Synthesis of macroporous poly(styrene-divinyl benzene) microspheres by surfactant reverse micelles swelling method

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Abstract

Macroporous poly(styrene-divinyl benzene) microspheres with pore size of about 500 nm were prepared by a new method, surfactant reverse micelles swelling method. The macroporous microspheres were prepared by convenient suspension polymerization. The difference from conventional suspension polymerization was that a higher concentration of surfactant was added in the oil phase. The effects of the amount and type of surfactants on the morphology of microspheres were investigated, and the formation mechanism was also discussed. Macropores were formed when the concentration of surfactant was much higher than critical micelle concentration (*cmc*). It was proposed that a large amount of reverse micelles formed by adding a large amount of surfactant in the oil droplet phase, and the reverse micelles could absorb water from the external aqueous phase. The water in the oil phase formed macropores after polymerization. The method developed in this study was convenient to prepare microspheres with larger pore size than the conventional method such as agglomeration method of nanoparticles.
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1. Introduction

Macroporous polymer particles are efficient materials for many separation processes, and they are widely used in chromatographic separation. With the development of biotechnology, more and more bioproducts with large molecule size such as protein and peptide need to be separated. But the pore size of most conventional macroporous polymer particles is in 100–300 Å, which have the problem of a longer time when separating these bioproducts due to their slow diffusion rate through the interior of the stationary phase particles [1,2]. Therefore, the polymeric particles with large pore size (larger than 10 or 20 times of solute molecule size) are desired.

Until now, a few preparation methods of microspheres with large pore size have been developed in addition to the conventional method of using soluble polymer as porogen. One of them is nanoparticles' agglomeration method. The polymer particles prepared by this method have two sets of pores: through pores (6000–8000 Å) and diffusive pores (800–1500 Å). These particles named as perfusion particles have been successfully used in the separation of biomolecules [3–8]. However, the preparation method of perfusion particles is complicated. The particles were prepared by two steps: the small nanoparticles were prepared at first, and then the small particles agglomerated to form a large particle with micron size. The through pores and diffusive pores were formed by the interstices between the small particles. Therefore, it was difficult to prepare and control. The second method is poly-HIPE (high internal phase emulsion polymer) method developed by Barby and Haq in 1985 [9]. In their study, the

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monolith polymer was prepared. HIPE is the emulsion with the volume concentration of the internal phase over 70%. After polymerization, the polymer has a macroporous structure containing interconnected cavities, which are formed by the internal water phase in HIPE. The diameter of the cavities is from one to tens of microns. Du et al. prepared poly(acrylamide) and poly(butyl acrylate) by HIPE method, and researched the polymerization conditions in detail, including the stable agent system, the viscosity of continuous phase, the pH value, electrolyte, temperature, etc. [10–12]. In 1996, Li and Benson developed the polyHIPE method by dispersing HIPE in external aqueous phase and spherical polyHIPE beads were formed after polymerization [13–16]. The third method is that inorganic particle is used as porogen. Sun [17–19] obtained polymers with the pore size of about 340 nm by this method. They found that the content of inorganic particles was 20–40% in order to get large pores. But the polymer beads tended to break because of the high content of the inorganic particles, and it is difficult to wash out the inorganic particles. Zhang et al. used silica colloidal crystal pellets as templates and prepared three-dimensionally ordered macroporous poly(*p*-methylstyrene) with pore size of 170 nm and each pore was connected to its neighbors by small channels of 72 nm [20].

In our initial study, the experiments were carried out based on the preparation method of polyHIPE beads in order to obtain macroporous P(ST-DVB) microspheres. It was found that the amount of surfactant in oil phase (monomer phase) played an essential role in the formation of macropores during investigating the effect of each factor on the morphology of microspheres. This gave us an illumination that macroporous microspheres may be obtained without preparing HIPE. So we tried to prepare macroporous microspheres only by adjusting the amount of surfactant in the oil phase. The macroporous microspheres with pore size of about 500 nm were obtained by this method. The macroporous polymer particles were obtained more easily than by conventional methods. The formation mechanism of macropores was also discussed in detail.

2. Experimental

2.1. Materials

Styrene (ST) and divinyl benzene (DVB) were of commercial grade (Beijing Chemical Reagents Co.). They were distilled under a vacuum to remove the inhibitor.

Benzoyl peroxide (BPO) (25% water, Beijing Chemical Reagents Co.) was used as an initiator. Hexadecane (HD) was of reagent grade (Wako Pure Chemical Industries, Ltd.), and was used as a hydrophobic additive to retard the monomer diffusing into the aqueous phase [21]. Sorbitan monooleate (Span 80) (Bangde Technology and Trade Co., Beijing) and sorbitan trioleate (Span 85, Shanghai Chemical Reagent Co.) were of reagent grade. PO-500 (hexaglycerin penta ester) was provided by Sakamoto Yakuhin Kogyo Co., Ltd. (Japan). Poly(vinyl alcohol) (PVA-217, degree of polymerization 1700, degree of hydrolysis 88.5%, Kuraray) was used as a stabilizer. Hydroquinone (HQ) was of analytical grade (Beijing

Chemical Reagents Co.) and was used as an inhibitor to prevent the secondary nucleation in the aqueous phase. Sodium dodecyl sulfate (SDS) was of the grade for biochemical use (Merck), and it is able to associate with PVA in solution and form complex at the interface, making the interfacial tension to decrease and the droplets more stable [22]. Na_2SO_4 was of reagent grade (Beijing Chemical Reagents Co.), and was used to adjust the electrolyte concentration of the aqueous phase. Ethyl alcohol was of commercial grade (Atozi fine Chemicals Co.), and was used to precipitate and wash the particles obtained. All these reagents were used as received. Water was deionized using ion-exchange resins.

2.2. Preparation of microspheres

A standard recipe is shown in Table 1. The mixture of monomer, crosslinking agent, HD and Span 80 dissolving initiator BPO was used as the dispersed phase (monomer phase). Water, where the stabilizer (PVA), surfactant (SDS), electrolyte (Na_2SO_4), and inhibitor (HQ) were dissolved, was used as the continuous phase (aqueous phase). An emulsion was prepared by dispersing the monomer phase into the aqueous phase in a four-neck glass flask equipped with an anchor-type agitator, a condenser, and a nitrogen inlet nozzle. After the emulsion was bubbled with nitrogen for 1 h, the nozzle was lifted up above the surface of the emulsion and the temperature was elevated to 75 °C for polymerization. The polymerization was carried out for 20 h under a nitrogen atmosphere. The polymer particles were washed by water and ethanol four times. The impurities in particles were further extracted by acetone for 24 h, and then the particles were dried in vacuum at room temperature. The yield of particles was calculated by the weight of dried polymer microspheres.

2.3. SEM observation

The diameter and surface features of polymer microspheres after drying were observed by a JSM-6700F scanning electron microscope (SEM) (JEOL, Japan). Microspheres were resuspended in distilled water and the dispersion was dropped on a piece of aluminum foil and dried at ambient atmosphere.

Table 1
Standard recipe for microspheres preparation

Ingredients	Weight (g)
Continuous phase	
PVA	1.0
HQ	**0.01**, 0.02, 0.10
Na_2SO_4	0.02
SDS	0.015
Water	100
Dispersed phase	
BPO	0.08, 0.12, **0.16**, 0.20
Total monomer (St and DVB)	4.0
DVB	**1.0**, 2.0
HD	0.2
Span 80	1.2, **1.6**, 2.0

Bold characters represent the standard recipe.

The sample was placed on a metal stub with double-sided conductive adhesive tape and was coated with a thin gold film under reduced pressure below 5 Pa with a JFC-1600 fine coater (JEOL, Japan).

2.4. Mercury porosimetry measurement

Mercury porosimetry measurements were conducted by an AutoPore IV 9500 mercury porosimetry (Micromeritics, USA). Experiments were conducted in accordance with the protocol given in the AutoPore IV 9500 operator's manual.

2.5. Analysis of particle size distribution

The particle size distribution and the average diameter were measured by laser diffractometry using Mastersizer 2000E (Malvern Instruments Ltd., UK).

3. Results and discussion

3.1. Effects of initiator and inhibitor on the particle yield

The effects of initiator (BPO) and water-soluble inhibitor (HQ) on the particle's yield were investigated at first. The yield of the microspheres increased with the increase of BPO feed amount as shown in Table 2. The particle yields were similar when BPO concentration was between 4% and 5%, therefore, 4% was selected in the standard recipe (Table 1).

HQ was added in the continuous water phase to inhibit the secondary nucleation in the water phase. It has been found that the secondary nucleation often occurred during suspension polymerization [23]. This would decrease the yield of microspheres and cause agglomeration of the particles. The main reason responsible for the secondary nucleation was as follows: the initiator or oligoradical containing initiator fragment in terminal was not hydrophobic enough, and it would escape into the aqueous phase easily during polymerization, resulting in the secondary nucleation in the water phase [23]. The effect of inhibitor (HQ) was also investigated in this new polymerization system, and the results are shown in Table 2. It was

observed in experiments that the aggregation of microspheres was reduced effectively when the amount of HQ increased from 0% to 0.02% (based on the amount of water), and the yield of polymer microspheres was similar (>80%). However, the yield decreased to 24% when the HQ amount was increased further. This was because HQ was slightly soluble in oil phase and restrained the polymerization in droplets. Therefore, 0.01% HQ was selected in the standard recipe as shown in Table 1.

3.2. Effect of surfactant amount on the morphology and size distribution of microspheres

It was observed that the amount of surfactant (Span 80) in oil phase had an important effect on the morphology of microspheres, as shown in Fig. 1. When the amount of Span 80 was below 30% (based on the total amount of ST and DVB), the pore size was not so large. When it was increased to 40%, the pore size became very large, around 500 nm as shown in Fig. 2. However, when the concentration of Span 80 exceeded 50%, the polymer beads were broken. The effects of Span 80 and other factors on the yield and the morphology of polymer microspheres are listed in Table 2. The particle size was mainly in the range of 30 −80 μm, the average particle size is also listed in Table 2. The macroporous structure was homogeneous on the particle surface and in the particle interior as shown in Fig. 3.

It is well known that crosslinking degree affects phase separation during polymerization, as a result the morphology of polymer, pore size distribution, specific surface area, and the mechanical strength of polymer, will be affected [24]. Therefore, it is necessary to investigate the effect of Span 80 amount on the macroporous structure at a higher crosslinking degree. The results are shown in Fig. 4, where the crosslinking degree was increased from 13.8% to 27.5% compared with Fig. 1, and the concentration of surfactant was changed from 40% to 70%. It was shown that the macroporous structure was formed at 60% of Span 80 when the crosslinking degree was 27.5%. When the Span 80 concentration attained to 70%, most of the polymer particles were broken. Therefore, more Span 80 was needed at a higher crosslinking degree for the formation of macropores.

Table 2
Effects of different factors on the yield and morphology of microspheres

Factor	Effect of surfactants											Initiator				Inhibitor			
	Span 80[b]			Span 80[c]				Span 85		PO-500		BPO				HQ[e]			
Amount[a] (%)	30	40	50	40	50	60	70	50	60	30	40	2	3	4	5	0	0.01	0.02	0.1
Morphology[d]	S	M	B	S	M	M	B	S	M	S	M	M	M	M	M	M	M	M	M
Yield (%)	81.9	88.5	77.1	86.5	88.9	95.3	83.7	85.6	87.3	89.2	87.9	65.8	79.9	83.2	85.0	84.0	83.2	80.6	24.0
Average particle size (μm)	41.2	42.6	–	43.5	41.7	42.8	–	43.2	44.3	42.9	41.6								

[a] Based on the total amount of ST and DVB.

[b] The crosslinking degree was 13.8% if it was not specified.

[c] The crosslinking degree was 27.5%.

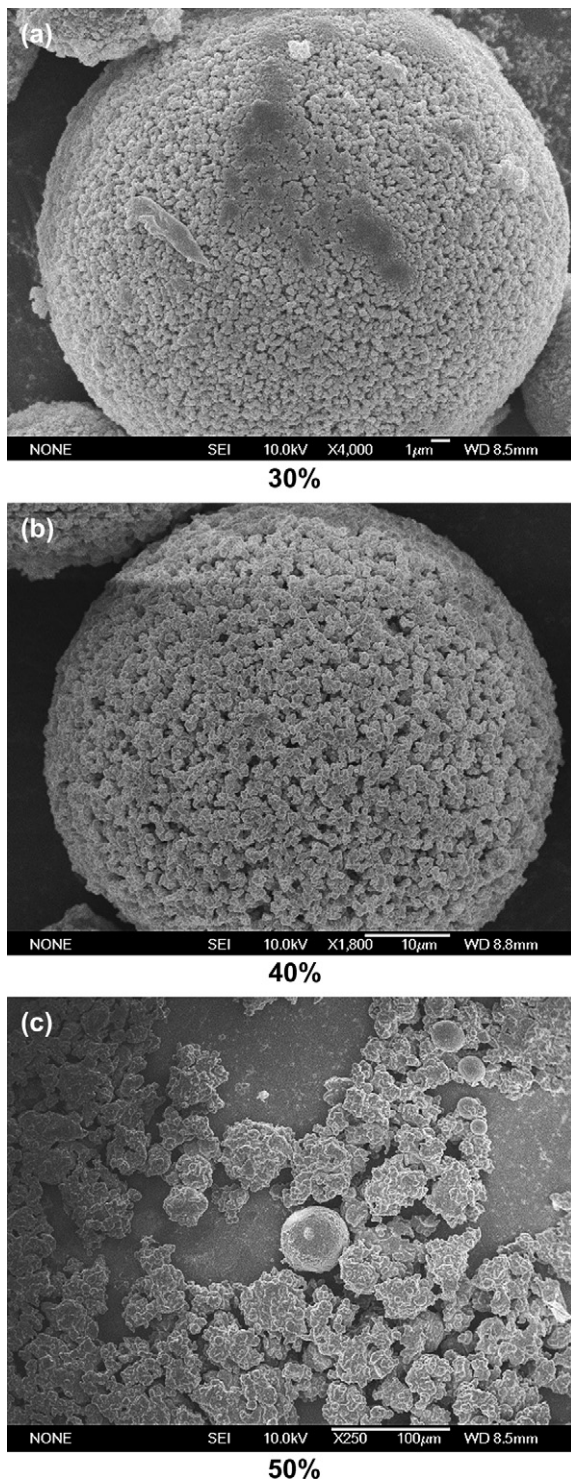[d] S—small pores; M—macroporous; B: broken.

[e] Based on the amount of water.

Fig. 1. Effect of amount of Span 80 on the morphology of microspheres (crosslinking degree was 13.8%).
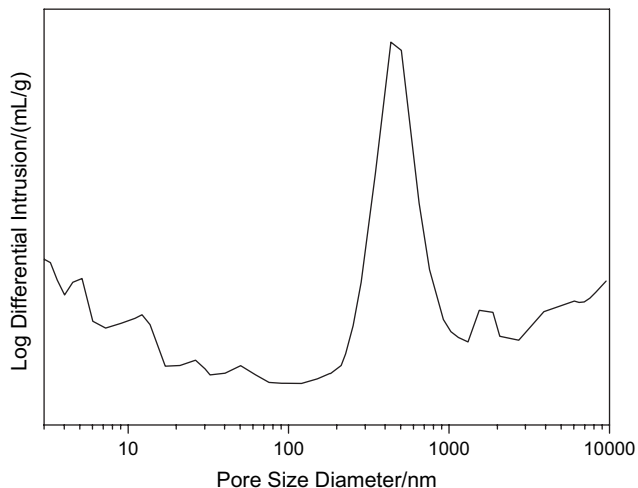

Fig. 2. Pore size distribution curve of the microspheres prepared with 40% Span 80.

polymerization system was the same with the standard recipe of the oil phase (dispersed phase) as shown in Table 1. The SEM photographs of the polymer prepared by bulk polymerization are shown in Fig. 5. The polymer was macroporous, and it suggested that phase separation occurred between the polymer phase and the surfactant phase during polymerization.

It is well known that when the concentration of surfactant in solution is increased to critical micelle concentration (*cmc*), numbers of surfactant molecules gather to form a micelle [25]. Reverse micelles are formed in non-aqueous solution above *cmc* just as the case in this study. When the surfactant concentration increases further above *cmc*, the number of reverse micelles increases and different types of reverse micelles will be formed as shown in Fig. 6. Spherical micelles are formed when the surfactant concentration is close to *cmc* (Fig. 6a) and the aggregation number (average number of surfactant molecules forming a micelle) is about 30–40. When the concentration of surfactant is 10 times higher than *cmc*, the aggregation number increases and a type of clubbed

3.3. Formation mechanism of macropores

As shown in Figs. 1 and 4, the formation of macropores was closely related with the surfactant concentration in the oil phase (monomer phase). In order to investigate the formation mechanism of macropores, we carried out bulk polymerization in absence of external water phase at first. The bulk
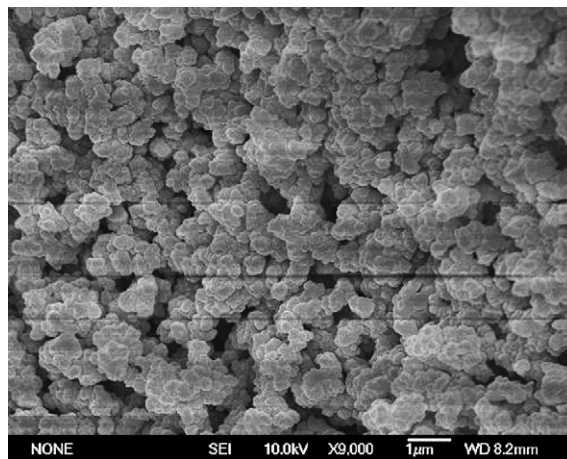

Fig. 3. SEM photograph showing the interior structure of the particles prepared with 40% Span 80.
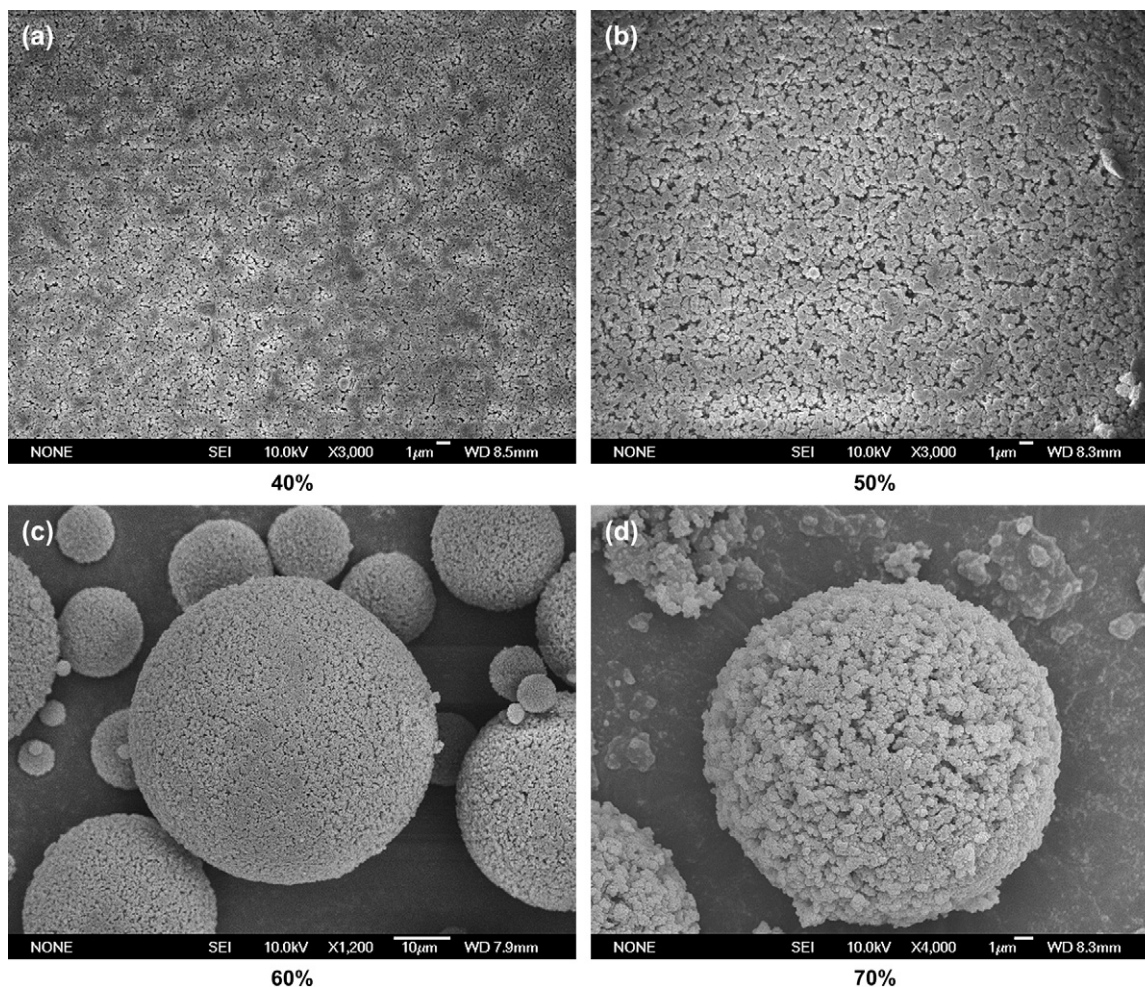
Fig. 4. Effect of amount of Span 80 on the morphology of microspheres at a higher crosslinking degree (27.5%).

reverse micelles is formed (Fig. 6b). The clubbed reverse micelles aggregate to a hexagonal cluster when the surfactant concentration increases further (Fig. 6c) and the lamellar reverse micelles are obtained at a rather high concentration of surfactant (Fig. 6d) [25]. Since the amount of Span 80 was 40% based on the total amount of ST and DVB in this study, the reverse micelle aggregates should be formed. Therefore, the surfactant phase was composed of a large amount of reverse micelle aggregates which were favorable for the formation of macropores.

Next, let's consider what happened when the oil phase was dispersed in the external aqueous phase. It is known that a reverse micelle has the ability of absorbing water [26,27]. Because there were a lot of reverse micelles existed in the oil droplets, they could absorb water from the external aqueous phase. This was responsible for the formation and enlargement of the macropores. The results of mercury porosimetry measurement of the polymer prepared by bulk polymerization and the microspheres prepared under the same condition but with the existence of the external aqueous phase were compared, and the data are shown in Table 3. It was evident that the results of total pore volume, total pore surface area, average pore size, and porosity increased apparently for sample B, suggesting the important role of the water absorbed. The

water-absorbing effect of reverse micelles was also confirmed by the comparison between the two samples prepared under different amounts of Span 80. When the amount of Span 80 increased from 30% to 40% (1.2–1.6 g), the peak value of pore size increased from 50 nm to 500 nm in the distribution curves of pore size (Fig. 7). The results of mercury porosimetry measurement are shown in Table 3. The total pore volume and the porosity increased to 2.65 mL/g and 83.6%, respectively. The apparent increase of the porosity and pore size could not only be achieved by the increase of the amount of surfactant, but also was mainly resulted from the absorbed water. Therefore, it can be concluded that the formation of macropores was related to two factors, the high concentration of reverse micelles existed in the oil phase and the water absorbed by the reverse micelles from the external aqueous phase as shown in Fig. 8. Candau and co-workers [28] found in the study of microemulsion polymerization that oil–water bicontinuous structure would form if the ratio of water/oil/surfactant was suitable. Since the perfect closed inclusion–exclusion curve was obtained in mercury porosimetry measurement of the sample in Fig. 1b which showed that the particles had good permeability, we assumed that whether the bicontinuous structure was possibly formed in this reaction system, however, it needs to be investigated further.
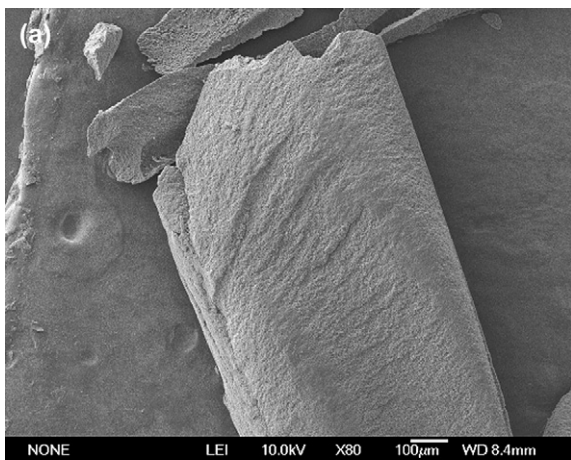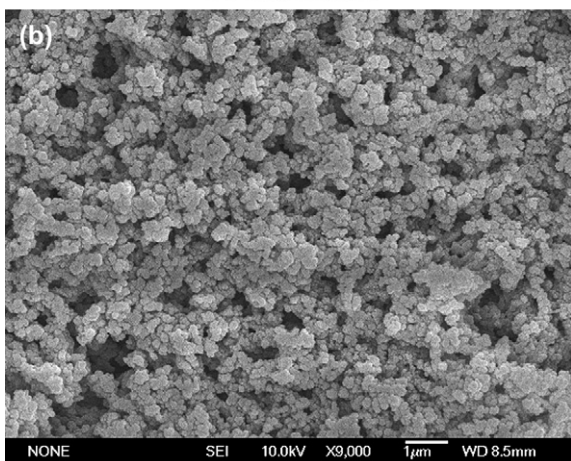
Fig. 5. SEM photographs of polymer prepared by bulk polymerization.

Table 3
Porosimetry measurement results of the microspheres prepared with different amounts of Span 80 and of the bulk polymer

Sample	Amount of Span 80 (%)	Total pore volume (mL/g)	Total pore surface area (m^2/g)	Average pore size (nm)	Porosity (%)
A	40	0.47	59.1	31.8	34.9
B	40	2.65	203.8	52.1	83.6
C	30	1.62	126.9	51.0	65.5

A: bulk polymer; B: microspheres prepared with the same recipe of bulk polymerization but with the existence of the outer aqueous phase.
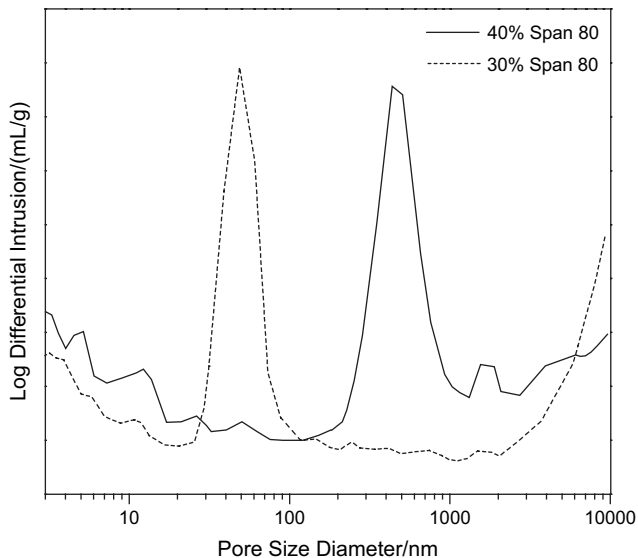

Fig. 7. Pore size distribution curves of microspheres prepared with different amounts of Span 80.

3.4. Effects of different surfactants

The effects of various surfactants (Span 85, PO-500) on the morphology of the particles were investigated. The effect of

Span 85 was similar with that of Span 80, that is, the pore size increased with the increase of Span 85 concentration. However, more Span 85 was needed in order to get similar pore size. For example, in order to get the microspheres
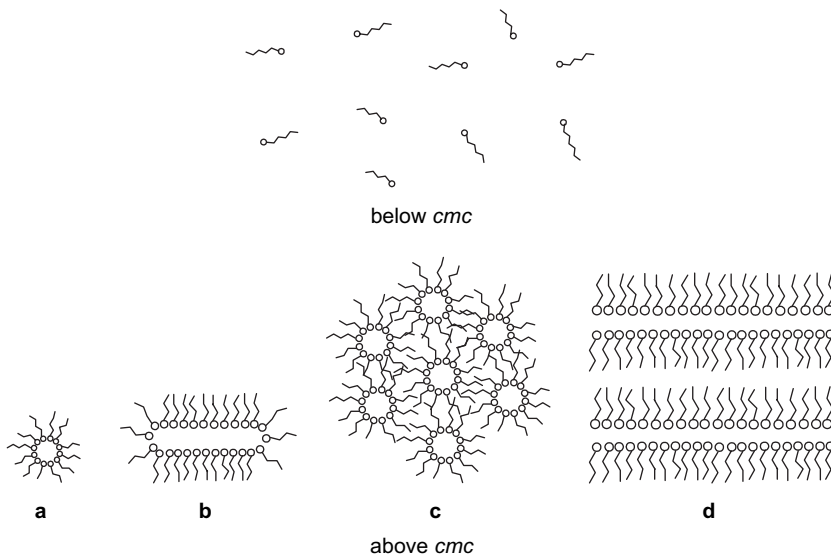

Fig. 6. Different types of reverse micelles formed with increase of surfactant concentration.
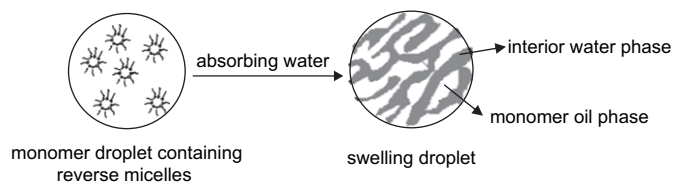
Fig. 8. Schematic image showing the formation of macropores in monomer droplet.

with large pore size showed in Fig. 9b, the necessary concentration of Span 85 was 60%, higher than the case of Span 80 (40%). This phenomenon was related to the hydrophobic−hydrophilic property of the surfactants. The HLB (Hydrophile−Lipophile Balance) of Span 85 is 1.8, it is lower than that of Span 80 (HLB 4.3). This implied that Span 85 was more hydrophobic, and its ability of absorbing water was weaker than that of Span 80 under the same concentration, therefore, relatively smaller pores were formed when using Span 85.

In the case of PO-500, the pore size also increased with the increase of concentration, and the pore size was about 1 μm

when the PO-500 concentration increased to 40%, but the microspheres were not spherical as shown in Fig. 10. This was probably because PO-500 (HLB 4.9) was more hydrophilic than Span 80 and the reverse micelles tended to move near the surface of the droplet. Thus, a lot of water was absorbed near the surface, so the surface was destroyed easily during polymerization.

4. Conclusion

Macroporous polymer microspheres were successfully prepared by adding a higher concentration of surfactant in oil phase. The recipe was optimized, the effects of the amount and type of surfactant on the morphology of microspheres were investigated, and the formation mechanism of macropores was also discussed. When the crosslinking degree was 13.8%, and the concentration of Span 80 was 40%, microspheres with pore size of about 500 nm were obtained. The pore size increased with the increase of Span 80 concentration in oil phase. The effect of Span 85 was similar with that of Span 80. However, more Span 85 was needed to get similar
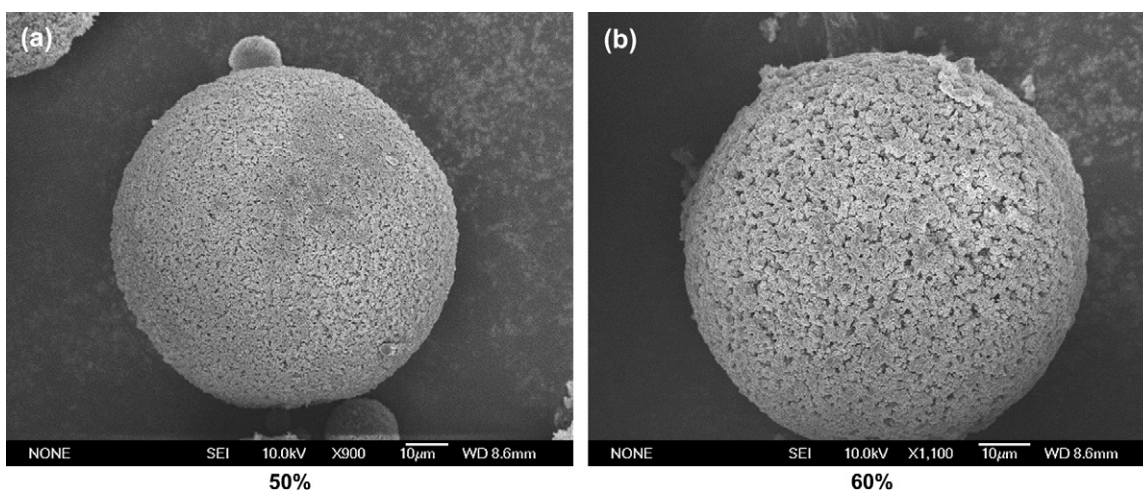

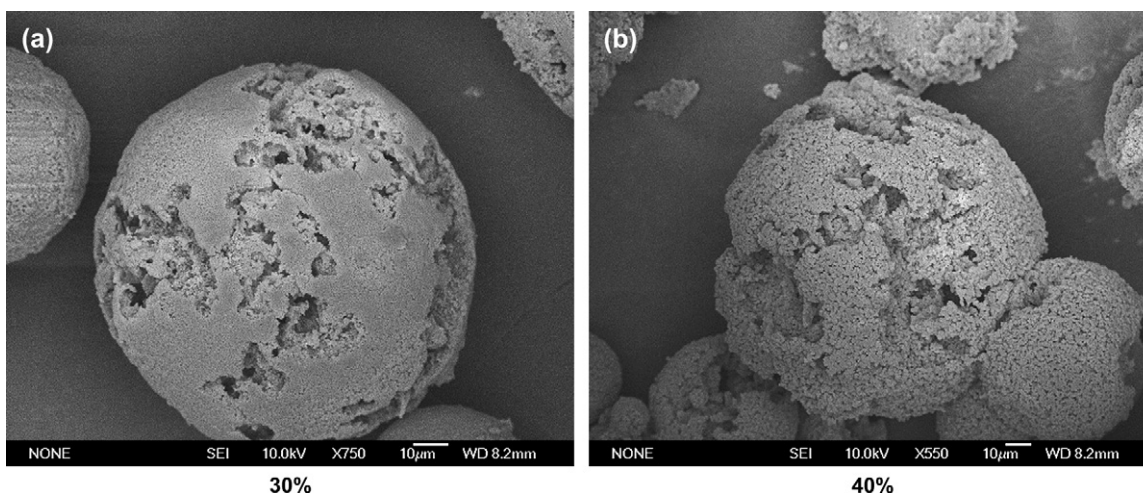Fig. 9. Microspheres prepared with different amounts of Span 85.

Fig. 10. Microspheres prepared with different amounts of PO-500.

pore size. The microspheres prepared by PO-500 were also macroporous, but the microspheres were not spherical due to the higher hydrophilic property of PO-500. The crosslinking degree also affected the structure, and more Span 80 was needed at higher crosslinking degree to get big pores.

The high concentration of surfactant in the oil phase was important for the formation of pores. After the oil phase was dispersed in the external aqueous phase, the reverse micelles in the oil droplets could absorb water from the aqueous phase. The absorbed water formed pores after polymerization.

This study provided a convenient method to prepare macroporous polymer particles for biomolecules separation, and they also can be used as microcarriers in fungal and animal cell cultures as well as in drug delivery after further modification of the surface.

Acknowledgment

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