Synthesis of Uniform Microspheres with Higher Content of 2-Hydroxyethyl Methacrylate by Employing SPG (Shirasu Porous Glass) Emulsification Technique Followed by Swelling Process of Droplets

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ABSTRACT: Relatively uniform microspheres containing a hydrophilic monomer, 2-hydroxyethyl methacrylate (HEMA), were prepared by employing a swelling method of uniform seed droplets. A uniform seed emulsion composed mainly of styrene (St) was prepared by the Shirasu porous glass (SPG) membrane emulsification technique; this was mixed with a secondary emulsion composed mainly of HEMA/St or HEMA/MMA (methyl methacrylate) prepared by a homogenizer for swelling. The swollen droplets obtained were polymerized at 75°C under a nitrogen atmosphere. The uniform microsphere with a higher content of HEMA was obtained successfully by the swelling method while it failed by a direct emulsification method. The effects of the composition of the oil phase and the inhibitor in the continuous phase on the incorporated fraction of HEMA, the morphology of particles, and monomer conversion were investigated. It was found that the incorporated fraction of HEMA increased with increasing its feed fraction, and more HEMA was incorporated into the microsphere when HEMA/MMA was used as the oil phase of the secondary emulsion rather than HEMA/St. Although the final conversion was very low when the feed fraction of HEMA was higher, it can be increased to more than 80% by using an adequate amount of ethylene glycol dimethacrylate (EGDMA) as a crosslinker and $NaNO_2$ as an inhibitor in the aqueous phase. Various microspheres with different morphologies such as spherical, snowmanlike, and popcornlike were observed, depending on composition of the oil phase. Furthermore, the porous microsphere with a high content of HEMA was obtained by employing hexanol (HA) as a porogen. J Appl Polym Sci **66:** 1325–1341, 1997

Key words: Shirasu porous glass; emulsification; swelling of droplets; hydrophilic monomer; 2-hydroxyethyl methacrylate

INTRODUCTION

In recent years, the studies on the preparation, characterizations, and applications of microspheres have come into prominence because they can be utilized in various fields, e.g., as a carrier of biomaterials and drugs,[1–3] in bioreactors of proteins,[4] DNA,[5] and cells,[6] and in conventional applications such as paints,[7] coating agents,[8–11] and adhesive agents.[12] Because the performances of microspheres are strongly dependent on the characters of microspheres when they are used in the above fields, the studies on the control of sizes and size distribution, compositions, morphologies of microspheres, and

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the amount and distribution of functional groups inside microspheres are being carried out extensively. Especially, monodispersed microspheres containing various functional groups and substances were often necessary for the above applications; studies on the establishment of simple preparative techniques of monodispersed functional microspheres also were noticed continuously.

Monodispersed microspheres with diameters of 0.01–1 μm can be prepared in aqueous media by miniemulsion,[13] emulsion,[14] and soap-free emulsion[15] polymerization. Although monodispersed large-size microspheres, e.g., from several to 10 μm, also can be synthesized by dispersion polymerization in organic media, there are few methods to prepare monodispersed large-size microspheres in aqueous media. A simple suspension polymerization method was often used to prepare large-size microspheres in the aqueous phase; the size distribution of microspheres obtained, however, is very broad. The seed polymerization method[16–18] can provide uniform large and various composite microspheres with different morphologies in the aqueous phase or organic solvent by employing a uniform small seed particle. This method was very attractive and was used extensively to prepare the well-defined microspheres for fine chemical applications. But it involved several steps and needed a long time, and it was difficult to incorporate a very hydrophilic monomer into microspheres if using water as a continuous phase. Instead of suspension polymerization by which only polydispersed microspheres were obtained, we developed an SPG emulsification technique followed by a polymerization process to prepare a uniform microsphere with diameters from several to above 100 μm in aqueous solution.[19–23] The diameter of a microsphere can be varied simply by using SPG membranes with different pore sizes. The coefficient of variation (CV value) which characterizes the size distribution of particles was around 10%. During the past several years, we discussed the effect of preparative conditions on the formation of a uniform microsphere, e.g., emulsification pressure, composition of the oil phase, type and amount of stabilizer and surfactant, and synthesis temperature. It was found that the hydrophobic microspheres of polystyrene (PSt) with a narrow size distribution can be prepared successfully by an SPG emulsification technique followed by a polymerization process[19] if the preparative conditions were proper. Furthermore, it was found that the direct SPG emulsification method was not suitable for the preparation of microspheres composed of a relatively higher hydrophilic monomer, such as methyl methacrylate (MMA). Because the SPG membrane is composed of hydrophilic Al_2O_3—SiO_2, its pore wall is wet easily by a hydrophilic monomer, generating a jetlike stream which leads to nonuniform droplets. Subsequently, we also devised a swelling method (two-step method) of droplets to prepare relatively hydrophilic poly(methyl methacrylate) (PMMA) microspheres. Thus, a uniform seed droplet composed of a hydrophobic unpolymerizable substance benzene (Bz) was prepared by an SPG emulsification technique at the first step, then it was mixed at the second step with the fine secondary emulsion composed mainly of MMA, which was prepared by using a homogenizer. The oil phase of the secondary emulsion will diffuse into the aqueous phase and be absorbed subsequently by the uniform seed droplets. Only by extracting Bz after polymerization can the pure hydrophilic PMMA microspheres be obtained. In such a type of seed polymerization, it can be expected that morphologies different from conventional seed polymerization, where the seed is composed of polymer, will be observed. Therefore, it is also very interesting and important to study about the different combinations of seed emulsion and secondary emulsion.

Based on the experiences of the preparation of PSt and PMMA microspheres, in this study, we tried to use these two methods (direct emulsification method and swelling method) to incorporate a more hydrophilic monomer, 2-hydroxyethyl methacrylate (HEMA), into microspheres and compared which method was more effective for the incorporation of HEMA. Furthermore, we also tried to manifest how to control the morphologies of the microspheres.

The difference between this particular swelling method and conventional swelling method (seed polymerization) was that the seed emulsion in this study was composed of monomer but not polymer. One may consider that the hydrophilicity of HEMA is very high; almost all of it will remain in the aqueous phase and will not be absorbed by the oil phase of the seed emulsion. This may be true when the seed is composed of the polymer, but it is not the case when the seed emulsion is composed of a monomer. In fact, HEMA is an amphiphilic substance; its miscibility with a monomer is much higher than that with a polymer. It will be partitioned into both the oil phase and the aqueous phase. The partition constant will depend strongly on the composition of the oil

phase, concentration of the surfactant and stabilizer, and other preparative conditions.

Incorporating HEMA into microspheres has many advantages; e.g., it has been reported that the materials with the PHEMA–PSt microphase separation structure have excellent biocompatibility.[24,25] These properties are necessary when microspheres are used as carriers of biomaterials and used in bioseparators. Furthermore, because the affinity of such hydrophilic microspheres with an inorganic substance such as magnetite is much better,[26] it can be used to prepare the magnetite microspheres or other organic–inorganic composite microspheres.

EXPERIMENTAL

Materials

Monomers

Styrene (St), methyl methacrylate (MMA), and divinylbenzene (DVB) were commercial grade (Kishida Chemical Co.) and were distilled under a vacuum to remove the inhibitor. DVB is a mixture of 55% isomeric DVB, 40% ethyl vinylbenzene, and 5% of saturated compounds. Ethylene glycol dimethacrylate (EGDMA) was commercial grade (Tokyo Chemical Industry Co.) and was used after distillation under a vacuum. 2-Hydroxyethyl methacrylate (HEMA) was commercial grade (Kishida Chemical Co.) and was used as purchased.

Solvents

Benzene (Bz), heptane (HP), and hexanol (HA) were reagent grade (Kishida Chemical Co.). Methyl alcohol was commercial grade (Kishida Chemical Co.). All were used as received.

Other Chemicals

Benzoyl peroxide (BPO) with a 25 wt % moisture content was reagent grade (Kishida Chemical Co.) and was used as an initiator. Hydroquinone (HQ) or sodium nitrite ($NaNO_2$) were reagent grade (Kishida Chemical Co.) and were used as inhibitors to prevent the secondary nucleation of polymer particles in the aqueous phase. Hexadecane (HD) was a reagent agent (Tokyo Chemical Industry Co.) and was used as a hydrophobic substance to stabilize droplets of the primary emulsion. Sodium lauryl sulfate (SLS) was of the grade for biochemical use (Merck). Poly(vinyl alcohol) (PVA-217, degree of polymerization 1700, degree of hydrolysis 88.5%) was provided by Kuraray and was used as a stabilizer. All these reagents were used as received. Water was purified by distillation followed by deionization using ion-exchange resins.

Apparatus

A particular apparatus for emulsification with an MPG module (microporous glass, a brand name of SPG) installed was purchased from Ise Chemical Co. The schematic diagram of this apparatus was shown elsewhere.[19]

One-Step Emulsification (Direct Method)

The detailed preparative conditions of the one-step emulsification (direct method) are shown in Table I. The mixture of St, HEMA, HD, and the crosslinker DVB dissolving initiator BPO were used as the dispersed phase (oil phase), and water, where the stabilizer PVA, surfactant SLS, Na_2SO_4, and inhibitor HQ were dissolved, was used as the continuous phase (aqueous phase). HQ was used to prevent the secondary nucleation of polymer particles in the aqueous phase because a part of the monomer, especially hydrophilic HEMA, will dissolve into the aqueous phase. Because it has been found in the previous studies[20,21] that adding a small amount of HD can improve the stability of the droplets, 10 wt % HD was added into the oil phase. The oil phase was pressed by nitrogen gas through the SPG membrane into the aqueous phase continuously. A droplet will be formed at the internal surface of the SPG tube due to the interface tension between the oil phase and the hydrophilic membrane. Then, PVA and SLS that dissolved in the aqueous phase will be adsorbed onto the surface of the droplet to stabilize it. The concentration of the monomer was always around 10 wt %. The feed fraction of HEMA in the total monomers was changed from 4 wt % (3.3 mol %) to 12.5 wt % (10.3 mol %). The detailed SPG membrane emulsification process was described elsewhere.[19,20]

Two-Step Emulsification (Swelling Method)

The detailed preparative conditions are shown in Tables II–V, including those of porous microspheres. The preparative procedure of the primary (seed) emulsion was the same as that in the direct method. The schematic diagram for the

Table I Preparative Conditions and Polymerization Results by Direct Emulsification Method

		Run No.		
		221	222	223
Preparative conditions[a]	Dispersion phase (g)			
	St	43	41.25	38.75
	HEMA	2.0	3.75	6.25
	DVB	5.0	5.0	5.0
	HD	5.0	5.0	5.0
	BPO	1.5	1.5	1.5
	HEMA/monomer (mol %)	3.3	6.2	10.3
Polymerization results	D_p (μm)	8.12	7.27	[b]
	CV (%)	9.17	9.60	Polydispersity
	Conversion (%)	66.9	52.0	71.0
	HEMA/monomer (mol %)	2.2	1.7	[b]

[a] The composition of the continuous phase for all three runs was the same (g): water 450; PVA 4.0; SLS 0.20; Na_2SO_4 0.20; HQ 0.15. Total weight of the dispersion and the continuous phase was about 500 g.
[b] Because the distribution of the diameter was very broad, the data were not measured.

swelling process is shown in Figure 1. The mixture of St, HD, and/or DVB dissolving BPO was used as the oil phase of the primary emulsion, and the water dissolving PVA, SLS, Na_2SO_4, and the inhibitor (HQ or $NaNO_2$) was used as the continuous phase of the primary emulsion. Sometimes, the inhibitor was added into the aqueous phase of the secondary emulsion for convenience. The secondary emulsion was prepared by using a conventional homogenizer with a rotation rate of 10,000 rpm for 15 min. The oil phase of the secondary emulsion was composed mainly of HEMA, St, or MMA. The water dissolving a small amount of SLS was used as the continuous phase. Sometimes, the crosslinker EGDMA was also added into the oil phase of the secondary emulsion in-

Table II Preparative Conditions and Polymerization Results by Swelling Emulsification Method (Secondary Emulsion: HEMA/St; Swelling Ratio S_r: 1.6)

		Run No.				
		205	206	207	243	244
Preparative conditions[a]	Secondary emulsion					
	Dispersion phase (g)					
	St	13.4	12.0	10.0	9.0	8.0
	HEMA	1.6	3.0	5.0	6.0	7.0
	HEMA/monomer (mol %)	3.3	6.2	10.3	12.5	14.7
Polymerization results	D_p (μm)	8.50	8.58	8.67	7.90	8.84
	CV (%)	13.70	11.34	13.67	9.43	10.14
	Conversion (%)	64.3	82.6	71.6	60.0	70.0
	HEMA/monomer (mol %)	2.3	2.4	4.5	8.5	7.3

[a] The composition of the primary emulsion for all five runs was the same. Dispersion phase (g): St 22.5; DVB 2.5; HD 2.5; BPO 0.75. Continuous phase (g): water 225; PVA 2.0; SLS 0.10; Na_2SO_4 0.10; HQ 0.075. The continuous phase of the secondary emulsion for all five runs was also the same (g): water 30; SLS 0.05. The total weight of the dispersion and continuous phases was about 300 g.

Table III Preparative Conditions and Polymerization Results by Swelling Emulsification Method (Secondary Emulsion: HEMA/MMA; Swelling Ratio S_r: 1.6)

		Run No.					
		257	247	248	258	279	273
Preparative conditions[a]	Primary emulsion						
	Dispersion phase (g)						
	St	22.5	22.5	22.5	22.5	22.5	22.5
	DVB	2.5	2.5	2.5	2.5	2.5	0
	HD	2.5	2.5	2.5	2.5	2.5	2.5
	BPO	0.75	0.75	0.75	0.75	0.75	0.75
	Secondary emulsion						
	Dispersion phase (g)						
	MMA	12.0	10.0	9.0	8.0	8.0	4.0
	HEMA	3.0	5.0	6.0	7.0	7.0	7.0
	EGDMA	0	0	0	0	0	4.0
	HEMA/monomer (mol %)	6.1	10.1	12.4	14.6	14.6	14.4
	Continuous phase (g)						
	Water	30	30	30	30	30	30
	SLS	0.05	0.05	0.05	0.05	0.05	0.05
	$NaNO_2$	0	0	0	0	0.03	0.03
Polymerization results	D_p (μm)	11.07	9.54	9.98	10.98	8.44	10.45
	CV (%)	12.59	11.13	12.41	11.47	14.11	12.15
	Conversion (%)	60.0	57.7	50.7	52.0	63.8	84.5
	HEMA/monomer (mol %)	4.3	7.7	8.4	13.1	[b]	15.6

[a] The composition of the continuous phase of the primary emulsion for all six runs was the same (g): water 225; PVA 2.0; SLS 0.10; Na_2SO_4 0.10; HQ 0.075. Total weight of the dispersion and continuous phase was about 300 g.
[b] Unmeasured, refer to text.

stead of DVB of the primary emulsion. The diluent was added into the oil phase of the primary or secondary emulsion according to its hydrophobicity or hydrophilicity. Then, the primary and secondary emulsions were mixed under mild stirring at 160 rpm. Because the stability of the secondary emulsion was lower due to its polydispersity and lack of SLS, and its hydrophilicity of the oil phase was higher than that of the primary emulsion, the oil phase will diffuse into the continuous phase, then be absorbed by the primary droplets until it disappeared completely. Because the size of the droplets of the primary emulsion was very uniform, the oil absorption rate was almost the same for every droplet. Therefore, the primary droplets would be swollen uniformly and maintain their monodispersity. The swelling ratio was varied from 1.6 to 4 for the preparation of usual particles, while it was also increased to 8 for that of porous particles. The swelling procedure was carried out for about 1 h under stirring.

A theoretical value of the swelling ratio (S_r) was defined as follows:

$$S_r = 1 + \frac{(\text{wt of monomers and/or solvents in the secondary emulsion})}{(\text{wt of monomers and/or solvents in the primary emulsion})} \quad (1)$$

Polymerization

The obtained emulsion was transferred to a four-neck glass separator flask equipped with a semicircular anchor-type blade, a condenser, and a nitrogen inlet nozzle. After the emulsion was bubbled with nitrogen gas for 1 h, the nozzle was lifted to above the surface of the emulsion and the temperature was increased to 75°C gradually for the polymerization. The polymerization process was carried out for 24 h under a nitrogen atmosphere.

Table IV Preparative Conditions and Polymerization Results by Swelling Emulsification Method (Secondary Emulsion: HEMA/MMA; Swelling Ratio S_r: 4)

		Run No.				
		265	266	276	274	267
Preparative conditions[a]	Primary emulsion					
	Dispersion phase (g)					
	St	10.0	10.0	10.0	10.0	0
	Bz	0	0	0	0	10.0
	HD	1.0	1.0	1.0	1.0	1.0
	BPO	0.5	0.5	0.5	0.5	0.5
	Continuous phase (g)					
	Water	90	90	90	90	90
	PVA	0.9	0.9	0.9	0.9	0.9
	SLS	0.04	0.04	0.04	0.04	0.04
	HQ	0.06	0.06	0	0	0.06
	Na_2SO_4	0.04	0.04	0.04	0.04	0.04
	Secondary emulsion					
	Dispersion phase (g)					
	MMA	18.0	16.0	12.0	8.0	9.0
	EGDMA	4.0	4.0	4.0	8.0	9.0
	HEMA	12.0	14.0	14.0	14.0	12.0
	HEMA/monomer (mol %)	22.5	26.7	29.7	29.6	33.8
	Continuous phase (g)					
	Water	166	166	170	170	170
	SLS	0.10	0.10	0.10	0.10	0.10
	$NaNO_2$	0	0	0.03	0.03	0
Polymerization results	D_p (μm)	11.24	10.27	9.66	10.45	12.39
	CV (%)	10.73	9.12	15.53	12.15	11.15
	Conversion (%)	47.6	36.7	48.2	89.1	60.0
	HEMA/monomer (mol %)	16.0	37.1	[b]	[b]	[b]

[a] Total weight of the dispersion and continuous phase was about 300 g.
[b] Unmeasured, refer to text.

Analyses

Optical Microscopic (OM) Observation

Droplets of the primary emulsion, those after swelling, and polymer particles were observed with an optical microscope. Diameters of about 300 droplets or particles were counted to calculate average diameters and size distribution.

SEM Observation

The surface features of polymer particles were observed by a JSM-35CFII (JEOL) scanning electron microscopy (SEM). The specimens for SEM observations were prepared by coating a thin gold film (about 60 Å in thickness) on sample under reduced pressure below 10^{-2} Pa with a JEE-3X vacuum evaporator (JEOL).

Measurement of Content of HEMA

The incorporated fraction of HEMA was measured by ^{13}C-NMR spectrometry. The ^{13}C-NMR spectra were recorded by a 500 MHz spectrometer (JEOL α-500) at 45°C with trichloromethane-d_3 ($CDCl_3$) as the solvent and locking agent. The NNE (hetero decoupling without nuclear Overhauser effects) irradiation mode was selected for quantitative ^{13}C-NMR measurement. The spectra were obtained using a spectral width of 33,898.31 Hz, an acquisition time of 0.97 s, and a pulse decay of 6.03 s. Spectra were obtained after accumulating 10,000 scans, by using a sample concentration of 15% (w/v). A typical spectrum was shown in Figure 2 (Run 273 of Table III). The areas of peaks at 61 ppm (— $CH_2\underline{C}H_2OH$ of HEMA), 128 and 136 ppm (— $\underline{C}_6H_5$ of St and DVB), and 20

Table V Swelling Degrees (S_d) of Microspheres with Different HEMA Contents

Run No.[a]	Crosslinker (Wt % in Total Monomer Fed)	HEMA Content (Mol % in Total Monomer)	Swelling Degree[b]
257	DVB (6.25 wt %)	4.3	1.68
247	DVB (6.25 wt %)	7.7	1.82
248	DVB (6.25 wt %)	8.4	1.89
258	DVB (6.25 wt %)	13.1	2.14
273	EGDMA (10 wt %)	15.6	2.24
274	EGDMA (20 wt %)	37.1[c]	3.19

[a] Refer to Tables III and Table IV.
[b] Measured in water.
[c] The value for Run 266 was used.

ppm $\begin{matrix}\diagdown\\ -\underline{C}\underline{C}H_3\\ \diagup\end{matrix}$ of MMA, EGDMA, and HEMA) were used to calculate the content of the HEMA unit (incorporated fraction of HEMA). When the particle was only composed of St, HEMA, and DVB, the following equation was used to calculate content of the HEMA content:

$$\text{HEMA content (mol \%)} = \frac{A(-CH_2\underline{C}H_2OH)}{A(-CH_2\underline{C}H_2OH) + A(-\underline{C}_6H_5)/6} \quad (2)$$

where A represents the area of the peak. When the particle was composed of St, MMA, HEMA, and EGDMA, the following equation was used to calculate the HEMA content:

$$\text{HEMA content (mol \%)} = \frac{A(-CH_2\underline{C}H_2OH)}{A\left(\begin{matrix}\diagdown\\ -\underline{C}\underline{C}H_3\\ \diagup\end{matrix}\right) + A(-\underline{C}_6H_5)/6} \quad (3)$$

where $\begin{matrix}\diagdown\\ -\underline{C}\underline{C}H_3\\ \diagup\end{matrix}$ was the total mount of $\begin{matrix}\diagdown\\ -CCH_3\\ \diagup\end{matrix}$ of HEMA, MMA, and twice EGDMA because one EGDMA contains two $\begin{matrix}\diagdown\\ -CCH_3\\ \diagup\end{matrix}$. Therefore, the feed fraction of HEMA was calculated by the following equation, in order to let it correspond to the measured value of eq. (3), i.e., one EGDMA was considered as two units of the monomer:

$$\text{Feed fraction of HEMA (mol \%)} = \frac{W_{HEMA}/M_{HEMA}}{W_{HEMA}/M_{HEMA} + W_{St}/M_{St} + W_{MMA}/M_{MMA} + 2W_{EGDMA}/M_{EGDMA}} \quad (4)$$

where W_i represents the weight (g), and M_i, the molecular weight of the monomer i.

Measurement of Conversion

The conversion of the monomer was determined gravimetrically. The polymer was precipitated by methyl alcohol from the serum, separated by centrifugation, dried in a vacuum, and weighted.

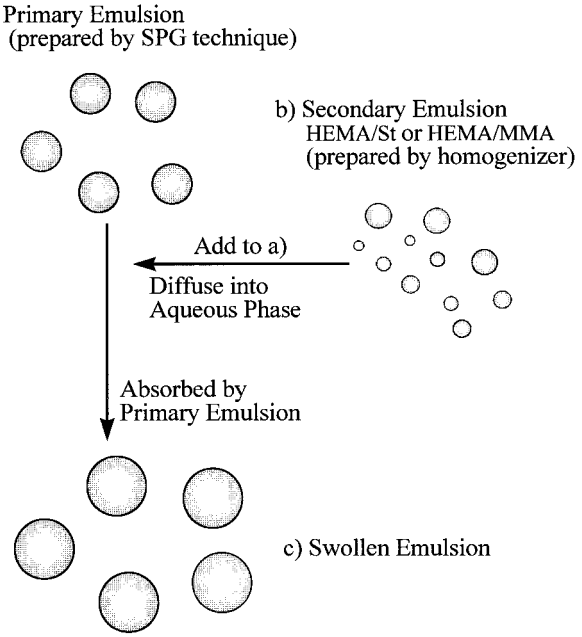

Figure 1 Schematic diagram showing the preparation of monodispersed droplets containing HEMA by the swelling method.

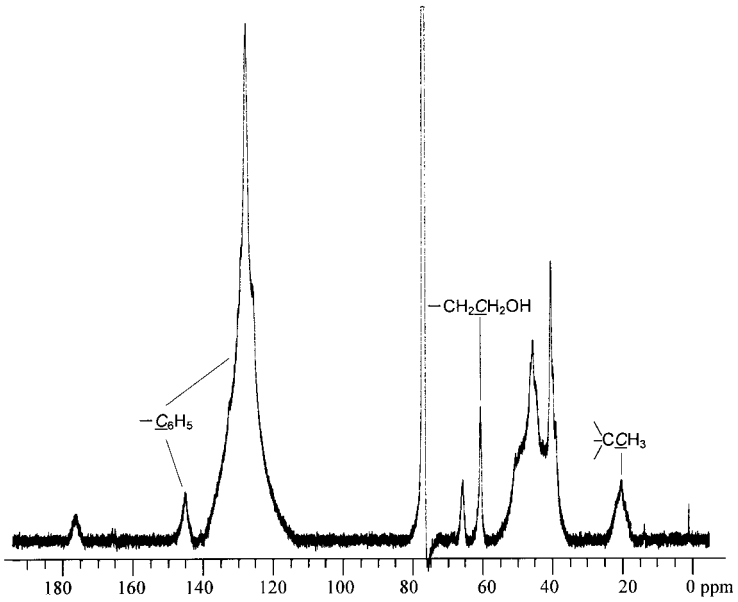

Figure 2 Example of ^{13}C-NMR spectrum of microspheres containing HEMA (Run 273). Solvent: $CDCl_3$; concentration: 15% (w/v); accumulation times: 10,000 scans.

Measurement of Swelling Degree

The swelling degree (S_d) was defined as the weight ratio of the wet to dry particle, which should be distinguished from the swelling ratio (S_r) in the swelling process of the droplets. The weight of the wet particle was measured as follows: After the particle was washed by methanol and dried, it was immersed into water and heated at 70°C for 3 h while stirring. Then, centrifugation was carried out with a rotation speed of 2000 rpm. After the serum was decanted, the weight of the wet particle containing water was measured.

RESULTS AND DISCUSSION

Incorporated Fraction of HEMA

To compare which method was more effective for incorporating HEMA into the microspheres, the one-step (direct method) and two-step (swelling method) emulsification techniques were employed. The results of the direct method are shown in Table I with the preparative conditions. The typical SEM micrographs or optical micrograph (OM) of the polymer particles are shown in Figure 3. When the feed fraction of HEMA was lower than 6.3 mol %, uniform microspheres were obtained as shown in Figure 3(a) and (b) for Runs 221 and 222, respectively, and the CV values of them were below 9.60%. When the feed fraction of HEMA was increased to 10.3 mol %, however, the size distribution of the droplets became very broad as shown in the OM of Figure 3(c). This is because the pore wall of the hydrophilic SPG membrane was easily wet by the hydrophilic HEMA, generating a jetlike stream which led to the nonuniform droplets.

The incorporated fractions of HEMA were measured for Runs 221 and 222 by ^{13}C-NMR spectrometry. Compared with the feed fraction of HEMA, the incorporated one for each sample was very low, only 2.2 and 1.7 mol % for Runs 221 and 222, respectively. There are two reasons responsible for this result: One was that a part of the HEMA was partitioned into the aqueous phase, in which polymerization would be inhibited by HQ. The other one was that a large amount of HEMA was adsorbed by the SPG membrane due to the affinity between HEMA and the membrane. The latter reason also suggested that the polydispersity of droplets of Run 223 resulted from the fact that the membrane was wet by HEMA, because a larger amount of HEMA would be adsorbed by the SPG membrane when the feed fraction was enhanced further. As a result, the interface tension between the membrane and the oil phase became smaller, leading to a nonuniform microsphere.

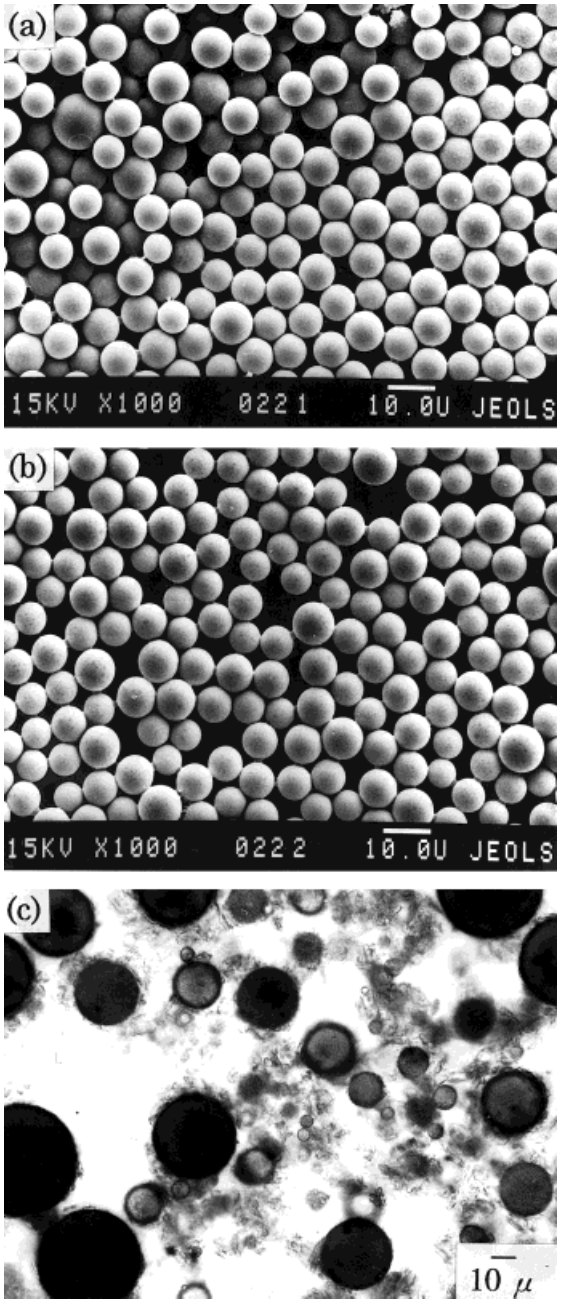

Figure 3 SEM micrographs or optical micrograph (OM) of microspheres prepared by the direct emulsification technique. HEMA/monomer in the feed (mol %): (a) 3.3 (SEM of Run 221); (b) 6.2 (SEM of Run 222); (c) 10.3 (OM of Run 223).

Therefore, to incorporate much more HEMA into the microspheres, the swelling method was employed. The results are shown in Tables II–IV together with the preparative conditions, and the relationships between the feed and the incorporated fraction of HEMA are summarized in Figure 4, together with the result of the direct method. When the oil phase was composed mainly of St and HEMA, DVB was used as the crosslinker and always added into the oil phase of the primary emulsion. When the oil phase was composed mainly of MMA and HEMA in preference to St, EGDMA was used as the crosslinker because it has been known that it copolymerizes with MMA or HEMA more readily than does DVB, and it always was added into the secondary emulsion. Usually, it is desirable that there exists a solubility difference in water between the primary and secondary oil phase in order to promote the secondary oil phase to collapse and, subsequently, to be absorbed by the primary emulsion rapidly. Because it was believed that the incorporated amount of HEMA depended largely on the partition constant and the composition of the secondary oil phase, the HEMA/St or HEMA/MMA was used as the secondary oil phase and the ratio of HEMA/St or HEMA/MMA was varied.

After the primary and secondary emulsions were mixed, the oil phase of the secondary emulsion will diffuse into the aqueous phase, then be absorbed by the stable primary emulsion. Every 15 min after two emulsions were mixed, a small amount of the mixed emulsion was sampled and observed by an optical microscope. The swelling process was completed within 15 and 30 min, re-

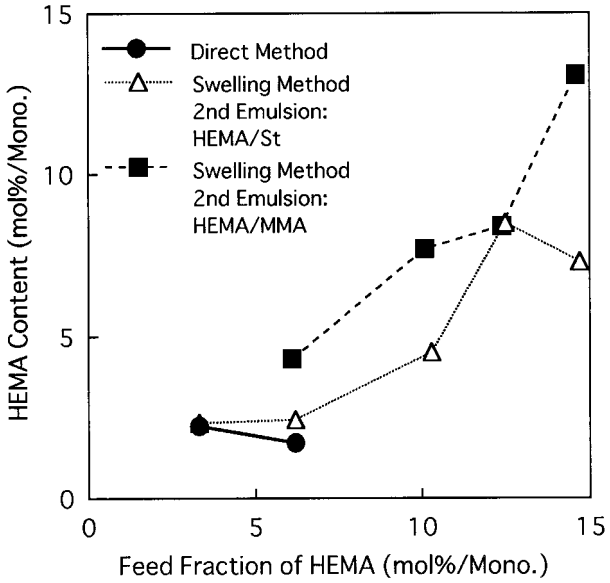

Figure 4 Relationship between feed and incorporated fraction of HEMA. (●) Direct method; (△) swelling method, secondary emulsion: HEMA/St, swelling ratio: 1.6; (■) swelling method, secondary emulsion: HEMA/MMA, swelling ratio: 1.6.

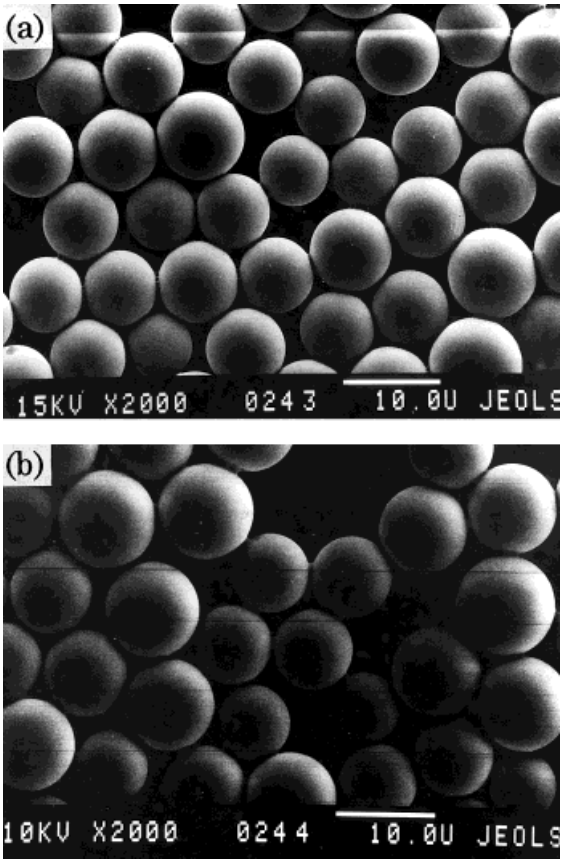

Figure 5 Typical SEM micrographs of microspheres prepared by the swelling method. Secondary emulsion: HEMA/St; swelling ratio: 1.6; HEMA/monomer in the feed (mol %): (a) 12.5 (Run 243); (b) 14.7 (Run 244).

spectively, for the HEMA/MMA and HEMA/St systems.

The typical SEM micrographs for HEMA/St and HEMA/MMA are shown in Figures 5 and 6, respectively when the swelling ratio (S_r) was 1.6. When the HEMA/St was used as the secondary oil phase, the uniform microsphere can be obtained even when the feed fraction of HEMA was increased to 14.7 mol % and the CV values were around 10% (Table II and Fig. 5). This indicated that the seed emulsion absorbed the oil phase of the secondary emulsion while maintaining its narrow size distribution. Although the incorporated fraction of HEMA increased as the feed fraction was enhanced, it attained a maximum value when the feed fraction was 10.3 mol % as shown in Figure 4 and did not increase as expected even when increasing the feed fraction of HEMA further.

On the other hand, when the HEMA/MMA was used as the oil phase of the secondary emulsion, it can be seen from Table III and Figure 6 that the uniform microspheres with around 10% of CV values also can be obtained. From Figure 4, it was obvious that the incorporated fraction of HEMA was higher than the case of HEMA/St as compared at each feed value. Furthermore, it attained

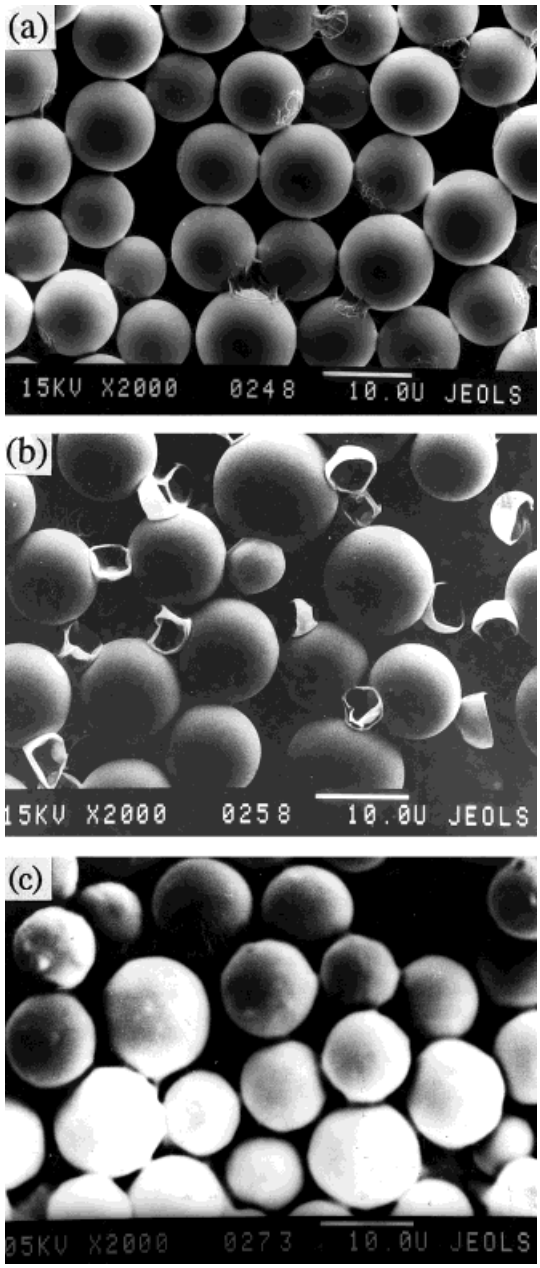

Figure 6 Typical SEM micrographs of microspheres prepared by the swelling method. Secondary emulsion: HEMA/MMA; swelling ratio: 1.6; HEMA/monomer in the feed (mol %): (a) 12.4 [Run 248, poly(St–MMA–HEMA–DVB) sphere]; (b) 14.6 [Run 258, poly(St–MMA–HEMA–DVB) sphere]; (c) 14.4 [Run 273, poly(St–MMA–HEMA–EGDMA) sphere].

13.1 mol % when the HEMA content in the feed was increased to 14.6 mol %; HEMA was incorporated into the microspheres quantitatively. This is because more HEMA was partitioned into the droplets due to its higher affinity with MMA than with St. There are many factors influencing the incorporated amount of HEMA, including the swelling process and copolymerization between HEMA and other monomers. Furthermore, the swelling process was very complicated, involving the dissolution process of the secondary oil phase and the absorption process by the primary emulsion. The partition constant of HEMA changed continuously as the swelling process progressed, because the composition inside and outside the primary droplets varied incessantly. In addition, as HEMA was consumed by the polymerization, a part of HEMA would be partitioned into the polymer–monomer droplets further, if there existed an affinity force between HEMA and polymer particle. Comparing the cases of HEMA/MMA and HEMA/St, the following description can be given: As MMA was absorbed by the seed emulsion, HEMA would be partitioned into droplets more easily than in the early stage of the swelling process without MMA inside the seed emulsion, because the affinity between HEMA and MMA was higher. In addition, even after the polymerization of HEMA and MMA was completed, a part of HEMA dissolved in the aqueous phase also can enter into particles because there exists affinity between poly(MMA–HEMA) and HEMA. So, a higher fraction of HEMA would be incorporated into the particles in the case of HEMA/MMA. On the other hand, in the case of HEMA/St, the amount of HEMA partitioned inside the primary droplets would attain a saturation value because the affinity between HEMA and St was lower. After polymerization, the PSt particle would not absorb HEMA further because the affinity between PSt and HEMA was very poor. As a result, the incorporated fraction of HEMA would show a lower value.

For sample of Run 258 where the incorporated fraction of HEMA was relatively high, an apparent phase separation was observed as shown in Figure 6(b). A snowmanlike microsphere was obtained. Because such a phenomenon was observed only when the HEMA content was relatively high (13.1 mol %), it can be attributed to the phase separation between the St-rich and the HEMA-rich polymer. This phenomenon also suggested that the HEMA-rich polymer was formed when the feed fraction of HEMA was higher. Because the phase separation between HD and PSt also occurred, HD and the HEMA-rich polymer composed the head of the snowman. After HD was extracted by methanol in the purification process, a hole on the HEMA-rich head of the snowman was formed. In our previous study, a one-eye particle was obtained when preparing a PMMA microsphere without employing a crosslinker,[20] i.e., there existed a hole on the surface of PMMA particle. However, a spherical microsphere without a hole was obtained by adding the crosslinker into the oil phase. This is because HD was expelled onto the surface completely due to the higher crosslinking density of the PMMA microsphere. In the sample of Run 258, a hole was found in the HEMA head of the snowman. It can be concluded that the crosslinking density of the HEMA-rich domain was relatively low and most DVB was used in the crosslinking of the St-rich domain.

To enhance the incorporated fraction of HEMA further, the swelling ratio was increased. In this case, because the droplet was richer in MMA and HEMA than in St, EGDMA was used as the crosslinker instead of DVB and was added into the secondary oil phase. As shown in Table IV, the swelling ratios of Runs 265 and 266 were increased to 4, while the compositions of the primary and secondary emulsions were almost the same as that of Runs 248 and 258 (Table III), respectively. As expected, the incorporated fractions of HEMA were increased to 16.0 and 37.1 mol %, respectively. Also, the snowmanlike microspheres were obtained for each sample as shown in Figure 7(a) and (b), because the contents of HEMA were higher.

Monomer Conversion

As described above, a higher fraction of HEMA can be incorporated into the microspheres by using HEMA/MMA as the secondary oil phase and enhancing the mixing ratio of the secondary to primary emulsion. Unfortunately, the final monomer conversions became lower compared with the case where HEMA/St was used, especially for the higher swelling ratios. The conversion was around 50–60% for a 1.6 swelling ratio and only about 40% for 4. There are two reasons to be considered: The first was that because the hydrophilicity of droplets became higher due to increase of HEMA a small amount of HQ would be partitioned into the droplets to inhibit the polymerization inside the droplets. Also, the second was that a large amount of HEMA-rich and MMA-rich oligomers

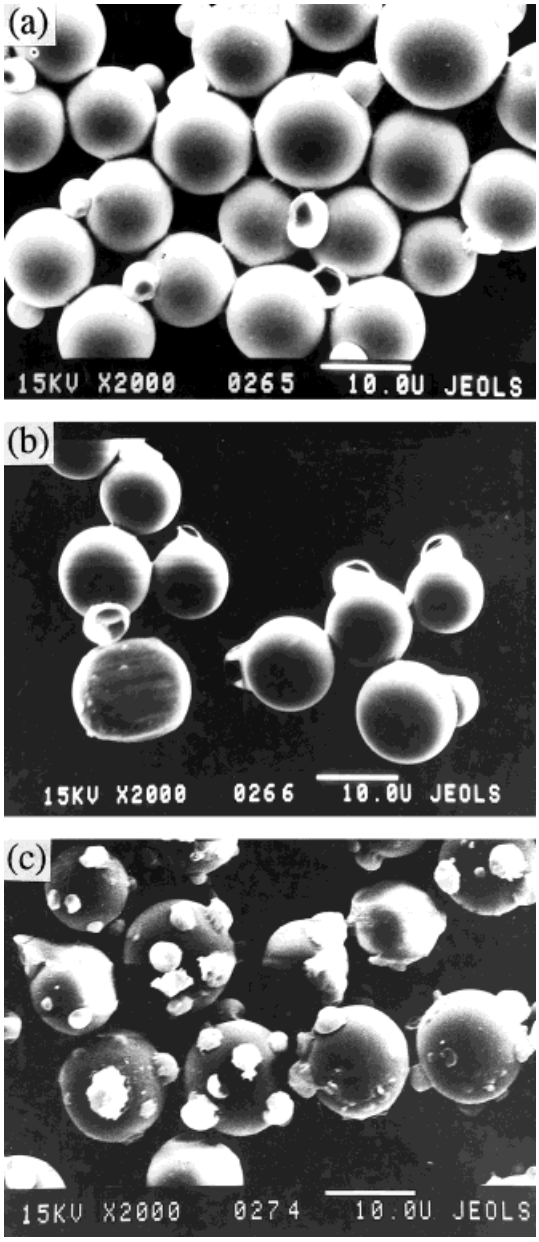

Figure 7 Typical SEM micrographs of microspheres prepared by the swelling method. Secondary emulsion: HEMA/MMA; swelling ratio: 4; HEMA/monomer in the feed (mol %): (a) 22.5 (Run 265, St : MMA : HEMA : EGDMA = 10 : 18 : 12 : 4); (b) 26.7 (Run 266, St : MMA : HEMA : EGDMA = 10 : 16 : 14 : 4); (c) 29.6 (Run 274, St : MMA : HEMA : EGDMA = 10 : 8 : 14 : 8).

formed would escape into the aqueous phase due to their hydrophilicity and cannot enter into droplets again due to their large size. To enhance the conversion, the inhibitor $NaNO_2$ was added into the aqueous phase instead of HQ for the runs where the HEMA contents were higher. Because the hydrophility of $NaNO_2$ is higher than that of HQ which contains a hydrophobic benzene ring, it can be expected that it will become more unlikely for $NaNO_2$ to enter into the droplets. When the swelling ratio was lower (1.6), the conversion was increased to 63.8% (Run 279) from 52.0% (Run 258) by using $NaNO_2$ instead of HQ as shown in Table III. More interesting, the conversion was increased to 84.5% (Run 273 of Table III) only by adding a part of EGDMA instead of DVB. This can be attributed to that the copolymerization among St, MMA, and HEMA was improved because the copolymerization of EGDMA with each of St, MMA, and HEMA was good. As a result, the HEMA-rich oligomer, MMA-rich oligomer, and unreacted HEMA were decreased, leading to a higher conversion. In this sample, the HEMA in the feed also was incorporated into the microsphere quantitatively. From the SEM micrograph of this sample [Fig. 6(c)], it was found that an almost spherical microsphere without an apparent phase-separation was obtained even when the incorporated fraction of HEMA was 15.6 mol % when EGDMA was used as the crosslinker. This result also suggested that the copolymerization among St, MMA, and HEMA was improved due to the addition of EGDMA, resulting in that the HEMA-rich polymer decreased and the phase separation was suppressed.

When the swelling ratio was 4, the conversion was increased very slightly even when using $NaNO_2$ as an inhibitor and EGDMA as a crosslinker (Run 276), compared with Run 266 as shown in Table IV. In this case, although the same amount (4 g) of EGDMA was added into the oil phase as in the case for 1.6 swelling ratio, a higher fraction of HEMA-rich and MMA-rich oligomers would be still formed because the feed fraction of HEMA was much higher. By increasing the feed amount of EGDMA to 8 g (Run 274), i.e., the EGDMA/HEMA ratio (=4/7) became the as same as that for the case of the 1.6 swelling ratio (Run 273, Table III), the conversion increased to 89.1%. Therefore, it can be concluded that it was necessary to increase the amount of EGDMA proportionally when the feed fraction of HEMA was increased. For this sample, the phase separation became more apparent as shown in Figure 7(c): A popcornlike microsphere was obtained. This was because the crosslinking density in the St-rich domain was enhanced further. With increasing of the crosslinking density inside the microsphere as the polymerization progressed, it became difficult for all the growing HEMA-rich polymers to

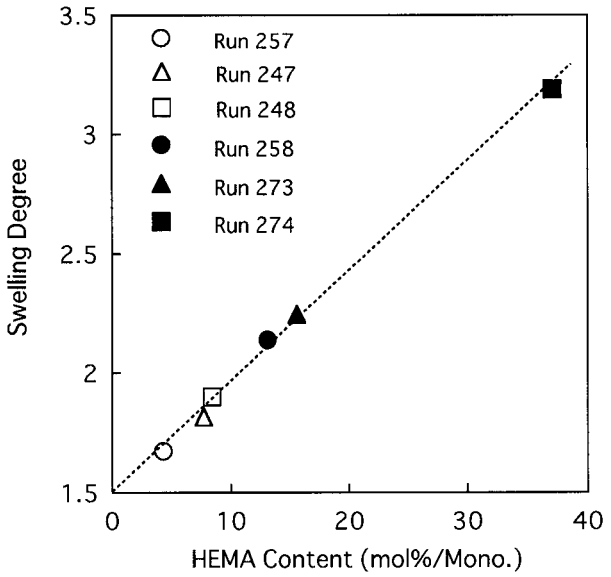

Figure 8 Relationship between HEMA contents and swelling degrees: (○) Run 257; (△) Run 247; (□) Run 248; (●) Run 258; (▲) Run 273; (■) Run 274. Refer to Tables III and IV.

diffuse to one domain. Sheu et al. found a similar phenomenon in seed polymerization using a highly crosslinked uniform particle as the seed.[27] They found that the morphology of the microsphere showed multiplets and a similar explanation was given by them.

Although a large amount of crosslinker was used for Run 274, the swelling degree (S_d) of the particle was higher than that of the other samples. Some data of the swelling degree (S_d) in the water for a series of samples are shown in Table V and the relationship between swelling degrees and HEMA contents is shown in Figure 8. Because the crosslinking density of Run 274 was very high and it did not swell sufficiently in trichloromethane-d_3, the HEMA content of Run 274 cannot be measured by ^{13}C-NMR spectrometry. The value of the HEMA content for Run 266 was presumed equal to that of Run 274, where feed fractions of HEMA of both samples were almost the same. It can be said that this assumption was reasonable by comparing the results of Runs 258 and 273 (Table III), where the feed and incorporated fractions of HEMA for both samples were very close, although the crosslinkers were different. From Figure 8, it was found that all the data lay on one straight line. This result also suggested that the crosslinker was used mainly in the crosslinking of the St-rich domain. The swelling degree of the microsphere was determined mainly by the low crosslinked HEMA-rich domain, and it increased as the HEMA content became higher due to the affinity between HEMA and water. Such an unusual phenomenon of the swelling degree in an inhomogeneous crosslinking structure also was found in previous studies.[28,29]

Morphologies of Microspheres

As described above, the microspheres with various morphologies were obtained. To investigate the relationship between the morphologies and compositions of the oil phase, the composition of the oil phase was changed further based on the above results. The results of the morphologies of the microspheres where HEMA/MMA was used as the secondary oil phase are summarized in Table VI.

It was mentioned above that the phase separation was observed and a snowmanlike microsphere was obtained when the HEMA content was higher (Run 258), while only a spherical microsphere was obtained at the lower HEMA content (Run 248). However, this phase separation was suppressed and a spherical microsphere can be prepared when EGDMA was used as a crosslinker instead of DVB (Run 273). This is because the copolymerizations of EGDMA with HEMA, MMA, and St were better, so the amount of the HEMA-rich polymer decreased. However, when increasing the feed fraction of HEMA further, the phase separation became apparent again although EGDMA was used (Run 266). In this case, the HEMA-rich polymer increased again due to the increasing of the feed amount of HEMA. Here, by increasing the EGDMA further, the phase separation became more apparent and a popcornlike microsphere (Run 274) was obtained due to the high crosslinking density of the St-rich domain.

To obtain a spherical particle with a high content of HEMA, benzene was used to prepare the primary emulsion instead of St (Run 267, Table IV) and Bz was extracted after polymerization. In this case, spherical particles were obtained as shown in Figure 9, although the feed fraction of HEMA was very high. This result also suggested that the phase separations in snowman- and popcornlike particles resulted from St-rich and HEMA-rich polymer.

Preparation of Porous Microspheres

When the microspheres containing HEMA were used in biomedical, bioseparation applications,

Table VI Relation Between Compositions of Oil Phase and Particle Morphologies

Run No.	Compositions of Oil Phase (Wt % in Total Feed Monomer)	Morphology
248	St (56.25 wt %) DVB (6.25 wt %) MMA (22.5 wt %) HEMA (15 wt %)	Spherical[a]
258	St (56.25 wt %) DVB (6.25 wt %) MMA (20 wt %) HEMA (17.5 wt %)	Snowman[a]
273	St (62.5 wt %) EGDMA (10 wt %) MMA (10 wt %) HEMA (17.5 wt %)	Spherical[a]
266	St (23 wt %) EGDMA (10 wt %) MMA (35 wt %) HEMA (32 wt %)	Snowman[b]
274	St (25 wt %) EGDMA (20 wt %) MMA (20 wt %) HEMA (35 wt %)	Popcorn[b]
267	Bz (25 wt %) EGDMA (22.5 wt %) MMA (22.5 wt %) HEMA (30 wt %)	Spherical[c]

[a] Refer to Table III and Figure 6.
[b] Refer to Table IV and Figure 7.
[c] Refer to Table IV and Figure 9.

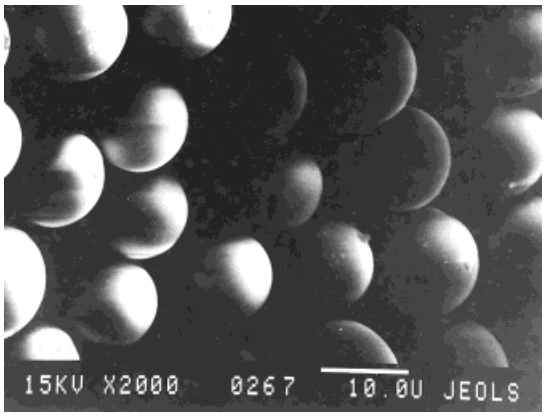

Figure 9 SEM micrograph of microsphere of Run 267 prepared using Bz as the oil phase of the primary emulsion. Swelling ratio: 4; Bz : MMA : HEMA : EGDMA = 10 : 9 : 12 : 9; HEMA/monomer in the feed (mol %): 33.8 mol %. Refer to Table IV.

the porous microspheres were usually necessary for the column packing and carriers to enhance the specific surface area of the microsphere. In principle, a porous structure can be obtained if a poor solvent (diluent) for the base polymer constituting a network is present in the recipe and a microphase separation occurs during polymerization. Delicate balancing among the monomers, diluent, and hydrophobic substance (HD) is required. In our previous studies, it was known that the porous microspheres of PSt and PMMA can be obtained[19–23]; however, the preparative conditions such as the type of diluent and its content were very different. In the case of PSt, e.g., poor solvents such as heptane (HP) and hexane were proper for the preparation of the PSt porous particle. But only a hollow microsphere was obtained when HP was used in the preparation of the PMMA microsphere,[23] and it was found that hex-

Table VII Effect of Diluent and Its Content on the Results of Porous Microspheres by Swelling Emulsification Method

		Run No.		
		269	272	271
Preparative conditions[a]	Primary emulsion			
	Dispersion phase (g)			
	St	4.0	5.0	5.0
	HP	6.0	0	0
	HD	1.0	0.5	0.5
	BPO	0.3	0.15	0.15
	Continuous phase (g)			
	Water	90	95	95
	PVA	0.9	0.9	0.9
	SLS	0.04	0.04	0.04
	Secondary emulsion			
	Dispersion phase (g)			
	MMA	8.1	8.1	0
	EGDMA	8.1	8.1	16.2
	HEMA	10.8	10.8	10.8
	HA	3	9	9
	Continuous phase (g)			
	Water	170	164	164
	SLS	0.10	0.10	0.10
	$NaNO_2$	0.03	0.03	0.03
	Swelling ratio	4	8	8
Polymerization results	D_p (μm)	12.82	18.09	15.10
	CV (%)	12.60	13.36	15.08
	Conversion (%)	72.1	67.2	81.3

[a] Total weight of dispersion and continuous phase was about 300 g.

anol or octanol which contains a hydrophilic endgroup was suitable for the preparation of the PMMA porous microsphere.[20] This is because the miscibility of HP with PSt and PMMA was very different. The miscibility of HP with PSt was higher than that with PMMA, so microphase separation occurred and the particles with micropores can be obtained. On the other hand, the miscibility between HP and PMMA was very poor; only a macrophase separation occurred. Also, because the hydrophilicity of PMMA was much higher than that of HP, HP preferred to stay in the center of particles to form a hollow particle.

Therefore, it is very important to investigate whether a porous microsphere can be obtained when the HEMA content is higher in the poly(St–MMA–HEMA) ternary system. The detailed results are shown in Table VII with the preparative conditions, and the SEM micrographs are shown in Figure 10. Because the microspheres are composed of St, MMA, and HEMA, the mixture of HP and HA or pure HA was selected based on the experiences of the preparation of porous PSt and PMMA microspheres. The feed fraction of EGDMA also was varied because the degree of phase separation depended strongly on the crosslinking density. When the mixture of HP and HA was used as a diluent, the particle was fragile and the surface of the particle was not clear although the porous microsphere can be obtained as shown in Figure 10(a). This is because the phase separation between HP and the poly(St–MMA–HEMA) copolymer was relatively strong due to the higher hydrophilicity of PMMA and PHEMA as the polymerization progressed. On the other hand, when pure HA was used as a diluent and the feed amount of EGDMA was comparable with HEMA, the clear porous microspheres were obtained as shown in Figure 10(b). When the feed fraction of

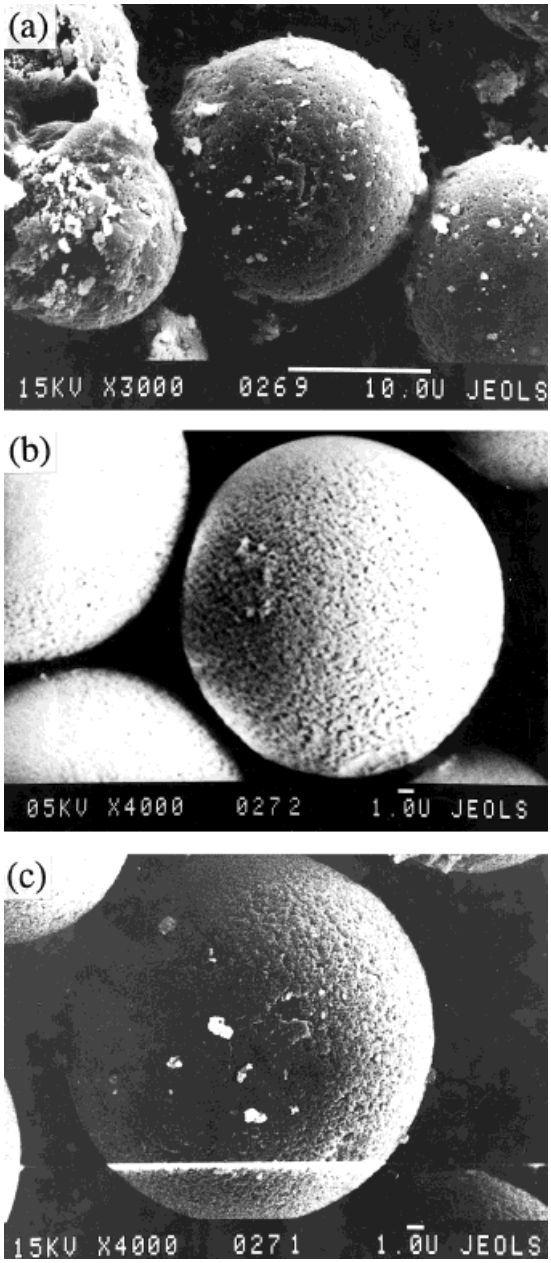

Figure 10 SEM micrographs of porous microspheres: (a) Run 269, diluent: HP : HA (2 : 1 wt ratio), St : MMA : HEMA : EGDMA (wt ratio) = 4 : 8.1 : 10.8 : 8.1; (b) Run 272, diluent: HA only, St : MMA : HEMA : EGDMA = 5 : 8.1 : 10.8 : 8.1; (c) Run 271, diluent: HA only, St : MMA : HEMA : EGDMA = 5 : 0 : 10.8 : 16.2.

EGDMA was increased further, the microspheres with relatively uniform micropores can also be obtained as shown in Figure 10(c). Because the degree of phase separation became higher between the highly crosslinked base polymer and the diluent, part of the particle collapsed and the surface of particle was not so clear.

As described above, the porous microsphere with a higher content of HEMA can be obtained by using an adequate amount of hexanol as a diluent and EGDMA as a crosslinker. Unfortunately, the content of HEMA of this sample cannot be measured by ^{13}C-NMR spectrometry because its crosslinking density was very high and did not swell in trichloromethane-d_3. However, it can be said that almost all of the HEMA fed was incorporated into the microspheres based on above results (Runs 258 and 273). Because a higher fraction of EGDMA was used in the preparation of porous microspheres, the copolymerizations between HEMA and other monomers were higher as mentioned above. As a result, HEMA can be incorporated into the microsphere quantitatively. The uniform microsphere containing HEMA with uniform fine pores is considered to be very useful for such applications as carriers of drugs in the drug delivery system (DDS), packing materials for bioseparation, carriers of enzymes, etc.

This study clarified that even a very hydrophilic monomer can be incorporated into microspheres by using the swelling method of uniform hydrophobic droplets which was prepared by the SPG emulsification method, if the composition of a secondary emulsion was proper and an adequate type and amount of crosslinker was used. This result proved further that the SPG emulsification technique and swelling method of droplets were the potential tools to prepare hydrophobic, hydrophilic, and very hydrophilic microspheres. Studies on the preparation of functional microspheres such as polydimethylaminomethacrylate and polyimide and the incorporation of functional materials such as magnetite into these particles are being carried out; the results will be reported in succession.

CONCLUSION

The uniform microspheres containing a very hydrophilic monomer HEMA can be prepared successfully by using a special swelling method of uniform hydrophobic droplets which were prepared by the SPG emulsification technique followed by a polymerization process, although the droplet with a high content of HEMA cannot be prepared directly by the SPG emulsification technique. The most adequate swelling conditions were clarified. Compared with the direct emulsification method, a higher fraction of HEMA can be incorporated into the microspheres by the

swelling method. Also, in the swelling method, a higher fraction of HEMA can be incorporated into the microspheres by using HEMA/MMA rather than HEMA/St as the secondary oil phase. A maximum fraction of 37.1 mol % of HEMA could be incorporated into microspheres. The problem of low final conversion where HEMA/MMA was used can be overcome by using an adequate amount of EGDMA as the crosslinker. Various microspheres with different morphologies such as a snowmanlike microsphere with a hole in the head, a popcornlike microsphere, a spherical microsphere with smooth surface, and a porous microsphere were obtained by varying the composition of the oil phase. Such microspheres with a high content of HEMA are very useful for various bioapplications and the preparation of polymer–magnetite composite microspheres.

REFERENCES

1. H. J. Sanders, *Chem. Eng. News,* **Apr. 1,** 31 (1985).
2. K. M. Scholsky and R. M. Fitch, *J. Control. Release,* **3,** 87 (1986).
3. H. Kawaguchi, H. Amagasa, T. Hagiya, N. Kimura, and Y. Ohtsuka, *Colloids Surf.,* **13,** 295 (1985).
4. S. Omi, K. Kaneko, M. Takesue, H. Tsujimura, A. Sato, and M. Iso, *J. Appl. Polym. Sci.,* **51,** 1239 (1994).
5. T. Imai, Y. Sumi, M. Hatakeyama, K. Fujimoto, H. Kawaguchi, N. Hayashida, K. Shiozaki, K. Terada, H. Hajima, and H. Handa, *J. Colloid Interf. Sci.,* **177,** 245 (1996).
6. Y. Kasuya, K. Fujimoto, H. Kawaguchi and M. Miyamoto, *Biomaterials,* **15,** 570 (1994).
7. Y. Nakao, M. Yabuta, and A. Tominaga, *Prog. Org. Coat.,* **20,** 369 (1992).
8. E. G. Bobalek, E. R. Moore, S. S. Kevy, and C. C. Lee, *J. Appl. Polym. Sci.,* **8,** 625 (1964).
9. J. Kumano, H. Hironori, and Hisakazu, *Polym. Prepr. Jpn.,* **29**(6), 1081 (1980).
10. K. Makuuchi, A. Katakai, and N. Nakayama, *Radiat. Phys. Chem.,* **18,** 623 (1980).
11. K. Makuuchi, A. Katakai, and N. Nakayama, *J. Coat. Tech.,* **55,** 28 (1983).
12. T. Shiraishi, T. Ishiguro, T. Yoneda, T. Kagiya, and T. Nomura, *Polym. Prepr. Jpn.,* **29**(3), 406 (1980).
13. C. M. Miller, J. Venkatesan, C. A. Sibeli, E. D. Sudol, and M. S. El-Aasser, *J. Colloid Interf. Sci.,* **162,** 11 (1994).
14. W. D. Harkins, *J. Chem. Phys.,* **13,** 381 (1945).
15. F. Hoshino, H. Kawaguchi, and Y. Ohtsuka, *Polym. J.,* **19,** 1157 (1987).
16. J. Ugelstad, P. C. Mork, K. H. Kaggerud, T. Ellingsen, and A. Berge, *Adv. Colloid Interf. Sci.,* **13,** 101 (1980).
17. M. Okubo, M. Shiozaki, M. Tsujihiro, and Y. Tsukuda, *Colloid Polym. Sci.,* **269,** 222 (1991).
18. M. Okubo, K. Kanaida, and M. Fujimura, *Colloid Polym. Sci.,* **269,** 1257 (1991).
19. S. Omi, K. Katami, A. Yamamoto, and M. Iso, *J. Appl. Polym. Sci.,* **51,** 1 (1994).
20. S. Omi, K. Katami, T. Taguchi, K. Kaneko, and M. Iso, *J. Appl. Polym. Sci.,* **57,** 1013 (1995).
21. S. Omi, K. Katami, T. Taguchi, K. Kaneko, and M. Iso, *Makromol. Symp.,* **92,** 309 (1995).
22. S. Omi, T. Taguchi, M. Nagai, and G.-H. Ma, *J. Appl. Polym. Sci.,* **63,** 931 (1997).
23. S. Omi, *Colloids Surf. A Physicochem. Eng. Aspects,* **109,** 97 (1996).
24. K. Kataoka, T. Okano, N. Yui, and Y. Sakurai, in *Bio-compatible Polymer,* The Society of Polymer Science, Japan, Ed., Kyoritushuppan, Tokyo, 1988.
25. M. Okubo, H. Hattori, and I. Azuma, in *6th Polymeric Microspheres Symposium (Japan),* Nov. 8–10, 1990, pp. 143–146.
26. M. Sakagawa, K. Fujimoto, and H. Kawaguchi, in *8th Polymeric Microspheres Symposium (Japan),* Nov. 9–11, 1994, pp. 79–82.
27. H. R. Sheu, M. S. El-Aasser, and J. W. Vanderhoff, *J. Polym. Sci. Polym. Chem. Ed.,* **28,** 629 (1990).
28. K. Arai, G.-H. Ma, and T. Hirata, *J. Appl. Polym. Sci.,* **42,** 1125 (1991).
29. G.-H. Ma and T. Fukutomi, *J. Appl. Polym. Sci.,* **43,** 1451 (1991).