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Synthesis of macroporous poly(glycidyl methacrylate) microspheres by surfactant reverse micelles swelling method

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Abstract

Macroporous poly(glycidyl methacrylate-ethylene dimethacrylate) [P(GMA-EDMA)] particles with pore size around 140–200 nm and poly(glycidyl methacrylate-divinylbenzene) [P(GMA-DVB)] particles with pore size of 450 nm were prepared by the surfactant reverse micelles swelling method. This method was similar with the conventional suspension polymerization, and the difference was that higher concentration of surfactant was added in the oil phase. When the oil phase containing surfactant was dispersed in aqueous phase, the surfactant reverse micelles in the oil droplets absorbed water from continuous phase. After polymerization, the large pores were formed by the absorbed water. The effects of the amount and type of surfactants, the cooperation of surfactant and diluents, and the crosslinking agent on the morphology of microspheres were investigated. This study provided a new and simple method to prepare microspheres with the pores of several hundred nanometers, which overcame the disadvantages found in the conventional preparation methods of macroporous microspheres.

Keywords: Macroporous polymers; Suspension polymerization; Surfactant reverse micelles; Phase separation

1. Introduction

Porous polymer particles are widely used in chromatographic separation due to its rigidity compared with soft beads such as agarose beads. With the development of biotechnology, more and more bioproducts with large molecular size such as protein, vaccine, and DNA plasmid need to be separated and purified. However, the pore size of polymer particles prepared by conventional method (utilizing phase separation between diluent and crosslinked polymer) usually is in the range of 100–500 Å, and it needs longer time to separate 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 of preparation methods of microspheres with large pore size have been developed. The first common method is that soluble polymer is used as porogen. Horák [3] prepared glycidyl

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methacrylate (GMA) particles with pore size of 750 nm by using poly(methyl methacrylate) (PMMA) or polystyrene (PST) as porogen. However, it is not easy to wash the polymer porogen out of the polymeric particles. The second one is nano-particles agglomeration method. The polymer particles prepared by this method had two sets of pores: through pores (600–800 nm) and diffusive pores (80–150 nm). These microspheres named perfusion particles have been successfully used in the separation of biomolecules [4–7]. However, the preparation of perfusion particles was complicated. The particles were built from porons to produce small poron clusters, and then to aggregate the clusters, and then to agglomerate the aggregates to form particles of macroscopic size, e.g., greater than 40 μm. The through pores and the diffusive pores were formed by the interstices between the small particles and their clusters. Therefore, the pores were difficult to control, and the reproducibility needs to be improved. Furthermore, this method was only used in ST-DVB system, and other monomer systems have not been reported. The third one is polyHIPE (high internal phase emulsion polymer) method developed by Barby and his coworkers in 1985 [8]. In their study, the monolith polymer was prepared. HIPE is the emulsion with the volume concentration of 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 too large, from one to tens of micrometer. In 1996, Li and Benson [9–12] developed the polyHIPE method by dispersing HIPE further in external aqueous phase and prepared spherical polyHIPE beads. The forth one is that inorganic particles is used as porogen. Sun et al. [13–16] prepared P(GMA-EDMA) microspheres with calcium carbonate granules and organic diluents as mixed porogen. The particles contained two sets of pores, micropores (smaller than 100 nm) formed by the organic diluents and superpores (500–7300 nm) formed by calcium carbonate. They also developed the double emulsion method, and prepared microspheres with pore size of 20–100 nm (micropores) and 300–4000 nm (superpores) [17], but the stability of the double emulsion should be carefully maintained.

In our previous study, we developed a new method of surfactant reverse micelles swelling method to prepare poly(styrene-divinylbenzene) [P(ST-DVB)] microspheres with pore size of 500 nm [18,19]. The formation mechanism of the macropores was also investigated. The advantage of this method is that the preparation process is very easy. The oil phase contained monomer (ST), crosslinking agent (DVB), surfactant, diluent and initiator (benzoyl peroxide). Due to the high surfactant concentration (40% of the total amount of ST and DVB), a lot of reverse micelles were formed in the oil phase. After the oil phase was dispersed in the aqueous phase, the reverse micelles in the oil droplets could absorb water from the aqueous phase and formed bicontinuous structure. The water phase in the oil droplets formed pores after polymerization. This method was convenient to prepare macroporous polymer particles. P(ST-DVB) microspheres are highly hydrophobic and there is no functional group on it, which will limit its application. In contrast, PGMA microspheres process the epoxy group which can be modified by a number of ligands under moderate conditions. Therefore, the preparation of macroporous PGMA particles by the surfactant reverse micelles swelling method was investigated in this study. In fact, we have ever used similar recipe with the case of P(ST-DVB) system to prepare macroporous P(GMA-EDMA) microspheres. However, it was found that the dispersity of the microspheres was poor, and the pore size of the P(GMA-EDMA) particles could not be as large as 400–500 nm, which is required for the separation of biomolecules with high molecular weight, and can be used in perfusion chromatography [1]. In order to obtain PGMA particles with large pores and good dispersity, we investigated the preparation conditions and the results of PGMA system, and compared them with P(ST-DVB) system in this study. It was found that the different combination of surfactant and organic diluents must be employed, compared with ST system.

2. Experiment

2.1. Materials

Glycidyl methacrylate (GMA) (>97.0%, Fluka), ethylene dimethacrylate (EDMA) (98%, Acros) and divinylbenzene (DVB) (commercial grade, Beijing Chemical Reagents Co.) were distilled under a vacuum to remove the inhibitor.

Benzoyl peroxide (BPO) (25% water, Beijing Chemical Reagents Co.) was used as an initiator. Isooctane (IO) (Wulian Chemical Co., Shanghai), 4-methyl-2-pentanol (MP) (Chinese Medicine Co.,

Beijing) were analysis grade and used as diluents. Sorbitan monooleate (Span 80) (Bangde Technology and Trade Co., Beijing) and sorbitan trioleate (Span 85) (Shanghai Chemical Reagent Co.) were reagent grade. Pentaoleic acid hexaglycerin ester (PO-500) was provided by Sakamoto Yakuhin Kogyo Co., Ltd. (Japan). Sorbitan sesquioleate (Arlacel 83) was provided by Sigma. Polyoxyethylene sorbitan monooleate (Tween 80) was provided by Kishida Chemical Co. (Osaka, Japan). Poly(vinyl alcohol) (PVA-217, degree of polymerization 1700, degree of hydrolysis 88.5%, Kuraray) was used as a stabilizer. Hydroquinone (HQ) was analytical grade (Beijing Chemical Reagents Co.) and was used as inhibitors to prevent the secondary nucleation in the aqueous phase. Sodium dodecyl sulfate (SDS) was of the grade for biochemical use (Merck). Na_2SO_4 was reagent grade (Beijing Chemical Reagents Co.), and was used to adjust the electrolyte concentration of the aqueous phase. Ethyl alcohol was a 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, diluents, and 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 flask equipped with an anchor-type agitator, a condenser, and a nitrogen inlet nozzle, the stirring rate of agitator was 160 rpm. 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 obtained were washed by water and ethanol, respectively. Then the particles were extracted by acetone for 24 h and dried under a vacuum at room temperature. The yield of particles was calculated by the weight of dried polymer microspheres as shown in Table 2. The main particle size was in the range of 30–100 μm. The average particle size and other structure characters of the samples are also shown in Table 2.

Table 1
Standard recipe for microspheres preparation

Ingredients	Weight/g
Continuous phase	
PVA	1.0
HQ	0.01
Na_2SO_4	0.02
SDS	0.015
Water	100
Dispersed phase	
BPO	0.16
GMA	**2.0**, 3.0
EDMA, DVB	1.0, **2.0**
Diluents	0.2, **0.3**, 1.0
Surfactant	0.5, **1.0**, 1.6, 2.0

Bold characters represent the standard recipe.

2.3. SEM observation

The diameter and surface features of the polymer microspheres after drying were observed by a JSM-6700F scanning electron microscope (SEM) (JEOL, Japan). Microspheres were re-suspended in distilled

Table 2
Effects of surfactants and diluents on the yield and morphology of microspheres

Factor	Surfactant[a]					Diluent[b]		
	Span 85	Alarcel 83	Span 80	PO-500	Span 80/Tween 80 (1:1)	IO	IO/MP (1:1)	MP
Concentration (%)	12.5	25	25	12.5	25	7.5	7.5	7.5
Yield (%)	81.3	85.2	87.0	82.0	79.4	77.5	78.8	79.0
Average particle diameter (μm)	–	47.8	52.3	48.5	38.5	51.4	48.5	50.8
Surface area (m^2/g)	–	156.1	152.2	169.1	–	48.8	85.3	124
Average pore size (nm)	–	93	94	73	–	122	92	47
Porosity (%)	–	89.8	88.4	75.6	–	60.7	75.2	89.2

[a] Diluent IO/MP = 1:1, 5%.
[b] Surfactant Span 80 = 25%, diluent = 7.5%; other condition is shown in Table 1.

water and the dispersion was dropped on a piece of aluminum foil and dried at ambient atmosphere. 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. Effect of different surfactants on the morphology of microspheres

It is well known that when the concentration of surfactant increases to critical micelle concentration (cmc), surfactant molecules will get together and form micelles. Reverse micelles are formed in non-aqueous solution just as the case of this study. When the surfactant concentration increased above cmc, different types of micelles would be formed in the oil phase, such as spherical, clubbed, hexagonal, and lamellar [20]. The type and number of reverse micelles would relate to the property of surfactant (especially the hydrophile–lyophile balance, HLB) added in the oil phase. When the oil phase containing reverse micelles was dispersed in continuous water phase to form oil droplets, the reverse micelles in the oil droplet would absorb water from the water phase, and the water-absorbing capacity of surfactant also depended on its HLB value. Therefore, the type and amount of the surfactant were important to the morphology and pore size of the microspheres. In order to obtain macroporous P(GMA-EDMA) particles, the effect of the concentration of surfactant in the oil phase was investigated at first, and all the samples shown in Fig. 1 were prepared at the maximum amount of surfactant, i.e. the particles would break if the amount of surfactant increased above it. The other factors such as the amount of the crosslinking agent and the initiator were fixed as shown in Table 1, based on the previous optimized results of the ST system [19].

As shown in Fig. 1, the sample prepared with Span 85 was microporous (~10 nm) although some locations on the particles showed macroporous (larger than 200 nm). It was because that Span 85 was too hydrophobic (HLB 1.8), only a small amount of water was absorbed into GMA-EDMA droplet. The microspheres prepared by Alarcel 83 (HLB 3.7) or Span 80 (HLB 4.3) were spherical, and their average pore sizes were 93 nm and 94 nm, respectively. The pore size of the particles prepared by PO-500 was 73 nm. The average pore sizes mentioned above were measured by mercury intrusion porosimetry. Though the HLB value of PO-500 is 4.9, the pore size of the sample prepared with PO-500 was smaller than the samples prepared with Alarcel 83 or Span 80. It was thought that this was related with the molecular structure of PO-500. It is a polyglyceryl fatty acid ester (pentaoleic acid hexaglycerin ester), however, Alarcel 83 and Span 80 are sorbitan aliphatic acid ester. The sample prepared with the mixture of Span 80 and Tween 80 (mass ratio 1:1) had a cavity in the center of the particle. The main reason was that the hydrophilicity of the mixture of Span 80 and Tween 80 was very high (HLB 9.65) and it absorbed a great deal of water into the oil droplets to form a large cavity. According to these results, the surfactant with HLB near 4 was suitable for P(GMA-EDMA) system. This result was consistent with that of P(ST-DVB) system. However, the maximal concentration of the surfactant which can be added in the GMA-EDMA oil phase was 25% (based on the total amount of monomer and crosslinker), less than that of ST-DVB system (40%). Corresponding to this, the water absorbed by the GMA-EDMA system was much less than ST-DVB system. Therefore, the pore size of the P(GMA-EDMA) particles was smaller than that of P(ST-DVB) particles. These results were related with the hydrophobicity of the two systems and the compatibility of the surfactants in them. For example, Span 80 is an oil-soluble surfactant, and its HLB is 4.3 which hydrophobicity is predominant over hydrophilicity. ST-DVB system is more hydrophobic than GMA-EDMA system. Therefore, more Span 80 can dissolve in ST-DVB system, then more water can be absorbed inside, forming larger pores.

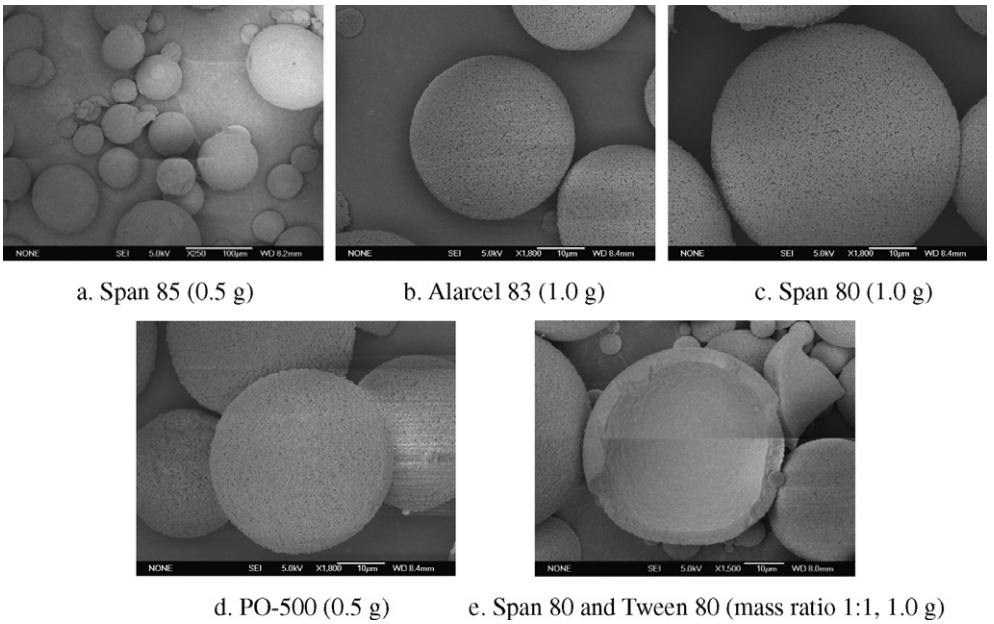


Fig. 1. Effect of different surfactants on the morphology of microspheres (Diluent: IO:MP = 1:1, 5%, other condition is shown in Table 1).

3.2. Effect of diluents on the morphology of microspheres

In the initial study, hydrophobic diluent (hexadecane) was added in the oil phase in order to retard the monomer diffusing into the aqueous phase [21] just as ST-DVB system. However, it was found that the common hydrophobic diluents such as hexadecane and heptane caused serious break-up and agglomeration of the particles although its concentration was only 5%. This was because that hexadecane and heptane are highly hydrophobic diluents, and the phase separation between the diluents and PGMA was much stronger than that in the case of PST, resulting in break-up or agglomeration of the particles. We referred to the experimental results of conventional microporous PGMA particles by Wang et al. [22], and chose isooctane, 4-methyl-2-pentanol and their mixture as diluent. As shown in Fig. 2 and Table 2, the dispersity of the particles prepared by 4-methyl-2-pentanol was well, but the average pore size was smaller (47 nm), compared with that prepared by isooctane (122 nm) or isooctane/4-methyl-2-pentanol mixture (92 nm). The pore size became larger with the increase of isooctane amount. Because isooctane is a poor solvent for PGMA, it was in favor of the formation of big pores. However, some particles in the sample prepared with isooctane agglomerated. Taking into account of the dispersity and the pore size, the suitable mass ratio of isooctane and 4-methyl-2-pentanol was selected as 1:1. These results showed that

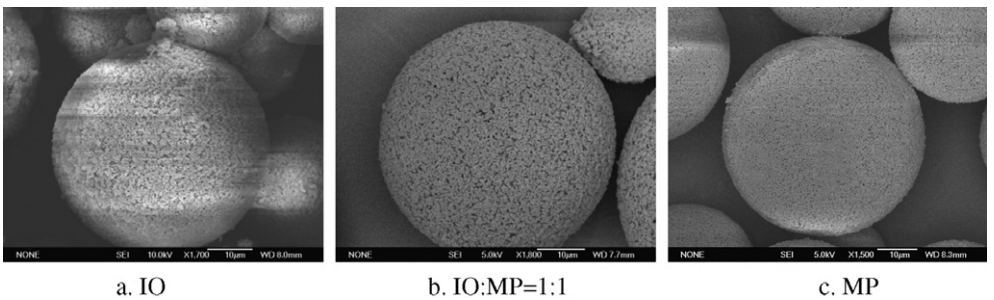
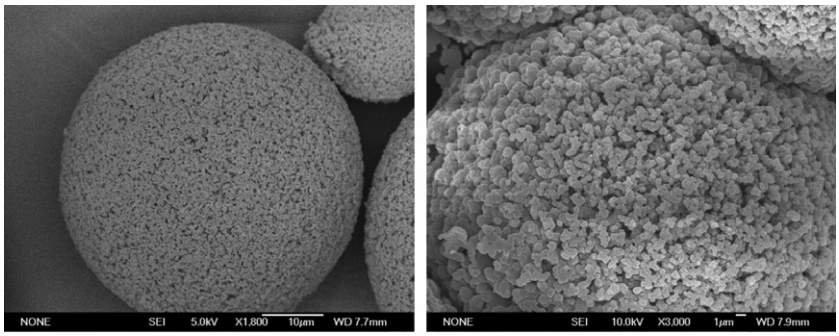


Fig. 2. Effect of different solvents on the morphology of microspheres (surfactant:Span 80:25%, diluent 7.5%, other condition is shown in Table 1).



a.7.5% b.10%

Fig. 3. Effect of the amount of diluent on the morphology of microspheres (surfactant:Span 80:25%, diluent IO:MP = 1:1, other condition is shown in Table 1).

the diluents were important to the structure of the particles. In the subsequent experiment, the effect of the diluents concentration was studied. As shown in Fig. 3, the diluent concentration should be below 7.5%, otherwise the particles would agglomerate. The pore size distribution curve of the optimized sample (Fig. 3a) is shown in Fig. 4, and the main pore size was in the range of 140–200 nm and the biggest pore size was near 900 nm. Other structure properties of the samples are summarized in Table 2.

3.3. Effect of the crosslinking agent on the morphology of microspheres

Though the concentration and the type of the surfactants and the diluents in P(GMA-EDMA) system were investigated, we did not obtain the particles with the pore size large enough as we expected

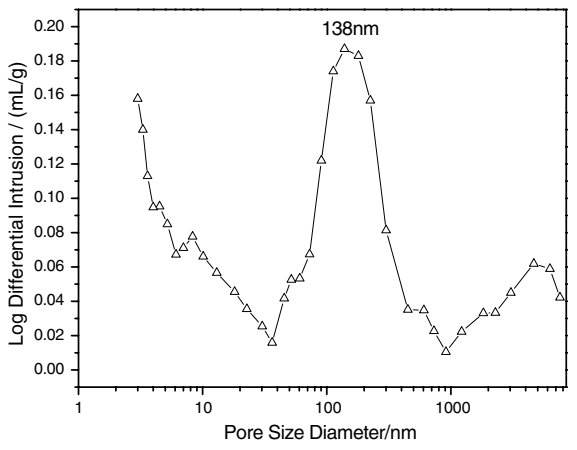


Fig. 4. Pore size distribution curve of the optimized P(GMA-EDMA) particles (the sample of Fig. 3a).

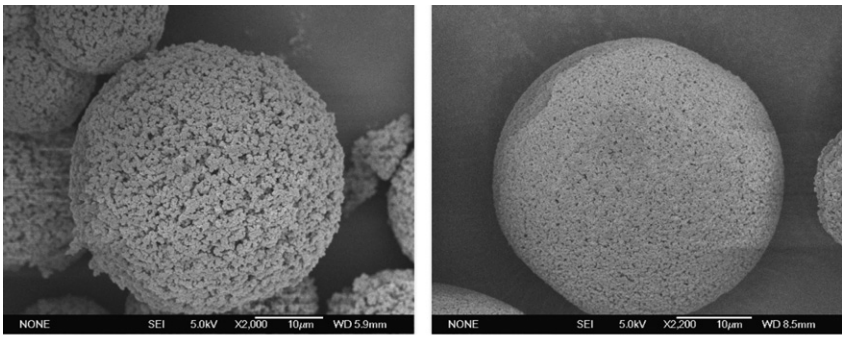
(larger than 400–500 nm). Compared with the results of ST-DVB system, it was thought that the main reason probably was the lower hydrophobicity of GMA-EDMA system, which weakened the phase separation between the water-surfactant phase and the polymer phase. Therefore, when more hydrophobic crosslinker DVB was used, it brought in changes. As shown in Table 3, the maximal amount of Span 80 and Alarcel 83 can be increased 40% respectively for GMA-DVB system. At the same time, the optimum ratio of isooctane and 4-methyl-2-pentanol was 3:2 in GMA-DVB system, compared with 1:1 for GMA-EDMA system; and the concentration of the diluents can be increased to 25%. That is, GMA-DVB system could contain more surfactant and higher hydrophobic diluents without break or aggregation of the particles. This was possibly related to the higher hydrophobicity of GMA-DVB system as described above. Macroporous P(GMA-DVB) particles with pore size of 450 nm were prepared under this condition (Fig. 5a).

Since DVB with 55% purity was used in this study, the real concentration of crosslinking agent in P(GMA-DVB) system was 27.5%. However, that

Table 3
Comparison between GMA-DVB and GMA-EDMA systems

Crosslinking agent	Amount of surfactant[a] (wt%)		Amount of diluents[a] (wt%)	Ratio of IO and MP[b] (wt/wt)
	Span 80	Alarcel 83		
EDMA	25	25	7.5	1:1
DVB	40	40	25.0	3:2

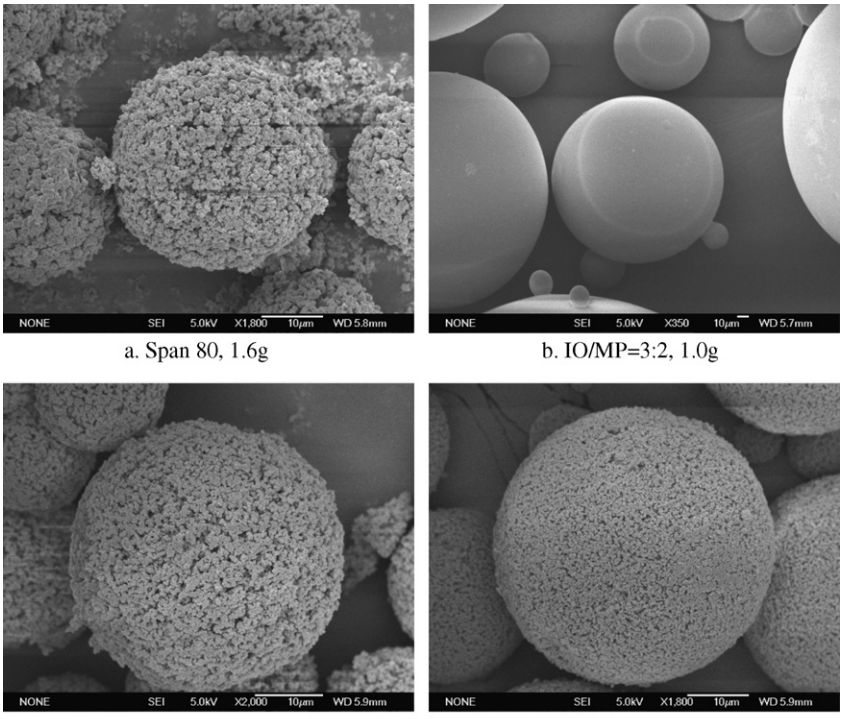
[a] The maximum amount can be added in the system.
[b] The maximum ratio of IO and MP in the system.



a. P(GMA-DVB) particles b. P(GMA-EDMA) particles

Fig. 5. SEM photos of P(GMA-DVB) particles and P(GMA-EDMA) particles with low concentration of crosslinking agent. (a) Concentration of crosslinking agent = 27.5%; surfactant:Span 80 = 40%; diluent: IO:MP = 1:1, 25%. (b) Concentration of crosslinking agent = 25%; surfactant:Span 80 = 15%; diluent: IO:MP = 1:1, 7.5%; other condition is shown in Table 1.

of P(GMA-EDMA) system was 50% (Table 1). It also can be thought that the formation of the large pores in the P(GMA-DVB) particle was possibly related with the low concentration of crosslinking agent. Therefore, the preparation of P(GMA-EGMA) particles with lower concentration of crosslinking agent (25%) was also carried out. It was found that the pores of the particles were evidently small. This was because the maximum concentration of surfactant was only 15% in this system, and the amount of the absorbed water was less correspondingly (Fig. 5b). From this result, it was confirmed that the formation of the larger pores in P(GMA-DVB) particle was mainly ascribed to the



a. Span 80, 1.6g b. IO/MP=3:2, 1.0g

c. Span 80, 1.6g, IO/MP=3:2, 1.0g d. Span 80, 1.6g, IO/MP=1:1, 1.0g

Fig. 6. Effect of the co-operation of surfactant and diluents on the morphology of particles (crosslinking agent DVB, concentration of crosslinking agent 27.5%, other condition is shown in Table 1).

higher hydrophobicity of P(GMA-DVB) system which can contain more surfactant and hydrophobic diluents as described above.

As shown in Fig. 6, the cooperation of the diluents and surfactant had an essential effect on the structure of particle. The pore size of the microspheres prepared only with Span 80 was very large, but some particles were broken (Fig. 6a). On the other hand, the pore size of those prepared only with the diluents was rather small (Fig. 6b). The diluent was contained in the part of monomer oil phase in swelling droplet, and the pores formed by phase separation between diluent and polymer were in accordance with the rules of phase separation. Therefore, the pores formed by diluent should be in the range of several to tens of nanometers, and the pores formed by the absorbed water could be in the range of several hundreds of nanometers. The particles prepared with the mixture of diluents and surfactant possessed the pore size between them. The mixture of isooctane and 4-methyl-2-pentanol with mass ratio of 3:2 (Fig. 6c) made the pores larger than those with mass ratio of 1:1 (Fig. 6d), because more isooctane (poor solvent of PGMA) was added. Their pore size distribution curves confirmed the difference of the pore size distribution of the two samples, one with the main pore size of 180 nm, another with that of 450 nm, as shown in Fig. 7. The intrusion–extrusion curve of the sample with pore size of 450 nm is shown in Fig. 8, it was closed which showed the sample had good permeability [23]. The inside of the microspheres was shown in Fig. 9, which indicated that it was also macroporous in the particles. Other

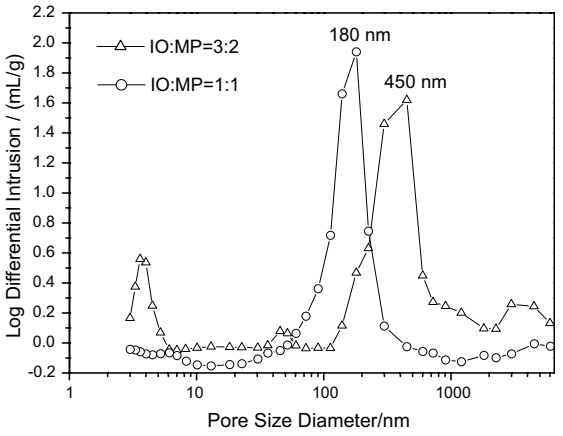


Fig. 7. Effect of different ratio of the mixed solvents on the pore size distribution (the samples of Fig. 6c and d).

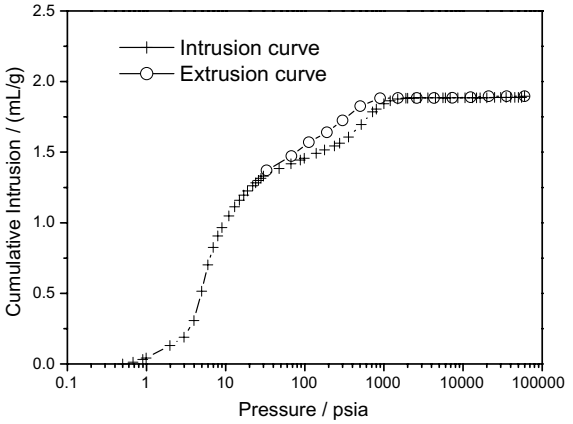


Fig. 8. Intrusion–extrusion curve of the sample with pore size of 450 nm (the sample of Fig. 6c).

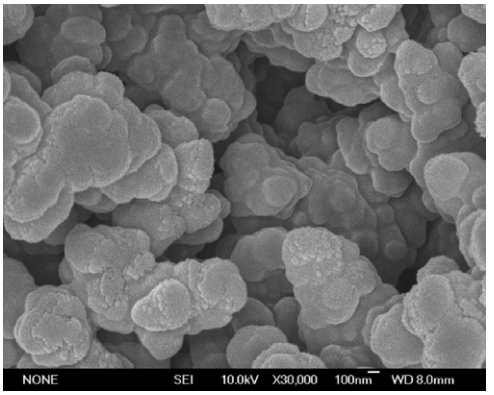


Fig. 9. Inside of the sample with pore size of 450 nm (the sample of Fig. 6c).

structure properties of the above two samples are shown in Table 4, and compared with the P(ST-DVB) microspheres with pores distributed around 500 nm which was prepared in a previous study [19]. It was shown that the surface areas of the

Table 4
Structure properties of the samples

Sample	Total pore volume (mL/g)	Total pore surface area (m^2/g)	Average pore size (nm)	Porosity (%)
A	3.06	89.5	139	72.7
B	3.10	81.8	150	75.3
C	2.65	203.8	52	83.6

A: P(GMA-DVB) particles, Span 80 = 40%, IO/MP = 1:1, 25% (based on the total amount of monomer and crosslinker).
B: P(GMA-DVB) particles, Span 80 = 40%, IO/MP = 3:2, 25%.
C: P(ST-DVB) particles, Span 80 = 40%, HD = 5%.

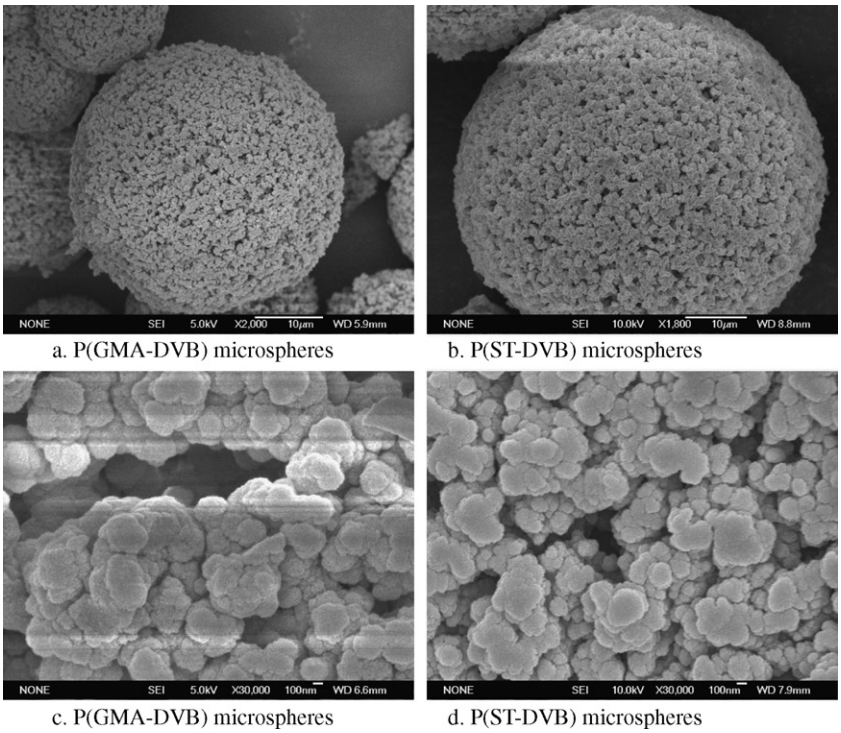


Fig. 10. Comparison of P(GMA-DVB) microspheres and P(ST-DVB) microspheres.

two PGMA samples were less than that of P(ST-DVB) sample. Okay et al. thought that the size of the primary particles determines the surface area of microspheres. Smaller the primary particles are, higher the surface area is [24]. From SEM photos of Fig. 10c and d, it can be known that the primary particles formed in P(ST-DVB) microspheres were smaller than that of P(GMA-EDMA) microspheres. This was why the surface area of the PGMA sample was less than that of P(ST-DVB) sample. The interstice of these small primary particles or their agglomerates in P(ST-DVB) microspheres formed a lot of small pores (smaller than 20 nm), which made the average pore size decrease to 52.1 nm, although most pores were distributed around 500 nm, as shown in Fig. 11.

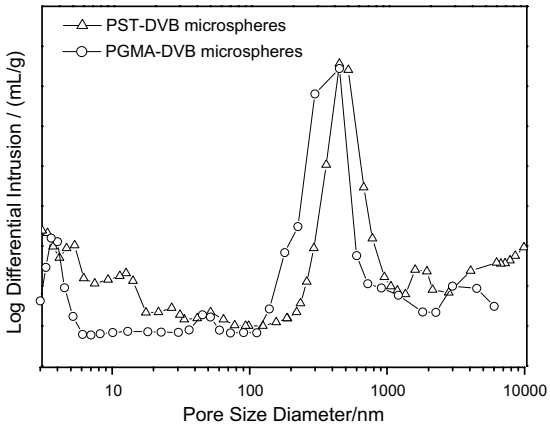


Fig. 11. Pore size distributions of P(GMA-DVB) sample and P(ST-DVB) sample.

4. Conclusion

In this study, macroporous PGMA beads were prepared by the surfactant reverse micelles swelling method. The surfactant with HLB near 4 such as Span 80 and Alarcel 83 was suitable for preparing macroporous PGMA particles. Compared with ST-DVB system where the large pores of 500 nm can be easily obtained just by adjusting the surfactant concentration in the oil phase, both surfactant and diluents were important for GMA system. By optimizing the preparation condition, the mixture of isooctane and 4-methyl-2-pentanol was selected as diluent in GMA system. The optimum ratio and concentration of them was 1:1 and 7.5% in GMA-EDMA system, and the particles with pore size of 140–200 nm were obtained. When using DVB as a crosslinking agent instead of EDMA,

higher concentration of surfactant and diluents can be added in the oil phase without break of the particles, resulting in the increase of the pore size in P(GMA-DVB) system. This was related with higher hydrophobicity of GMA-DVB system. The necessity of co-operation of the surfactant and diluents was confirmed in GMA-DVB system. The surfactant was important for the formation of larger pores, and the diluents were related with the formation of smaller pores. The microspheres with pore size of 450 nm were obtained in the GMA-DVB system. The macroporous PGMA particles will have great potentials in separation of biomolecules, enzyme immobilization and so on, because it can be modified further.

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