Preparation and Characterization of Large Porous Poly(HEMA-*co*-EDMA) Microspheres with Narrow Size Distribution by Modified Membrane Emulsification Method

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ABSTRACT: Porous poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) [p(HEMA-*co*-EDMA)] microspheres have important applications in chromatography. In this study, p(HEMA-*co*-EDMA) microspheres with controllable size were successfully prepared by modified membrane emulsification technique. The CV value of the obtained microspheres was as low as 14.4%. An oil phase mainly composed of toluene was permeated through a membrane with uniform pores into an aqueous phase to form fairly uniform seed emulsion. The secondary emulsion composed of HEMA and EDMA was prepared by a homogenizer. Then, the two emulsions were mixed together. HEMA and EDMA diffused into the aqueous phase, and then were absorbed by the seed droplets. The swollen droplets were further polymerized to prepare monodisperse porous microspheres. It was found that HEMA/EDMA ratio and toluene/monomer ratio showed a significant influence on swelling degree, diameter, and morphology of the obtained microspheres. The swelling degree of the microspheres increased with the increase of toluene/monomer ratio, whereas the average diameter increased with decrease of the ratio. Microspheres with controllable size (28–73 μm) and porosity were obtained, by varying HEMA/EDMA ratios and toluene/monomer ratios. It can be expected that the p(HEMA-*co*-EDMA) microspheres with controllable size and porosity will provide many advantages in their applications. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 105: 1632–1641, 2007

Key words: HEMA; membrane emulsification; particle size distribution; phase separation; swelling

INTRODUCTION

In recent years, the demand for hydrophilic media has rapidly increased with the development of biotechnology. Porous poly(2-hydroxyethyl methacrylate) (pHEMA) microspheres are an excellent media for the applications in chromatographic separation and immobilization of bioactive molecules: (1) pHEMA possesses abundant hydroxyl groups on its surface, so it can be modified to meet the requirement of different chromatographic applications, such as the stationary phases in ion exchange chromatography, hydrophobic interaction chromatography, as well as affinity chromatography,[1,2] (2) it was reported that pHEMA had excellent biocompatibility because of its hydrophilic surface, so it can be used in the immobilization of bioactive molecules.[3,4] As supports for the separation, it is important that microspheres possess uniform size and controllable porosity. Furthermore, it is reported that large microspheres with narrow size distribution showed many advantages, such as low performance pressure, high flow rate, high separation resolution, and thereby low costs.[5]

Large microspheres with several 10 μm can be prepared by suspension polymerization; however, the microspheres obtained by this method show a broad size distribution. Various seeded polymerization techniques were developed to overcome the drawback of broad size distribution of conventional suspension polymerization. Ugelstad et al.[6,7] proposed an activated swelling method to prepare large monodispersed polymer miscrospheres. The basic idea for this reputable invention was a two-step polymerization procedure. At the first step, the seed microspheres with submicron-size were prepared by emulsion polymerization. Then, the small polymer particles were dispersed in water; they would absorb oil-soluble organic compounds and monomers to swell to droplets with micron-size. Finally, the second-step polymerization was carried out. Various

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large monodispersed microspheres can be prepared by the seeded polymerization, and some of them have been used as chromatographic media. His pioneering work has been extended to many other interesting areas such as preparation of monodispersed paramagnetic beads and applications in immunology, cellular biology, microbiology, molecular biology, medical diagnostics, and DNA technology. However, it took a long time to let seed microspheres with submicron-size swell to several 10 μm sizes. Okubo et al.[8] developed a dynamic swelling method of dispersion polymerization, using the seed microspheres of 1.6 μm to prepare monodispersed polystyrene (PST) microspheres with diameter as large as 7.7 μm. Because of the dynamic technique, the microspheres with micron-size can be obtained more easily. However, this method also required two polymerization steps. Omi and Ma et al.[9–11] have developed a membrane emulsification technique followed by a suspension polymerization to prepare fairly uniform microspheres. The porous glass membrane with uniform pores consists of hydrophilic substance SiO_2-Al_2O_3. In this method, hydrophobic oil phase containing monomer and initiator was permeated through the uniform pores into the aqueous phase under controlled pressure to form uniform droplets. After the emulsification process, the emulsion was polymerized. Fairly uniform PST microspheres with several 10 μm were successfully prepared by a single-step emulsification polymerization process. The coefficient of variation (CV value) that characterizes the size distributions of the microspheres was smaller than 15%.

The necessary condition for preparation of uniform droplets by membrane emulsification technique is that the oil phase should be hydrophobic. This is because that the membrane is composed hydrophilic substance that is easily wetted by the hydrophilic monomer such as HEMA, generating polydispersed droplets. To obtain droplets with narrow size distribution by membrane emulsification method, the interfacial tension between the membrane and dispersion phase should be high enough. However, HEMA is a hydrophilic monomer, which may wet both hydrophilic glass membrane and other hydrophobic membranes, so the direct membrane emulsification method was not suitable for the preparation of pHEMA microspheres. Therefore, it is a big challenge to prepare pHEMA microspheres with narrow size distribution by utilizing a special membrane emulsification technique.

In this study, p(HEMA-*co*-EDMA) microspheres with several 10 μm and narrow size distribution were prepared by combining membrane emulsification technique and swelling process of droplets. The main difference between this method and Ugelstad's technique was that the seed in this study was composed of not polymer, but hydrophobic diluent. The detailed procedure was as follows: seed droplets with narrow size distribution mainly composed of toluene (used as diluent) were prepared by the membrane emulsification technique; secondary droplets composed of HEMA and EDMA were prepared by a homogenizer, then, the two emulsions were mixed together. Because HEMA and EDMA in the secondary droplets showed higher solubility in water than toluene, they diffused into the aqueous phase, and then were rapidly absorbed by the seed droplets to form swollen droplets. The final swollen droplets were still uniform because the size distribution of the seed droplets was narrow. Finally, the swollen droplets were polymerized and pHEMA microspheres with narrow size distribution were obtained. Toluene and unreacted monomers were removed and pores were left in the micropheres during the process of washing and extraction. The advantage of the modified membrane emulsification method developed in this study was that not only the size distributions of microspheres were uniform, but also the size and porosity of the microspheres were easily controlled by varying the (seed droplets)/(secondary droplets) ratio. In this study, the effects of toluene/monomer and HEMA/EDMA ratios on the size and size distribution, surface morphology, and porosity were investigated.

EXPERIMENTAL

Materials

HEMA was purchased from Tianjin Institute of Chemical Reagents (Tianjin, China) and distilled under vacuum to remove the inhibitor. EDMA was purchased from Sigma-Aldrich (Germany) and purified by the same method. Benzoyl peroxide (BPO) and Toluene were purchased from Beijing Chemical Reagents Company (Beijing, China) and used as an initiator and diluent. Poly(vinyl alcohol) (PVA-217, degree of polymerization 1700, degree of hydrolysis 88.5%) was kindly provided by Kuraray (Japan) and used as a stabilizer. Sodium dodecyl sulfate (SDS) was purchased from Merck (Germany) and used as a surfactant. Hexadecane (HD) was purchased from Wako Pure Chemical Industry, LTD and used as a hydrophobic substance to stabilize droplets of the seed emulsion. Hydroquinone (HQ) was provided by Beijing chemical reagents company and used as an inhibitor to prevent the secondary nucleation of droplets. Water was produced via reverse osmosis water system (Millipore, USA). All other chemicals of analytical grade were purchased from Beijing chemical reagents company and used as received.

Preparation of p(HEMA-*co*-EDMA) microspheres

The preparation of p(HEMA-*co*-EDMA) microspheres was divided into four steps: preparation of the seed

TABLE I
Preparative Conditions of Seed Emulsion and Secondary Emulsion

	Secondary emulsion[a] dispersion phase (g)		Seed emulsion[b] dispersion phase (g)		
Run no	HEMA	EDMA	Toluene	BPO	HD
A1	8	4	3	0.050	0.3
A2	8	4	4.5	0.075	0.45
A3	8	4	6	0.100	0.6
A4	8	4	12	0.200	1.2
B1	4	4	3	0.050	0.3
B2	4	4	6	0.100	0.6[c]
B3	4	4	8	0.133	0.8
C1	4	8	4	0.067	0.4
C2	4	8	6	0.100	0.6
C3	4	8	12	0.200	1.2
D1	4	12	3	0.050	0.3
D2	4	12	6	0.100	0.6
D3	4	12	12	0.200	1.2
D4	4	12	16	0.267	1.6

[a] The composition of the continuous phase in the secondary emulsion for all 14 runs except run B2 was the same (g): water 100; SDS 0.02; $NaSO_4$ 0.3; HQ 0.06.
[b] The composition of the continuous phase in the seed emulsion for all 14 runs was the same (g): water 100; PVA 1.00; SDS 0.04; $NaSO_4$ 0.04.
[c] The composition of the continuous phase in the secondary emulsion for run B2 was (g): water 100; SDS 0.02; $NaSO_4$ 0.3; HQ (seven different addition: 0, 0.03, 0.06, 0.09, 0.15, 0.3, 0.6).

emulsion, preparation of the secondary emulsion, the swelling of seed droplets to form swollen droplets, and the polymerization of the swollen droplets.

The detailed recipe for preparation of the two emulsions is shown in Table I. The seed emulsion was prepared by membrane emulsification technique. The schematic diagram of the apparatus is shown in Figure 1. The dimension of tubular porous glass membrane was 2 cm (L) × 1 cm (Ø), and the average pore size of the membrane was 5.2 μm in diameter. The dispersion phase (oil phase) containing toluene, BPO, and HD was stored in a Teflon tank, which was connected to a nitrogen gas inlet, and the continuous phase (containing water, PVA, SDS etc.) was stirred gently with a magnet bar at 160 rpm. The dispersion phase was permeated through the pores of the membrane by the pressure of nitrogen gas into the continuous phase to form droplets with narrow size distribution, which were used as seeds and stabilized by stabilizer (PVA) and surfactant (SDS).

The secondary emulsion was prepared by a RJT018 emulsification homogenizer (Beijing He Mo Mechano-Electronics Institute, China) with a rotation rate of 10,000 rpm for 1 min. The dispersion phase was composed of HEMA and EDMA. The continuous phase was composed of water, SDS, Na_2SO_4, and HQ. Na_2SO_4 was used as electrolyte to decrease the solubility of the monomer mixture in the aqueous phase.[12]

The seed emulsion and secondary emulsion were mixed under a mild stirring of 160 rpm. The latter would be absorbed by the former by the diffusion process through the aqueous phase. That is, HEMA and EDMA in the secondary emulsion diffused into the aqueous phase because of their slight solubility

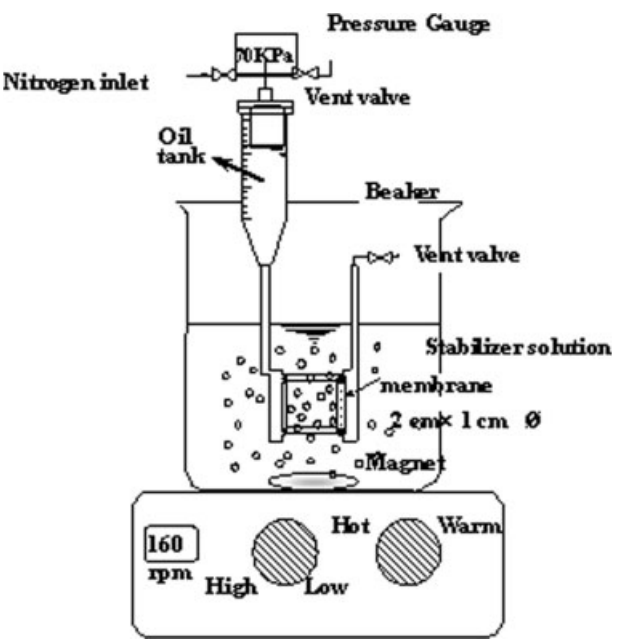

Figure 1 Schematic diagram of a miniature apparatus for membrane emulsification.

in the water, then were absorbed by the seed droplets to form swollen droplets. The swelling course was monitored by an optical microscope, until the secondary droplets disappeared completely. After the swelling process, 100 g (2 wt %) PVA solution was added to adjust the total PVA concentration of the final emulsion to 1 wt %.

The final emulsion was transferred to a four-neck glass separator flask in a thermostatic water bath. The emulsion was flushed by bubbling nitrogen gas for 1 h. Then, the temperature was increased to 75°C to carry out polymerization for 6 h. After polymerization, the microspheres were recovered by filtration, and then, were washed with water and ethanol repeatedly until the filtrate was transparent. By this process, the secondary new particles of submicrons can be removed except those adhered on the large particles. After that, a Soxhlet Extraction Apparatus was used to extract the remaining diluent and unreacted monomers inside the microspheres (circulation of 12 h, 78°C). Finally, the microspheres were dried in a vacuum oven at 65°C overnight.

Characterization of p(HEMA-*co*-EDMA) microspheres

Yield of the microspheres

Yield of the microspheres was calculated by the following eq. (1):

$$\text{Yield} = \frac{W_d}{W_i} \tag{1}$$

where W_d is the weight of dry microspheres after purification, W_i is the total weight of monomers initially added.

Size and size distribution of the microspheres

The dry p(HEMA-*co*-EDMA) microspheres were dispersed in water and measured by Laser Diffraction using a Coulter LS230 (Coulter Electronics, USA). The size of wet microspheres was expressed as volume-mean diameter, and the CV (coefficient variation) value, defined as eq. (2), was used to characterize the size distribution.

$$CV = \frac{\left(\sum_{i=1}^{n} \frac{(d_i - \overline{d})^2}{N}\right)^{1/2}}{\overline{d}} \tag{2}$$

where d_i is the diameter of the ith diameter, $\overline{d}$ is the volume-mean diameter and N is the total number of microspheres measured.

Surface morphology of the microspheres

The surface morphology of p(HEMA-*co*-EDMA) microspheres was observed by a JSM-6700F (JEOL, Japan) scanning electron microscope (SEM). The specimens for SEM were prepared by mounting sample on metal stubs with double-sided conductive adhesive tape and coating a thin platinum film (about 6 nm in thickness) on sample under reduced pressure below 5 Pa with a JFC-1600 fine coater (JEOL, Japan).

Pore properties of the microspheres

An Autosorb-1 automatic surface area and pore size analyzer (Quantachrome, USA) was used to determine the pore properties of the microspheres. In the vacuum sorption system, ultra pure nitrogen gas and liquid nitrogen gas were used as adsorbate and coolant. Specific surface area was determined by the multipoint Brunauer–Emmett–Teller (BET) method. Total pore volume and average pore diameter were determined by Barrett–Joyner–Halenda (BJH) method.[13]

Swelling behaviors of the microspheres

The swelling degree (S_d) of the microspheres was defined as the weight ratio of the wet to the dry microspheres as eq. (3).

$$S_d = \frac{W_w}{W_d} \tag{3}$$

where W_w is the weight of wet microspheres, W_d is that of dry microspheres. W_w was measured as follows: the dry microspheres were immersed into water in a centrifugal tube and shaken on a vibrator for well swelling (37°C ± 0.5°C, 120 rpm and 24 h). Then, the water was removed by centrifugation (4000 rpm, 15 min) and the wet microspheres were weighed.

RESULTS AND DISCUSSION

Optical microscopic (OM) observation of the swelling process of seed droplets

Droplets before, during (1 and 20 min) and after swelling course (60 min) were observed with optical microscope. The typical optical micrographs showing the variation of the droplets size during the swelling process are shown in Figure 2. It can be seen that the secondary droplets disappeared and size of the final droplets became larger after the swelling course. This course proceeded quickly because of the great difference of stability between the two emulsions and different water solubility of organic compounds in the two emulsions.

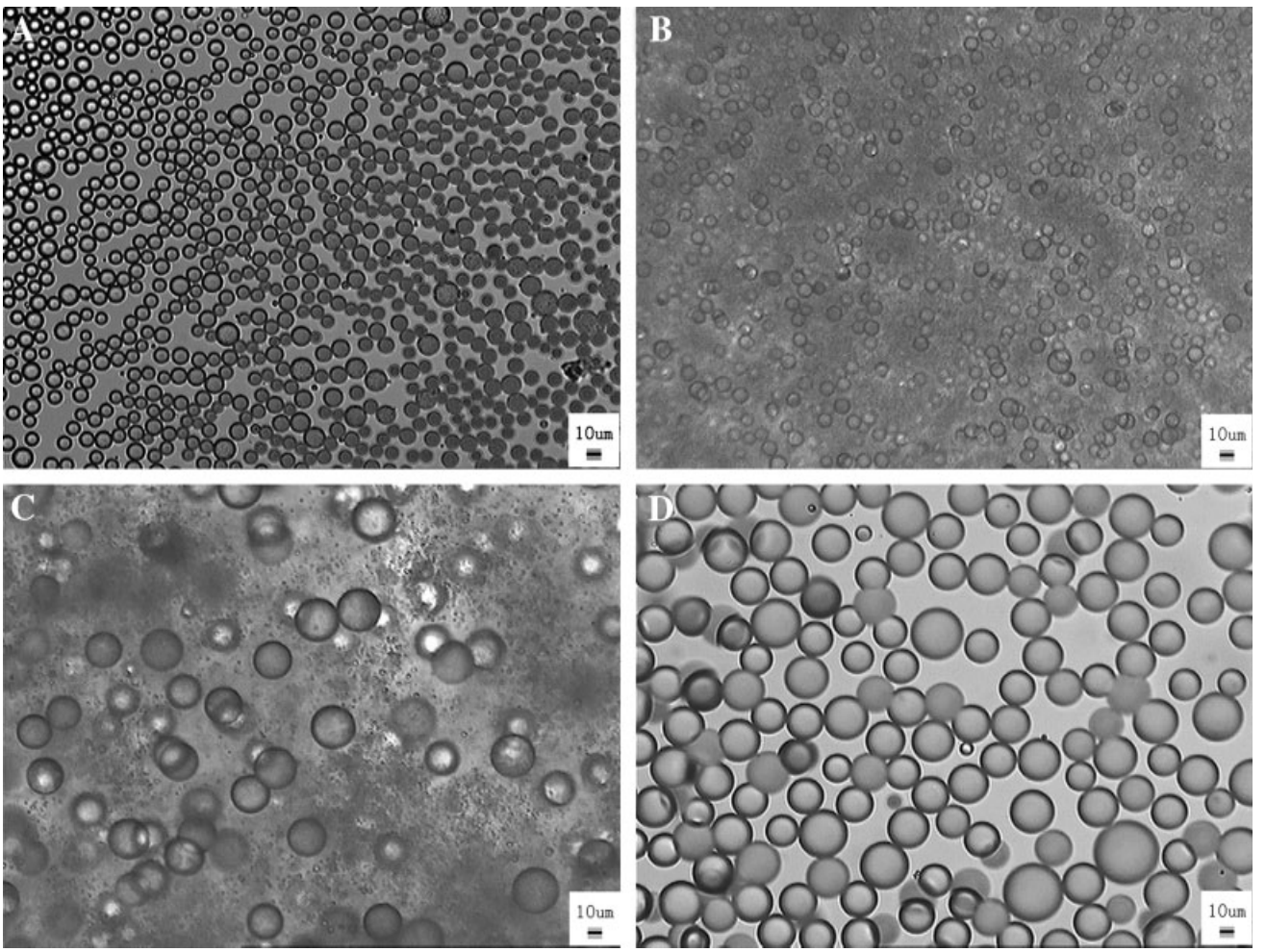

Figure 2 The typical optical micrographs during the swelling process of droplets. (A) is seed droplets; (B–D) are swollen droplets at 1, 20, and 60 min after adding secondary emulsion, respectviely.

The secondary droplet was quite unstable. Ostwald ripening (diffusional degradation) occurred easily. That is, monomer in smaller droplet diffused into the aqueous phase because it showed a slight solubility in aqueous phase, and then was absorbed by a larger seed droplet. At last, the smaller droplet became smaller and smaller, and larger droplet got larger and larger.

On the other hand, the seed droplet was stable and above Ostwald ripening was retarded, mainly because of two factors: (1) HD addition; and (2) uniform size of the droplets.

It has been found that HD can retard the diffusion of monomer into the aqueous phase because it showed low solubility in water phase (solubility: 5.9×10^{-6} g/L).[14] Ugelstad et al. compared the stability of droplet with and without adding HD, they found that the average droplet size did not change apparently in the former case during the storage of the emulsion, while that became larger and larger with the storage time in the latter case.[14] Furthermore, because the diameters of seed droplets were uniform, Ostwald ripening can be avoided because every droplet showed similar specific surface area.

In fact, HD was added because it played another role in the swelling process of droplets. Ugelstad found that adding a small amount hydrophobe in the seed promoted the swelling rate, so that, micron-sized microspheres can be obtained by this technique (famous activated swelling method).[7]

Because of above reasons, the monomer in the smaller secondary droplets diffused into aqueous phase quickly, and then absorbed by stable seed droplets, to form swollen droplets with narrow size distribution.

Effect of HQ concentration on the yield of the microspheres

It has been presented that HQ was an efficient inhibitor to prevent the secondary nucleation in the aqueous phase.[15] In this study, the amount of HQ addition was optimized. The HQ addition was varied from 0 to 0.6 g based on the water (100 g) in the

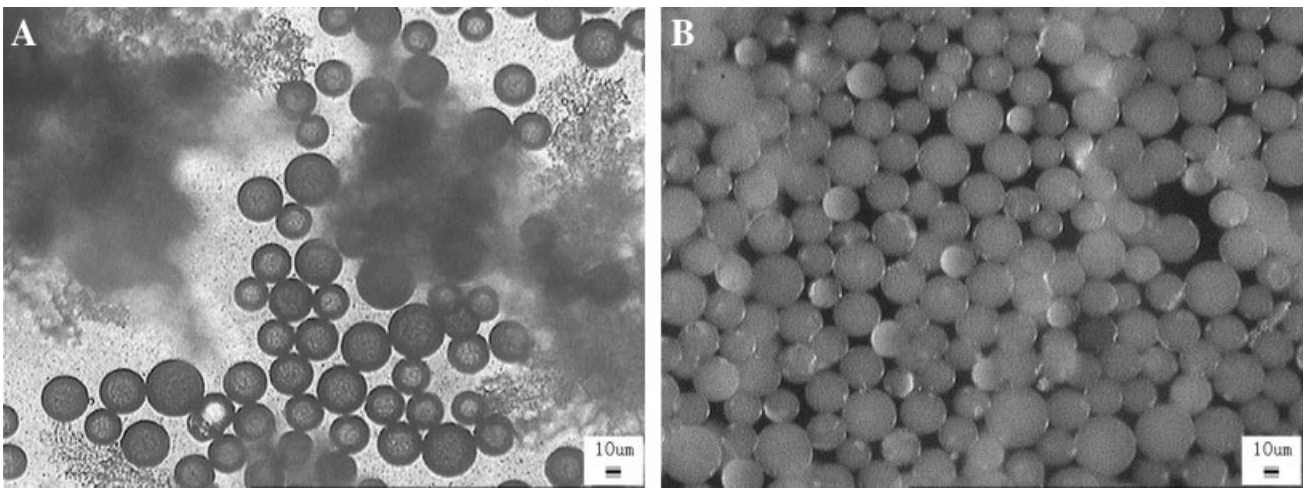

Figure 3 Optical micrographs of p(HEMA-*co*-EDMA) microspheres. HQ concentration based on the water in the secondary emulsion: (A) 0 and (B) 0.06%.

secondary emulsion. Optical micrographs and size distributions of p(HEMA-*co*-EDMA) microspheres prepared without HQ addition and with 0.06% HQ addition are shown in Figures 3 and 4, respectively. Figure 3(A) showed that a lot of small particles aggregated with large p(HEMA-*co*-EDMA) microspheres when no HQ was added, whereas the small particles disappeared when 0.06 wt % HQ was added as shown in Figure 3(B). By measuring the size distributions of the microspheres with Laser Diffraction, the CV value was 26.1% when HQ concentration was 0.06 wt %, whereas it increased to 87.8% when no HQ was added. This was because a large amount of secondary new particles were formed when HQ was not used. These particles acted as a linker between the large microspheres, which resulted in the formation of aggregates. In the initial stage of polymerization process, BPO initiated polymerization of HEMA and EDMA in the droplets to form oligomeric radicals, a part of oligomeric radicals escaped out of oil droplets into aqueous phase, and continued to react with monomers that dissolved in the aqueous phase to form the secondary new nuclei. As a result, a large number of small new particles with submicron-size were obtained if HQ was not added. However, when HQ was used, the secondary nucleation was prevented effectively. This phenomenon was observed even in the case of styrene, because styrene also shows a slight solubility in the aqueous phase, the secondary nucleation has been determined quantitatively for styrene system.[11]

As expected, HQ concentration in the continuous phase also affected the yield of microspheres (B2 series in Table I). The results are shown in Figure 5. It was evident that the yield of the microspheres decreased with increase of HQ concentration. The

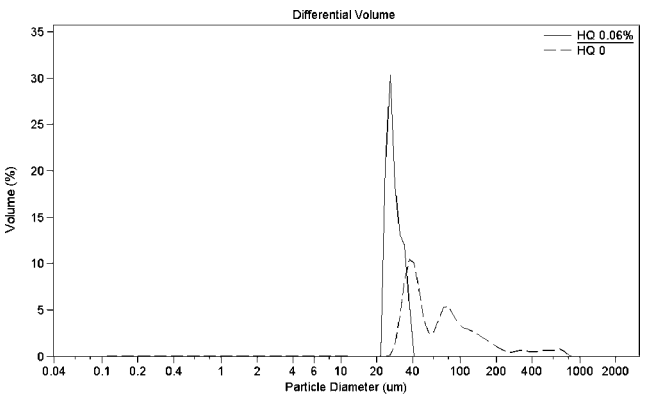

Figure 4 Size distributions of p(HEMA-*co*-EDMA) microspheres prepared without HQ addition and with 0.06% HQ addition.

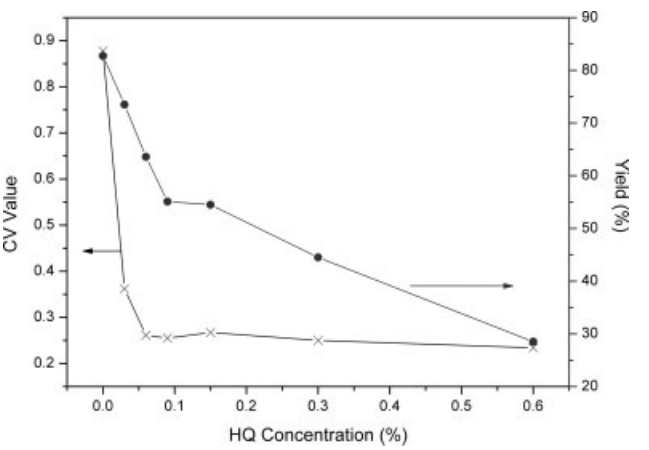

Figure 5 Effect of HQ concentration on size distributions and the yield of p(HEMA-*co*-EDMA) microspheres (based on run B2).

maximum yield attained to 83% when HQ was not added. However, when HQ concentration was under 0.06 wt %, the CV value became higher with the decrease of HQ concentration although the yield of the microspheres was at high level. This was because the secondary nucleation was not effectively prevented as mentioned above. On the contrary, the yield of the microspheres was under 45% when HQ concentration exceeded 0.3 wt %, because that a part of HQ diffused into the inside of the droplets due to its slight solubility in the oil phase, and inhibited the polymerization there. In the range of HQ from 0.06 to 0.6 wt %, the secondary nucleation could be effectively prevented with the CV value of around 25%, and the yield of the microspheres could be maintained at a reasonable value. Therefore, the appropriate concentration of HQ in the secondary emulsion was fixed at 0.06 wt % for high yield and low CV value of microspheres. The CV value could be further improved by decreasing the swelling ratio, which will be described in Size and size distribution of p(HEMA-*co*-EDMA) microspheres.

Size and size distribution of p(HEMA-*co*-EDMA) microspheres

In our previous studies,[10,11,15] membrane emulsification technique has exhibited advantages in the preparation of emulsion with narrow size distribution. The pHEMA porous microspheres with narrow size distribution can be synthesized through the swelling process of seed droplets and subsequent suspension polymerization in this study. Table II shows the size and size distributions of wet p(HEMA-*co*-EDMA) microspheres. It was known that the volume-mean diameter of the microspheres decreased with the increase of toluene content when the ratio of HEMA/EDMA was constant. This result can be understood as follows: the diameter of the seed droplets prepared by a membrane with the same pore size was the same, thus, the diameter of the swollen droplets increased with the ratio of monomer mixture/toluene.

Among the 14 runs, the largest volume-mean diameter of the microspheres attained to 73.5 μm (run D1) with a CV value of 30.5% when the ratios of HEMA/EDMA and toluene/(monomer mixture) were 4/12 (g/g) and 3/16 (g/g), respectively. However, the lowest CV value was 14.4% (run D4) with the average diameter of 28.2 μm when the two ratios were 4/12 (g/g) and 16/16 (g/g), respectively. This was because the seed droplets still showed a size distribution (CV value: 5%); larger droplets with higher surface area could absorb more monomers and resulted in broader size distributions when the swelling ratio increased.

From Table II, it was noticed that there was difference in the average particle diameter when the same amount of toluene was introduced. As aforementioned, the initial seed droplets were stable and less coalescence or breakup of droplets occurred during the swelling process, that is to say, the number of seed particles was constant. Accordingly, there were three factors that caused the diameter difference of wet microspheres. (1) The difference in the ratio of (monomer mixture)/toluene (i.e., secondary droplets/seed droplets). As aforementioned, the higher the ratio of (monomer mixture)/toluene was, the larger the average diameter became. (2) The difference in the crosslinker concentration. The solubility of HEMA in water was higher than that of EDMA, as a result, there was more monomer dissolved in

TABLE II
Size Distribution and Porosity of the p(HEMA-co-EDMA) Microspheres

Run	Volume-mean diameter[a] (μm)	CV (%)	Total pore volume (ml/g)	Average pore diameter (nm)	Specific surface area (m^2/g)
A1	62.3	33.9	0.1999	9.97	80.2
A2	46.4	17.3	0.3179	18.75	67.8
A3	–[b]	–	–	–	–
A4	–	–	–	–	–
B1	49.9	27.0	0.2567	12.30	83.5
B2	43.8	26.1	0.4055	12.57	129.0
B3	–	–	–	–	–
C1	57.4	31.2	0.0882	7.45	47.4
C2	39.1	29.0	0.2084	16.85	49.5
C3	28.5	19.1	0.4234	44.97	37.7
D1	73.5	30.5	0.0020	0	0
D2	54.3	18.7	0.1584	6.80	93.5
D3	43.8	18.7	0.4534	20.60	88.0
D4	28.2	14.4	0.4536	26.00	69.9

[a] It is apparent value of wet particles in water.
[b] The collapsed microspheres were not measured.

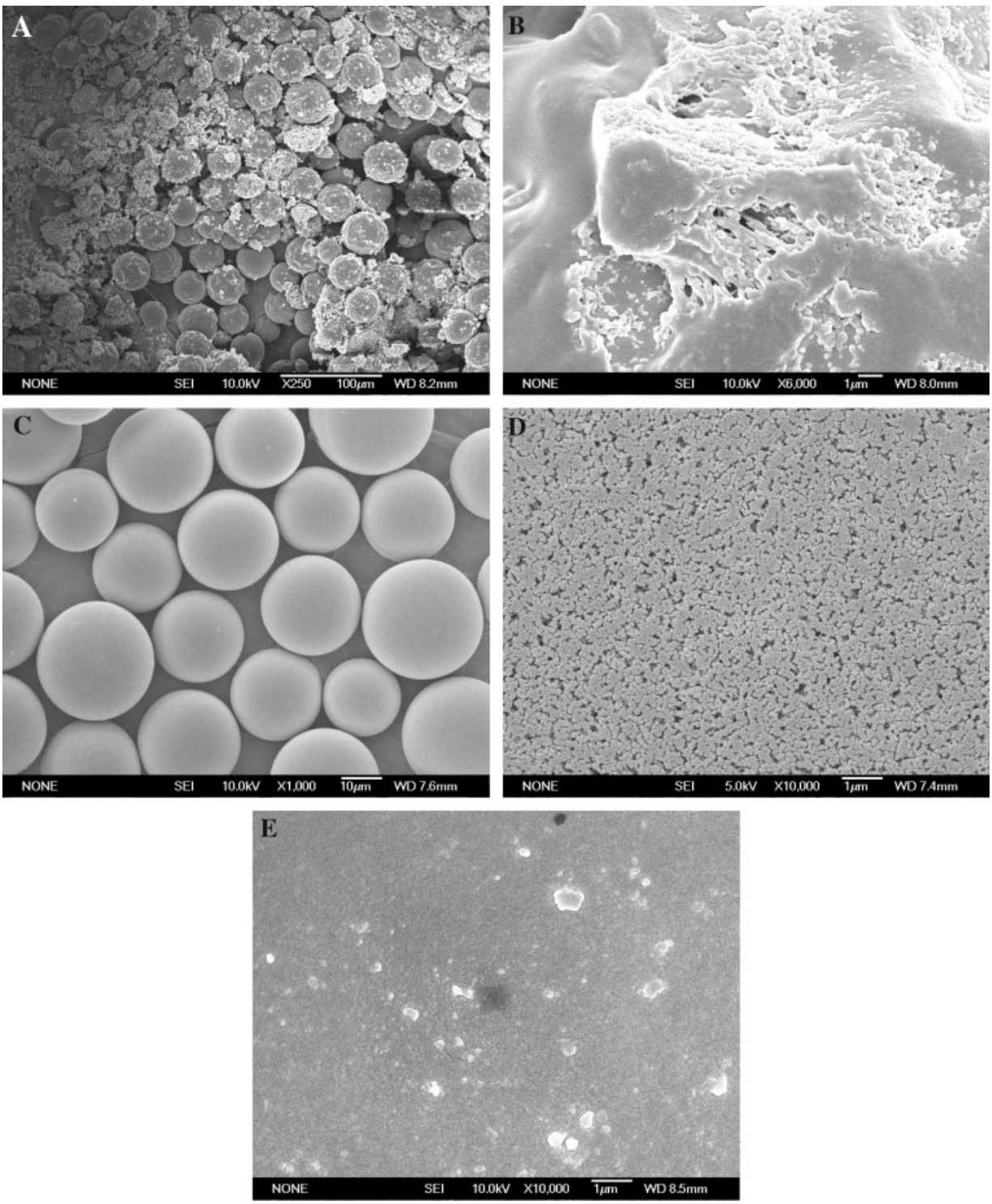

Figure 6 SEM photographs of p(HEMA-*co*-EDMA) microspheres: (A, B) for collapsed microspheres (run A3), C and D for porous microspheres (run C3), and E for microporous microspheres (run D1).

water when low EDMA concentration was introduced, that is to say, less monomer was absorbed by the seed droplets, which caused a decrease in the actual (monomer mixture)/toluene ratio. Furthermore, the wet particle absorbed more water to show a higher wet diameter value when EDMA was lower. (3) The difference in final conversion. The conversion for each monomer was different. As a result, the final yields of all the reactions were not constant, but varied with the EDMA concentration.

In our study, the more EDMA was introduced, the higher the final yields was.

Morphologies of the p(HEMA-*co*-EDMA) microspheres

It was known that microspheres with different pore sizes, induced by phase separation during the polymerization, were useful as chromatographic media for the separation of biomolecules with different structures. Daniel[16] had studied the effect of inert components on the porosity of polymer materials. They proposed that phase separation induced by polymerization had much to do with the porosity. In this article, we focused on how the EDMA/HEMA and toluene/monomer ratios affected the phase separation during the polymerization by influencing the chain length of polymer and their solubility in the polymerization mixture.

The typical SEM photographs showing the surface morphologies of the final microspheres are shown in Figure 6. When EDMA concentration was fixed at 33% (EDMA/HEMA = 4/8), the particles collapsed at a high toluene content (toluene/monomer mixture = 6/12) as shown in Figure 6(A,B) (Run A3); however, the particles kept intact without collapse at a lower toluene content (toluene/monomer mixture = 4.5/12). Similarly, at a higher EDMA concentration (EDMA/HEMA = 4/4), the same phenomenon was observed, but the upper toluene content, at which the particles began to collapse, was higher. We suggested that high toluene content (i.e. low monomers content) would reduce the molecular weights of the polymer chains and their solubility in the polymerization mixture, accordingly, precipitation of polymer chain occurred at a low conversion of monomer. Premature phase separation would more easily bring in structure break (i.e. collapsed microspheres). However, with the increase of crosslinker content, a 3Dnetwork with higher molecular weight was obtained and the copolymerization continued to take place until the polymer chain precipitated from the reaction mixture. As a result, the polymer structure was too firm to break up. For example, when EDMA concentration was as high as 67% (EDMA/HEMA = 8/4), the microspheres were porous and uniform as shown in Figure 6(C,D) (Run C3). The same trend was observed in series D (EDMA/HEMA = 12/4). However, the surface of the microsphere became compact, and the pores couldn't be observed by SEM as shown in Figure 6(E) (Run D1) when toluene content was low (toluene/monomer mixture = 3/16). This was because a network with nearly infinite molecular weight was formed and the small amount of toluene was pushed out of the reaction system.

From the above, it can be concluded that the morphology of the microspheres is determined by not only the crosslinker (EDMA) concentration but also the porogen (toluene) composition. Only when the ratio of toluene/(monomer mixture) was between a lower and an upper limit at a specified EDMA/HEMA ratio, porous microspheres with narrow size distribution can be obtained.

The results of Nitrogen adsorption analysis about interior morphology of the final microspheres are shown in Table II. It can be seen that the average pore diameter of the microspheres increased with the increase of toluene concentration. In this case, larger pores that contributed less to the specific surface area were formed and resulted in lower specific surface area. The result of run B2 that the specific surface area increased with the increase of average pore diameter was a particular case. This was because the average pore diameter of the microspheres in run B2 was close to that in run B1, but the total pore volume of run B2 was much higher, which resulted from more toluene in the swollen droplets. As a result, the specific surface area increased. It can also be seen from Table II that the highest total pore volume, largest average pore diameter, and highest specific surface area were 0.4536 mL/g, 44.97 nm, and 129.0 m^2/g, when the ratios of HEMA/EDMA/toluene (g/g/g) were 4/12/6, 4/8/12, and 4/4/6, respectively. The optimum recipe can be selected according to the requirement of different applications. The typical adsorption isotherm of the final product (run C2) is shown in Figure 7. It was clear that the adsorption curve and desorption curve did not overlap but formed a close loop. It can be assumed that the pores were cylindrical and continuous because of the close adsorption-desorption loop.[17,18] These continuous pores that benefit the unblocked in-out of analyte would play a major role in the success of a separation.

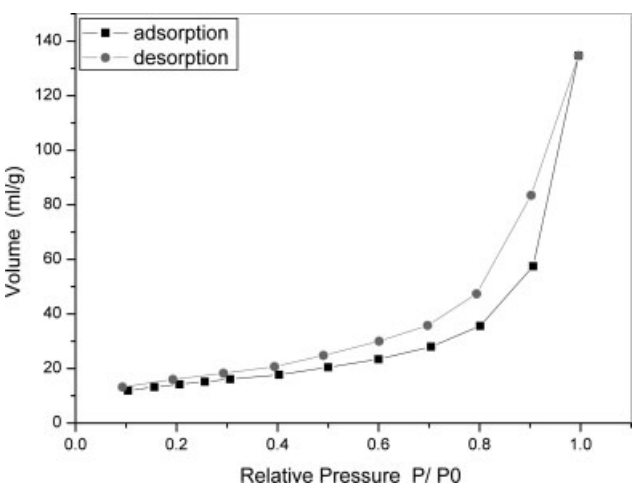

Figure 7 Adsorption isotherm of the representative p(HEMA-*co*-EDMA) microspheres (run C2).

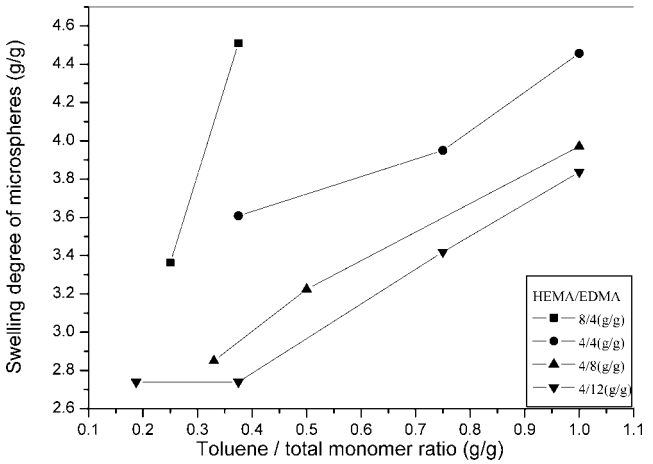

Figure 8 Effect of toluene concentration and crosslinking degree on the swelling degree of p(HEMA-*co*-EDMA) microspheres.

Swelling behaviors of p(HEMA-*co*-EDMA) microspheres

It was considered that pHEMA could absorb and retain water because of its 3D network and abundant hydroxyl groups on the surface.[2] It is necessary to investigate the swelling behavior if we want to use pHEMA microspheres as chromatographic media. As can be observed in Figure 8, the toluene and EDMA content affected the swelling behavior apparently. The swelling degree of the microspheres increased with the increase of toluene content. The largest swelling degree reached to 4.5. This was because that the porosity of the microspheres was enhanced by the increase of toluene concentration, as shown in Table II. The microspheres with higher porosity can absorb more water in the equilibrium state. Additionally, the microspheres began to collapse when toluene/(monomer mixture) ratio was as high as 1/2 at low EDMA content (HEMA/EDMA = 8/4), therefore, real swelling degree can not be obtained from these microspheres.

From Figure 8, it was also known that the swelling degree of all microspheres decreased with increase of crosslinker concentration as expected. The minimum swelling degree was 2.75 when EDMA/HEMA ratio was 12/4.

CONCLUSIONS

p(HEMA-*co*-EDMA) microspheres with controllable size and porosity were successfully prepared by combining membrane emulsification technique and swelling process of the droplets. The factors that affected the characteristics of the microspheres were clarified. Porous microspheres with narrow size distribution can be obtained only when the toluene/monomer ratio was between a lower and an upper limit at a special EDMA/HEMA ratio. By changing the HEMA/EDMA/toluene ratio, the diameter and porosity can be controlled. The results showed that the largest volume-mean diameter attained to 73.5 μm when the ratio of HEMA/EDMA/toluene (g/g/g) was 4/12/3, and the maximum total pore volume, average pore diameter, and specific surface area were 0.4536 mL/g, 44.97 nm, and 129.0 m^2/g, when the ratio of HEMA/EDMA/toluene (g/g/g) were 4/12/6, 4/8/12, and 4/4/6, respectively. The large porous crosslinked p(HEMA-*co*-EDMA) microspheres with narrow size distribution can be used as chromatographic media for the separation of proteins and natural products under low pressure after further modification. The higher resolution will be expected because of its narrow size distribution and controllable porosity.

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