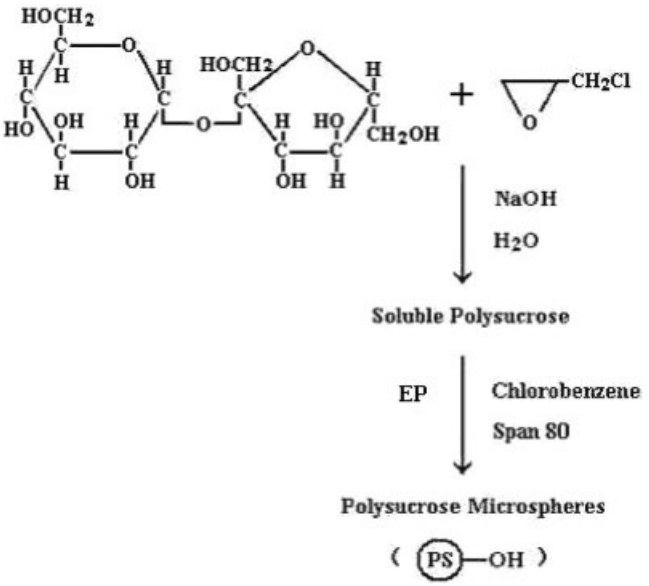

Figure 1 Synthesis route for the preparation of hydrogel polysucrose microspheres

(analar) were purchased from Tianjin Kewei Reagents (Tianjin, China). Bovine serum albumin (BSA) was obtained from Sigma Chemical Reagents (St. Louis, MO). All the other reagents were of analytical grade.

Synthesis of soluble polysucrose

NaOH (30 g, 50% aqueous solution w/w) was introduced into a solution of sucrose (80 g), deionized water (25 g), and epichlorohydrin (35 g). The mixture was stirred at room temperature for 2 h while pH was maintained at 13 with before-mentioned NaOH solution. After 2 h, the reaction was allowed to proceed for another 2 h at 60°C. A standard hydrochloric acid (HCl) solution was added dropwise to this mixture until pH 7. The products obtained were fractioned by stepwise precipitation method.[13] The soluble polysucrose with a number–average molecular weight (M_n) of 3–4 $\times$ 10^5 were selected for further polymerization after pretreated with ion exchange resins (001 $\times$ 7, 122, 201 $\times$ 7, and 330). The M_n of soluble polysucrose was determined by Waters-410 GPC Instrument. PEO samples were standard for calibration and H_2O was used for mobile phase with a flow rate of 0.5 mL/min at 25°C.

Synthesis of hydrogel polysucrose microspheres

In a typical process, soluble polysucrose (5 g), deionized water (20 g), NaOH solution (2 mL, 50% aqueous solution w/w), and epichlorohydrin (1.69 mL) were mechanically stirred in a 250-mL flask at room temperature. Then, chlorobenzene (160 mL) and span80 (3 g) were introduced into the mixture to make a W/O suspension. Hydrogel polysucrose microspheres were formed during this reversed suspension polymerization at 70°C for 2 h in an oil thermostat bath. Following this step, the temperature was raised to 90°C, and the polymerization lasted for another 4 h. At the end of the reaction, the polysucrose microspheres were first washed with ethanol to extract oil from the beads and then washed by large amounts of deionized water.

Characterization of hydrogel polysucrose microspheres

Fourier transform infrared (FTIR) spectra were recorded on a Bio-Rad FTS135 spectrometer (BIO-RAD, USA). The dry samples were powdered and mixed with KBr and pressed into pellets under reduced pressure. The morphology of dried PS microspheres was observed using a Philips XL_30 scanning electron microscope (Philips, The Netherlands). Samples were sputter coated with a thin layer of gold to enhance the surface contrast and reduce surface charging. The particle size distribution of polysucrose microspheres was measured with a Mastersizer S particle size analyzer (Malvern Instrument, UK). Hydrated and dry densities of polysucrose microspheres were measured with a 6-mL pycnometer, using deionized water and heptane as steeps respectively. The hydroxyl content of polysucrose microspheres was determined by a nonaqueous titration.[14] The equilibrium water content (EWC) was calculated as water content of swollen beads divided by the mass of those beads after drying in vacuum at 60°C.[15]

Protein adsorption

BSA was used as a model protein to test the adsorption characteristics of the polysucrose microspheres.

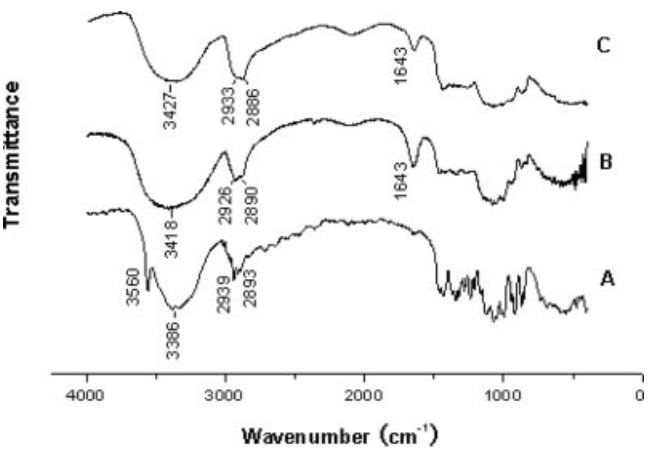

Figure 2 FTIR spectra of sucrose, soluble polysucrose, and hydrogel polysucrose microspheres. A, sucrose; B, soluble polysucrose; C, hydrogel polysucrose microspheres.

TABLE I
Properties of Hydrogel Polysucrose Microspheres with Different Crosslinker Amounts[a]

EP/soluble polysucrose weight ratio in feed	D (v, 0.5) (μm)	$D^{3,4}$ (μm)	Hydrated density (g/mL)	Dry density (g/mL)	EWC (%)	Hydroxyl content (mmol/g)
0.4 : 1	443.36	449.40	1.00 ± 0.04	0.91 ± 0.03	94.13 ± 0.21	19.04 ± 0.18
0.5 : 1	398.96	406.93	1.01 ± 0.03	0.95 ± 0.02	90.09 ± 0.18	18.84 ± 0.15
0.6 : 1	339.94	345.69	0.99 ± 0.03	1.11 ± 0.02	86.35 ± 0.13	17.90 ± 0.12
0.7 : 1	313.98	318.61	1.00 ± 0.02	1.13 ± 0.02	84.02 ± 0.38	17.13 ± 0.11
0.8 : 1	290.62	295.27	1.01 ± 0.01	1.22 ± 0.01	79.61 ± 0.35	15.48 ± 0.15

[a] Preparation conditions: [soluble polysucrose] = 20%; W/O ratio (v : v) = 1:5; temperature = 70°C ± 2°C; stirring speed = 240 ± 10 rpm.

All the adsorption experiments were conducted at 25°C in Tris–HCl buffer solution (0.01 mol/L). Typically, dried microspheres (0.05 g) were added into BSA solution (5.0 mL) of different concentrations (ranging from 0.5 to 3.0 mg/mL) for 24 h in a shaking incubator (pH 7.6, NaCl 0.2 mol/L). After centrifugation, the optical density at 280 nm of the supernatant solutions was recorded, and the equilibrium concentration and the amount of protein adsorbed were calculated by mass balance and BSA standard curve.

In kinetic experiments, capped tubes each containing dried micropheres (0.05 g) and BSA solution (5.0 mL) with a definite concentration were kept in a shaking incubator at 25°C. The tubes were taken out successively for supernatant protein concentration measurement. By this procedure, the time course of BSA concentration decrease was determined.

RESULTS AND DISCUSSION

Preparation of hydrogel polysucrose microspheres

The synthesis route for the preparation of hydrogel polysucrose microspheres is shown in Figure 1. In the first step, sucrose and EP reacted continually to form a long-chain molecule due to the multiple active hydroxyl groups of sucrose. Substitution might happen between these resultant molecular chains and produce chain propagation yielding the branching oligomers. So the oligomers (soluble polysucrose) might contain linear or comb polymers.

In the second step, under the alkaline condition, reversed suspension polymerization of soluble polysucrose and EP in chlorobenzene containing Span 80 was carried out for preparation of crosslinked microspheres. Water phase (aqueous solution of soluble sucrose oligomers, EP, and NaOH) was dispersed into droplets enwrapped by oil phase (chlorobenzene) and Span 80 as dispersion agent. After the temperature was raised above 70°C, crosslinking reaction between soluble polysucrose and EP took place in these suspension droplets to obtain the crosslinked polysucrose microspheres.

The FTIR spectra of sucrose, soluble PS oligomers, and crosslinked PS microspheres were shown in Figure 2, respectively. It was observed that the sharp peak at 3560 cm^{-1}, which is assigned to primary hydroxyl groups, existed only in spectrum of sucrose and disappeared in the spectra of soluble PS and crosslinked PS microspheres. It indicated that the all of the primary hydroxyl groups were consumed in the polymerization. It revealed that the polymerization of sucrose

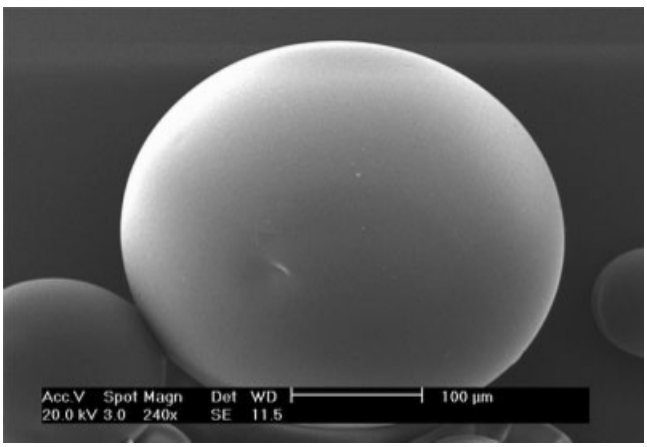

Figure 3 SEM images of hydrogel polysucrose microspheres (polysucrose-1).

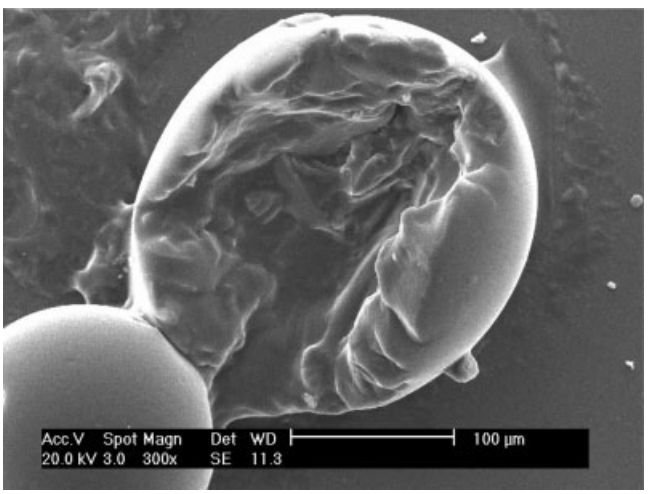

Figure 4 SEM image of interior structure of hydrogel polysucrose microspheres (polysucrose-1).

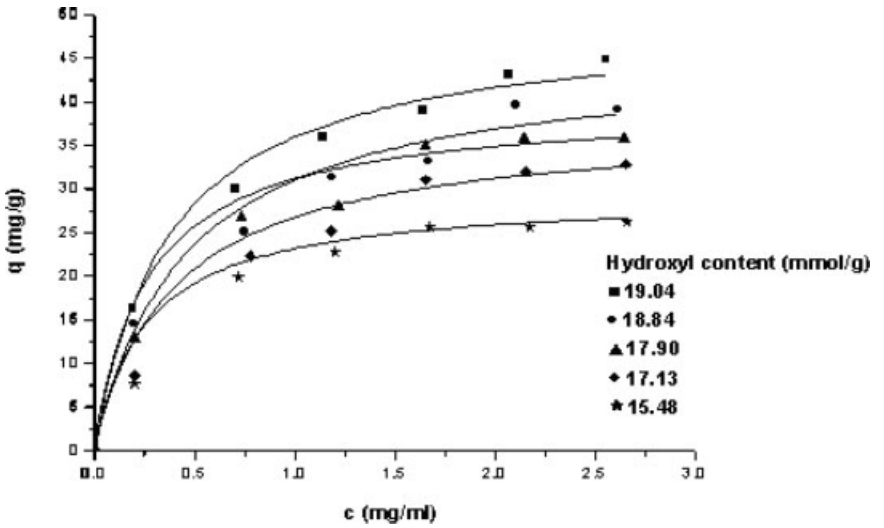

Figure 5 Effect of hydroxyl content on BSA adsorption capacity of polysucrose microspheres. (■) $q_m = 49.28$, $K_d = 0.37$; (●) $q_m = 46.25$, $K_d = 0.47$; (▲) $q_m = 40.44$, $K_d = 0.27$; (◆) $q_m = 37.36$, $K_d = 0.41$; (★) $q_m = 29.18$, $K_d = 0.27$.

with EP first took place at primary hydroxyl groups due to the fact that primary hydroxyl groups of sucrose have the most reactivity. The peak at 3418 cm^{-1} of soluble PS, which is assigned to secondary hydroxyl groups,[16] displays a decrease in its intensity when compared with that of sucrose and this peak of PS was much weaker. This indicated that some secondary hydroxyl groups were consumed during the reaction. The peaks at 2933 and 2886 cm^{-1} in spectra of crosslinked PS microspheres, which are assigned to methylene groups,[17] show an increase in their intensities when compared with those of sucrose and soluble PS. It is attributed to more methylene groups introduced by EP. The chemical structure of crosslinked PS microspheres is similar to that of soluble PS, which is confirmed by their almost same FTIR spectra.

Structure and physical properties of polysucrose microspheres

The hydrogel polysucrose microspheres were found to be in large spherical shape and swellable in water. Five polysucrose microspheres were obtained by altering the amounts of crosslinker during the crosslinking reaction. Some properties of these five sets were listed in Table I. It is shown that the microspheres grew smaller and more compactly with the increasing amount of EP.

The hydroxyl content of polysucrose microspheres decreased with the increasing of EP, which is shown in Table I. It could be expected that the functional groups on the hydrogel microspheres would remain unchanged after the reactions, because each EP mol-

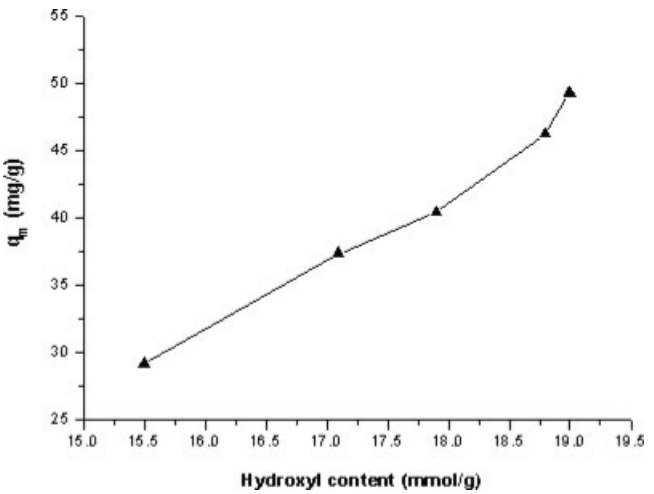

Figure 6 Impact of hydroxyl content on adsorption capacity of polysucrose microspheres.

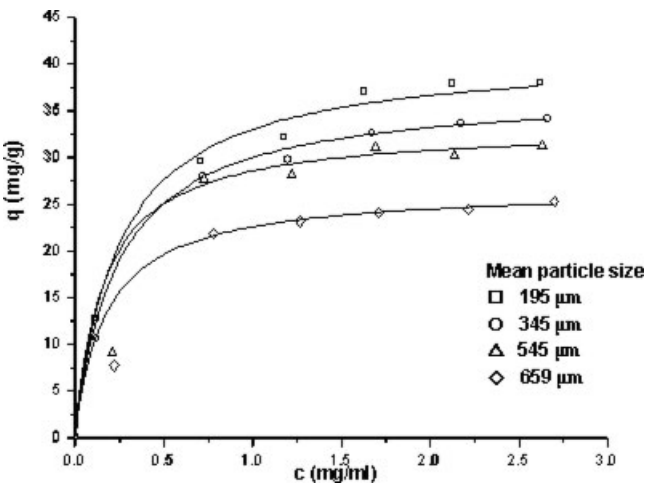

Figure 7 Influence of the mean particle size on BSA adsorption capacity to hydrogel polysucrose microspheres. (□) $q_m = 41.16$, $K_d = 0.25$; (○) $q_m = 37.18$, $K_d = 0.24$; (△) $q_m = 33.27$, $K_d = 0.17$; (◇) $q_m = 26.68$, $K_d = 0.18$.

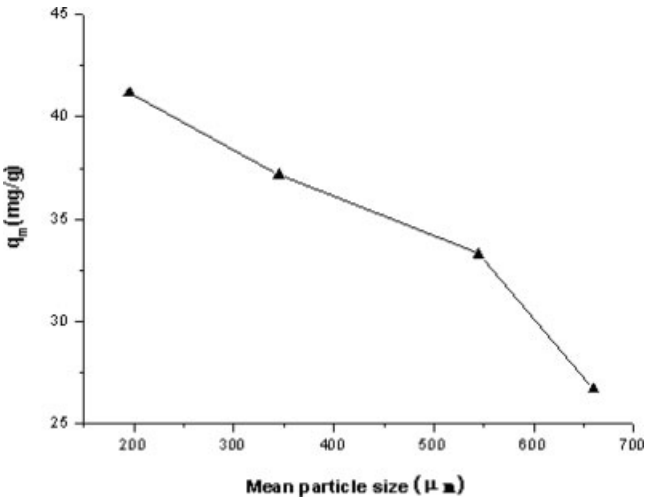

Figure 8 Effect of the mean particle size on adsorption capacity of polysucrose microspheres.

ecule reacted with two hydroxyl groups from different sucrose/soluble polysucrose molecules, yielding one hydroxyl group. Therefore, the increase of amounts of used EP would result in decrease of the hydroxyl content in the crosslinked PS microspheres. And there was only a small difference in equilibrium water content (EWC) because their hydroxyl contents were quite close to each other.

Morphology of polysucrose microspheres

Figure 3 shows the SEM image of hydrogel polysucrose microspheres. It is found that all the particles maintained a spherical morphology and the smooth surface. The interior structure of microspheres is a typical feature of hydrogel, which is shown in Figure 4. Owing to the hydrogel essence of polysucrose microspheres, BSA could hardly penetrate into the inner structure of the microspheres.

Protein adsorption

Adsorption equilibrium

The adsorption of BSA to polysucrose microspheres can be described by the Langmuir equation:

$$q = \frac{q_m c}{K_d + c} \tag{1}$$

where c (mg/mL) is the equilibrium concentration of BSA in bulk solution, q (mg/g) is the adsorbed density of protein to the microspheres, q_m is the saturation capacity, and K_d is the dissociation constant. Parameters in the Langmuir equation were estimated by fitting the equation to the experimental results using the least-square regression.[18]

Hydroxyl content of hydrogel polysucrose microspheres plays an important role in the adsorption capacity of the beads. Hydrogel polysucrose microspheres with different hydroxyl contents were obtained by changing the amount of EP during the reaction. Figure 5 shows the adsorption curves of polysucrose microspheres with different hydroxyl contents. The influence of hydroxyl content on adsorption capacity of the microspheres is shown in Figure 6. It is revealed that the adsorption capacity increased with the increasing content of the hydroxyl.

The influence of the mean particle size on the adsorption capacity of polysucrose microspheres was also studied. The curves of BSA adsorption to micro-

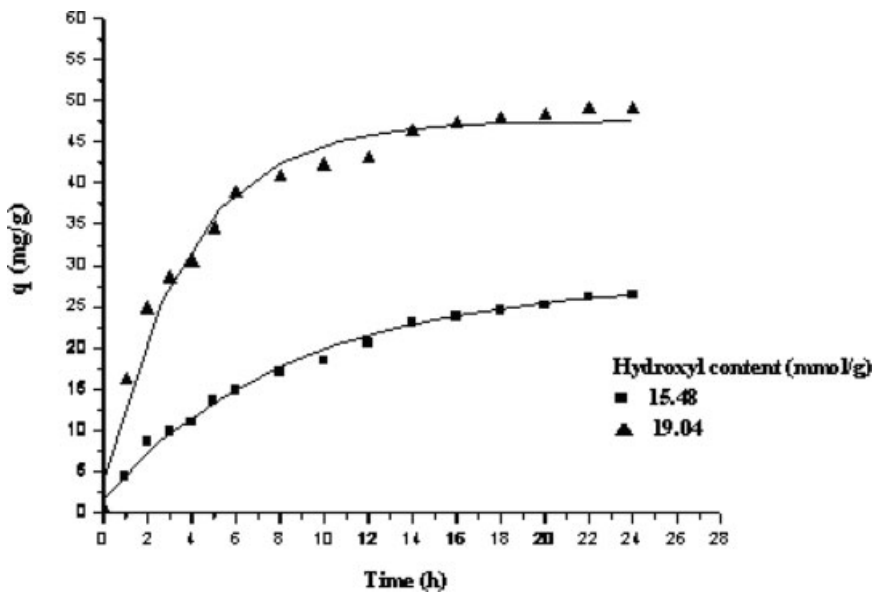

Figure 9 Adsorption kinetics of BSA to hydrogel polysucrose microspheres with different hydroxyl contents.

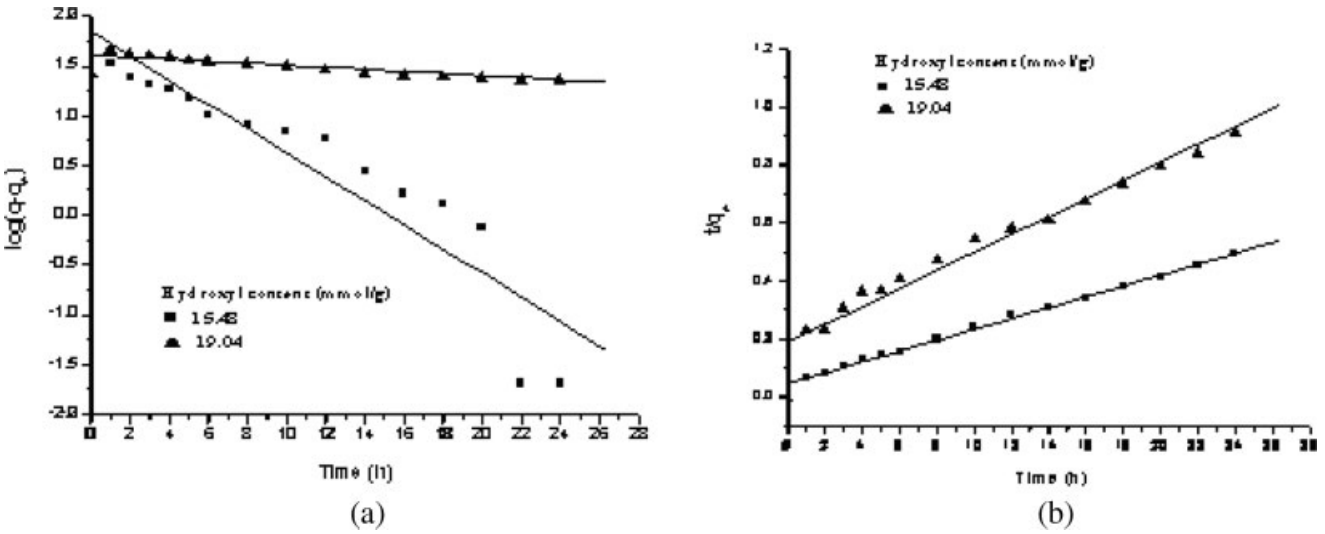

Figure 10 The first-order (a) and the second-order (b) kinetic models of polysucrose microspheres, respectively.

spheres with four different particle sizes were displayed in Figure 7. The effect of particle size on adsorption capacity of the microspheres is shown in Figure 8. It is demonstrated that the binding capacity depended heavily on the mean particle size.

Adsorption kinetics

The experimental results of adsorption kinetics were demonstrated in Figure 9. The first- and second-order kinetic models of Lagergren had been used to investigate the adsorption rate.[19] The first-order rate expression of Lagergren is:

$$\frac{dq}{dt} = k_1(q - q_e) \tag{2}$$

where q_e (mg/g) is the adsorbed density of protein at time t, and k_1 (min^{-1}) is the rate constant of first-order adsorption. The integrated form of eq. (2) is:

$$\log(q - q_e) = \log q - k_1 t/2.303 \tag{3}$$

A straight line of log ($q - q_e$) versus t suggests the applicability of this kinetic model. To make eq. (3) fit the experimental data, the equilibrium adsorption capacity, q, must be known. In many cases, q is unknown and as adsorption tends to become unmeasurably slow, the amount of adsorbed BSA is still significantly smaller than the equilibrium amount. Furthermore, in most cases the first-order equation of Lagergren does not fit the whole range of time very well,[20] which is shown in Figure 10(a).

Then the second-order kinetic model was employed. It is expressed as:

$$\frac{dq}{dt} = k_2(q - q_e)^2 \tag{4}$$

where k_2 (g/mg min) is the rate constant of second-order adsorption. The integrated linear form of eq. (4) is:

$$\frac{t}{q_e} = \frac{1}{k_2 q^2} + \frac{t}{q} \tag{5}$$

It is demonstrated from Figure 10(b) that the second-order kinetics is applicable, the plot of t/q_e against t gave a linear relationship and there was no need to know any parameter beforehand. The calculation results indicate that the polysucrose microspheres with higher hydroxyl content adsorbed BSA more rapidly than those with lower ones, which is listed in Table II.

Recycling of polysucrose microspheres

After adsorption, polysucrose microspheres were washed with 1.0 mol/L NaCl solution. Then, 0.1 mol/L NaOH solution was employed as a regeneration

TABLE II
Adsorption Kinetics of Polysucrose Microspheres with Different Crosslinker Amounts

Hydroxyl number (mmol/g)	q_{exp} (mg/g)	k_1 (min^{-1})	R^2	q_{cal} (mg/g)	k_2 (10^3 g/mg min)	R^2
19.04	49.28	0.121	0.981	53.16	29.380	0.985
15.48	28.68	0.090	0.967	32.10	12.456	0.993

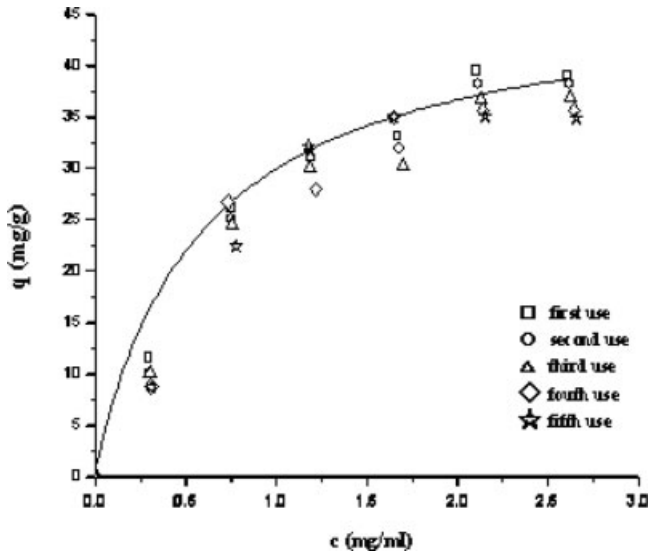

Figure 11 Recycling of polysucrose microspheres.

agent. The adsorption capacity of the regenerated beads was then reinvestigated. Figure 11 shows that there was little loss in adsorption capacity of BSA after reuse for five times, which confirms good recycling ability of polysucrose microspheres in short term.

CONCLUSIONS

In this study, A series of novel hydrogel Polysucrose microspheres with an average diameter of 200–500 μm have been prepared by a two-step way including synthesis of soluble polysucrose followed by a reversed suspension crosslinking reaction. It is found that these spherical beads have smooth surface and hydrogel interior structure. These hydrogel polysucrose microspheres are easily swellable in water and their equilibrium water content values are as high as 94.13%. One of the important characteristics of these microspheres is quite high hydroxyl content (15.48–19.04 mmol/g), which makes them suitable for protein adsorption. Their adsorption capacity was determined by using BSA as a model protein. It is shown that the microspheres had a capacity as high as 49.28 mg/g. Adsorption kinetics of the microspheres was also investigated with the first- and second-order kinetic models of Lagergren. Short-term stability of the microspheres was confirmed by recycling the microspheres in batch adsorption.

References

1. Godshell, M. A. Int Sugar J 2001, 103, 378.
2. Mantovani, G.; Vaccari, G. Int Sugar J 2001, 103, 372.
3. Marcy, K. ACS Symp Ser 1977, 41, 328.
4. Pankaj, M. Immunology 1997, 20, XI.
5. Oman, H.; Akerblom, E.; Richter, W.; Johansson, S. G. Int Arch Allergy Immunol 1992, 98, 220.
6. Dordick, J. S.; Martin, B.; Linhardt, R. J.U.S. Pat 5,474,915(1995).
7. Chen, X.; Martin, B. D.; Neubauer, T. K.; Linhardt, R. J.; Dordick, J. S.; Rethwisch, D. G. Carbohyd Polym 1995, 28, 15.
8. Patil, N. S.; Li, Y.; Rethwisch, D. G.; Dordick, J. S. J Polym Sci, Part A: Polym Chem 1997, 35, 2221.
9. Ferreira, L.; Vidal, M. M.; Geraldes, C. F. G. C.; Gil, M. H. Carbohyd Polym 2000, 41, 15.
10. Liu, X. C.; Dordick, J. S. J Polym Sci, Part A: Polym Chem 1999, 37, 1665.
11. Chen, J.; Park, K. Carbohyd Polym 2000, 41, 259.
12. Yu, Y. H.; Sun, Y. J Chromatogr A 1999, 855, 129.
13. Yang, J.; Song, Z. F.; Zhang, Z. G.; Hou, X.International Symposium on Polymer Chemistry, Changchun, China, 2004, pp 139–143.
14. Sun, J.; Wu, B. L. Non-Aqueous Titration; Science Publishers: Beijing, 1983.
15. Wang, F. Universal Ion Exchange Technology Manual. Scientific and Technical Documents Publishing House: Beijing, 2000.
16. Bellamy, L. J. The infrared spectra of Complex Molecules; Chapman & Hall: London, 1975.
17. Nakanshi, K.; Solomon, P. H. Infrared Absorption Spectroscopy; Holden-Day: San Francisco, 1977.
18. Zhou, X.; Xue, B.; Bai, S.; Sun, Y. Biochem Eng 2002, 11, 13.
19. Zümriye, A. Biochem Eng 2001, 7, 79.
20. McKay, G.; Ho, Y. S. Water Res 1999, 33, 578.