Preparation of Uniform-Sized Polystyrene−Polyacrylamide Composite Microspheres from a W/O/W Emulsion by Membrane Emulsification Technique and Subsequent Suspension Polymerization

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ABSTRACT: Uniform-sized polystyrene/polyacrylamide (PST−PAAm) composite microspheres were prepared from a water/oil/water (W/O/W) emulsion by using a glass membrane emulsification technique. An AAm aqueous solution was emulsified into an oil phase containing ST, initiator, Span 85, and PST by ultrasonification to obtain W/O primary emulsion. Then, the W/O emulsion was pressed through the uniform pores (5.25 μm) of a SPG (Shirasu porous glass) membrane into an outer aqueous solution to form uniform-sized W/O/W emulsion. PST was added to increase the viscosity of oil phase, retarding the phase separation between inner water phase and oil phase during the membrane emulsification process. After the W/O/W emulsion was polymerized, the uniform-sized composite particles around 20 μm were obtained. It was found that the spherical PAAm microdomains were distributed inside and on the surface of a composite particle; the number of PAAm domains on the surface was controllable by varying cross-linking density of the PST phase.

Introduction

The uniform-sized polymer particles with the diameter from several micrometers to 100 μm have been utilized for various applications such as spacers for a liquid crystal display,[1] toners,[2] carriers for immobilization of enzymes,[3] and packing media for chromatography.[4] However, the preparation of uniform micron-sized particles was not so easy. There are few methods to prepare relatively large particles around or more than 10 μm in diameter by one-step polymerization in the aqueous phase. Emulsion polymerization, soap-free emulsion polymerization, miniemulsion polymerization, and microemulsion polymerization only provide submicron-sized uniform microspheres. Although microsized uniform microspheres is able to be prepared by dispersion polymerization, organic solvent is usually used, and it is difficult to prepare large particle above 5 μm. Conventional suspension polymerization can provide large particles; however, the size distribution of the particles is very broad. Fractionation must be carried out to obtain the microspheres with the desired size.

Vanderhoff et al. employed a repeated seeded polymerization process in a gravity-free space and successfully obtained uniform spheres up to 30 μm.[5] Ugelstad developed an "activated swelling method" to prepare microsized microspheres by using seeded polymerization.[6] The detailed process is as follows. A water-insoluble low-molecular-weight compound or oligomer (Y compound) is incorporated into the seed particle at the first step; then the seed particle containing Y compound is swollen by monomer at the second step. They found that the seed particle can absorb much more monomer (3000-fold monomer of the seed) after incorporating Y compound;[7] therefore, a lager particle can be obtained by postpolymerization. By using water-insoluble Y compound, the thermodynamical limitation of monomer swelling in a seed particle presented by Morton et al.[8] was overcome.

Okubo et al. developed a "dynamic swelling method" to prepare a uniform large particle (several micrometers) in the polar organic media by seeded dispersion polymerization. First, they dispersed the seed, monomer, and hydrophobic initiator into the methanol/water mixture and then continuously added water slowly into the system to decrease the solubilities of the monomer and initiator in the medium and allow them to be absorbed by the seed particles. By this method, a 1.8 μm sized monodispersed polystyrene particle absorbed 100 times the styrene monomer; finally, a 6.1 μm sized monodispersed polystyrene particle was obtained by subsequent polymerization. Furthermore, they devised a cooling process after the dynamic swelling process to further decrease the solubility of the monomer in the medium. As a result, a 7.7 μm sized monodispersed particles was obtained.[9]

Membrane emulsification technique is an attractive technique for preparing emulsions because the uniform-sized droplet can be prepared with low energy and low shear. Shirasu porous glass (SPG) membrane is a special glass membrane, which possesses a very uniform pore size distribution. The fabricated process of SPG membrane was developed by Nakashima et al.;[10] the SPG membrane was commercially available now with a free choice of the nominal pore size ranging from 0.1 to 18 μm. The membrane is composed of hydrophilic $SiO_2-Al_2O_3$. The O/W emulsions with uniform-sized oil droplets can be obtained due to the uniformity of the pore size, by pressing the oil phase through the pores of the membrane into the aqueous phase containing stabilizer and surfactant, under an adequate pressure. Fairly uniform droplets with the diameter from submi-

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cron to above 100 μm are able to be obtained. The coefficient of variation (CV) is usually around 10% due to narrow distribution of pore size of the membrane.

The authors developed various uniform-sized large microspheres by combining SPG membrane emulsification technique and polymerization process or other solidification process of the droplets, such as polystyrene microspheres,[11] poly(methyl methacrylate) microspheres,[12] poly(styrene-*co*-2-hydroxyethyl methacrylate) microspheres,[13] poly(styrene-*co*-(*N*,*N*-dimethylamino)ethyl methacrylate) microspheres,[14] magnetite−polymer microspheres,[15] TiO_2−polymer microspheres,[16] poly(urethane urea) microspheres,[17,18] and so forth. All above studies were about polymerization after oil droplets (O/W emulsion) were prepared.

In this study, we attempted to prepare uniform hydrophilic−hydrophobic composite particle by pressing W/O primary emulsion through the pores of the membrane into the aqueous phase to form uniform-sized W/O/W emulsion. As a first step, an acrylamide (AAm) inner aqueous solution was dispersed in a styrene solution to form a stable W/O emulsion, the W/O emulsion was then permeated through the membrane pores into an outer aqueous solution containing stabilizer, and the resulting W/O/W emulsion was polymerized.

This is a first attempt to prepare uniform-sized hydrophilic−hydrophobic composite microspheres by combining W/O/W process and SPG emulsification technique. One difficulty by this combined process is that W/O primary emulsion should be very stable during the SPG emulsification process. Another problem is that AAm monomer and PAAm microdomains prefer to diffuse into the outer phase during the polymerization. Furthermore, it is unknown how the size distribution and morphology are affected by polymerization conditions.

Experimental Section

Materials. All the chemicals were reagent grade and were purchased from Wako Pure Chemical Industries, Ltd., unless specified.

Styrene was distilled under a reduced pressure. Divinylbenzene (DVB, 55% purity, and the rest consisting of 40% ethylvinylbenzene and 5% saturated compounds) and ethylene glycol dimethacrylate (EGDMA) were washed with 5% sodium carbonate solution three times and with distilled and deionized (DDI) water five times and then dried with 4 Å molecular sieve. They were stored in a freezer before use. Acrylamide (AAm) and *N*,*N*′-methylenebis(acrylamide) (MBAAm) were used as received.

A water-soluble initiator, potassium persulfate (KPS), and an oil-soluble initiator, 2,2′-azobis(2,4-dimethylvaleronitrile) (ADVN), were used as received. Poly(vinylpyrrolidone) K-30 (PVP, MW = 40 000) and sodium lauryl sulfate (Merck, biochemistry grade) (SLS) were used as a mixed stabilizer in an aqueous phase. Sorbitan trioleate (Span 85) was used as an oil phase stabilizer. Sodium sulfate, anhydrous, was used as an electrolyte. Hydroquinone (HQ), sodium nitrite ($NaNO_2$), and diaminophenylene (DAP) were used as water-soluble inhibitors to prevent secondary nucleation in the outer aqueous phase. Phosphotungstic acid was used as a staining agent of AAm domains. Methanol (commercial grade) was used for precipitating polymers from an emulsion sample.

Self-made polystyrene (PST) was added in the oil phase to adjust the viscosity of the oil phase. The PST with lower molecular weight ($M_w = 3.1 \times 10^4$) was prepared by solution polymerization, and those with higher molecular weights were prepared by initiator-initiated emulsion polymerization ($M_w = 8.9 \times 10^4$) and thermally initiated emulsion polymerization ($M_w = 8.9 \times 10^5$).

Table 1. Standard Recipe for Preparing W/O/W Emulsion

phase/ingredient	weight/g
(W) inner aqueous phase	
water	2.0
AAm	2.0
MBAAm	0−0.2 (max10 wt % of AAm)
KPS	0, 0.01
(O) oil phase	
styrene	12.6
DVB or EGDMA	0−1.26 (max 10 wt % of styrene)
ADVN	0.3
Span 85	0.8
PST	0.075
(W) outer aqueous phase	
water	225
PVP	2.0−4.0
SLS	0.075
sodium sulfate	0.2
HQ, $NaNO_2$, or DAP	0.1

Preparation of W/O/W Emulsion. A standard recipe for the preparation of W/O/W emulsion is shown in Table 1. An inner aqueous solution (W) dissolving AAm, and desired amounts of KPS and MBAAm was mixed with an oil solution (O) composed of styrene, ADVN, Span 85, PST, and a desired amount of the cross-linking agent (DVB or EGDMA). The mixture was treated with an ultrasonic homogenizer (UH-150, SMT Co., Japan) for 3 min and then fed in an oil tank of the SPG membrane emulsification kit (Ise Chemical Co. Ltd.). The stainless steel module, in which a cylindrical glass membrane was inserted, was immersed in a 300 mL beaker containing an outer aqueous solution (W).[9−11] By applying a pressure to the oil tank, the W/O primary emulsion was permeated through the pores of the membrane into the outer aqueous phase to form the uniform-sized W/O/W emulsion. The detailed SPG emulsification process referred to the preparation of O/W single emulsion.[19] The membrane with a pore size of 5.25 μm was used in this study, and the pressure was controlled around 0.06 ± 0.01 kgf/cm^2.

Polymerization. After the emulsification was completed, the W/O/W emulsion was transferred to a 500 mL glass separator flask equipped with a nitrogen inlet, a condenser with a nitrogen outlet, and a half-moon blade for agitation. The nitrogen gas was gently introduced in the emulsion for 1 h to remove dissolving air. Then, the nitrogen inlet was lifted up to above the emulsion surface, the temperature was raised to 70 °C, and the polymerization was carried out for 20 h.

Analysis. Monomer conversion was determined gravimetrically. After the polymerization, 6 g of samples was taken from a final emulsion, and the polymer was precipitated by adding methanol. Then, the polymer was separated by a centrifuge, washed with methanol more than three times, and dried in a vacuum drier at room temperature. The monomer conversion was calculated from an averaged dry polymer weight.

An optical microscope (BHC-313, Olympus) (OM), a scanning electron microscope (JSM-5300, JEOL) (SEM), and a transmission electron microscope (H700H, Hitachi) (TEM) were used for the observations of particle morphologies. 300 monomer droplets and polymer particles were picked up from OM photographs, and their diameters were measured to calculate the average diameter and its distribution. The size distribution was expressed by a CV (coefficient of variation) value, which is defined as

$$\mathrm{CV} = (\sum (d_i - d)^2/N)^{1/2}/d$$

where d_i is the diameter of the ith particle, d is the number-average diameter, and N is the total number of particles counted.

For SEM observation, the specimens were prepared by diluting the final emulsion with 3-fold DDI and then dropping it on an aluminum film attached on a brass stub. Then, a thin

gold film was coated on the specimen under reduced pressure below 8 Pa with a JFC-1200 fine coater (JEOL).

The TEM specimens were prepared by cutting the ultrathin films (ca. 100 nm in thickness) from the particles embedded in epoxy resin with an RMC MT-7000 ultra-microtome (ATOM TECH Ltd., England) and setting them on the copper meshes. The PAAm domain was stained by immersing an ultrathin film (on the copper mesh) in a 2−4 wt % phosphotungstic acid solution.

The composition of PAAm/polystyrene composite particle was measured qualitatively with a FT-IR (Avatar 360, Nicolet) spectroscopy by employing a KBr tablet method.

Results and Discussion

General Descriptions of Emulsification. The necessary conditions for preparation of W/O/W double emulsion with narrow size distribution by SPG emulsification technique are that the W/O emulsion primary emulsion should be stable during the emulsification, and the interfacial tension between the W/O emulsion and the pores of the membrane should be high. To improve the stability of W/O primary emulsion, that is, to prevent the phase separation between the inner water phase and oil phase, it is necessary to add a high amount of emulsifier in the oil phase. However, it will decrease the interfacial tension between the W/O emulsion and the SPG membrane. It was found that the concentration of Span 85 in the total amount of W/O emulsion should be limited below 5 wt % to obtain W/O/W emulsion with uniform size. Otherwise, the pores of the membrane will be wetted, resulting in a jetlike steam to form polydispersed droplets. At this concentration of Span 85, the phase separation between the inner water phase and oil phase occurred within 15 min. However, it needed more than 1 h to finish the membrane emulsification process.

Therefore, a small amount of PST with high molecular weight was added in the ST monomer (oil phase). It was considered that an addition of PST could increase the viscosity of the oil phase, so that the phase separation between the inner water phase and oil phase would be retarded. Three kinds of PST with the molecular weights of $M_w = 3.1 \times 10^4$, 8.9×10^4, and 8.9×10^5 g/mol were tested. It was found that adding a small amount (0.075 g) of PST with the highest molecular weight ($M_w = 8.9 \times 10^5$ g/mol) in the ST oil phase (Table 1) yielded a remarkably stable W/O emulsion; phase separation was not observed within 7 days. The viscosity of the oil phase was 251 mPa s and decreased to 76 mPa s for the W/O primary emulsion. Adding a small amount of PST in the oil phase has another advantage; it can retard ST monomer from diffusing into the outer aqueous phase (Oswald ripening degradation) to keep the stability of W/O/W emulsion during the subsequent polymerization because PST is a water-insoluble polymer. El-Aasser et al. found that adding polymer in the monomer phase can prevent monomer from diffusing into the aqueous phase (Oswald ripening degradation), thus preventing the secondary nucleation in the aqueous phase during the W/O miniemulsion polymerization.[20] Although Oswald ripening degradation can also be prevented by adding water-insoluble hexadecane (HD) in the oil phase,[21] we found that the low density of HD accelerated the phase separation of W/O primary emulsion. However, adding PST can overcome theses difficulties.

After the SPG emulsification of W/O primary emulsion, W/O/W emulsion with uniform droplet size was obtained. A typical OM photograph of an W/O/W emulsion (run 03, Table 2) is shown in Figure 1. The average diameter of monomer droplets of run 03 was 21.4 μm, and the coefficient of variation (CV) was 7.0%, indicating that W/O/W with narrow size distribution of droplet can also be prepared by SPG membrane emulsification if the primary emulsion is stable and oil phase is hydrophobic enough.

Table 2. Effect of Water-Soluble Inhibitor on the Polymerization Results of Composite Particles[a]

run no.	inhibitor	d_e/μm	CV of d_e/%	d_p/μm	CV of d_p/%
03	HQ	21.4	7.0	14.9	11.0
06	$NaNO_2$	20.4	9.2	1.5	40.0
07	DAP	22.3	10.5	20.6	11.5

[a] d_e = number-average diameter of droplet before polymerization. d_p = number-average diameter of polymer particle after polymerization. Other conditions: KPS = 0; DVB or EGDMA = 0; MBAAm = 0; PVP = 2 (g); the amount of inhibitor = 0.1 (g). Refer to Table 1.

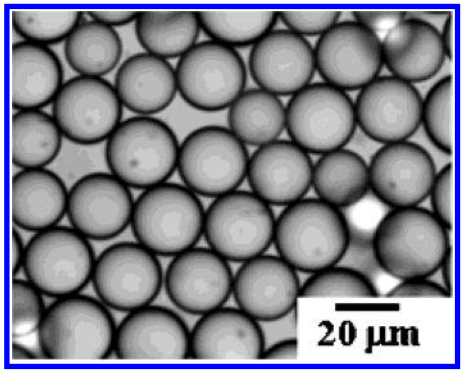

Figure 1. Typical OM photograph of W/O/W emulsion prepared by the SPG emulsification technique.

Effect of Water-Soluble Inhibitor on the Preparation of Composite Particles. It was found that the monomer would diffuse into the aqueous phase to generate the secondary nucleation besides the polymerization inside the droplets even when the monomer such like styrene had quite low solubility in the water. Therefore, it is necessary to add the water-soluble inhibitor into the aqueous phase to inhibit the secondary nucleation. Three kinds of water-soluble inhibitors were tested separately. The preparative results are shown in Table 2, and the SEM photographs are shown in Figure 2. It was found that the diameter decreased largely, from 21.4 μm of the droplet to 14.9 μm of the particles when HQ was used. This result suggested that a large amount of AAm and ST diffused into the aqueous phase to form the secondary particles. When $NaNO_2$ was used, the diameter decreased to 1.5 μm and a lot of coagulum formed, suggesting that $NaNO_2$ was not effective for the inhibition of the secondary nucleation in the AAm/St polymerization system. The SEM observation was unable to be carried out because of the large amount of coagulum. On the other hand, when the DAP was used, the diameter of the particle did not change much compared with that of droplet before polymerization. The droplet size before polymerization was 22.3 μm with a CV value of 10.5%, and the diameter of polymer particle was 20.6 μm with a CV value of 11.5%. Furthermore, the surface of the composite particle was smooth compared with the case of HQ.

Therefore, DAP was used as a water-soluble inhibitor in the following experiments. On the other hand, although the secondary nucleation was prevented ef-

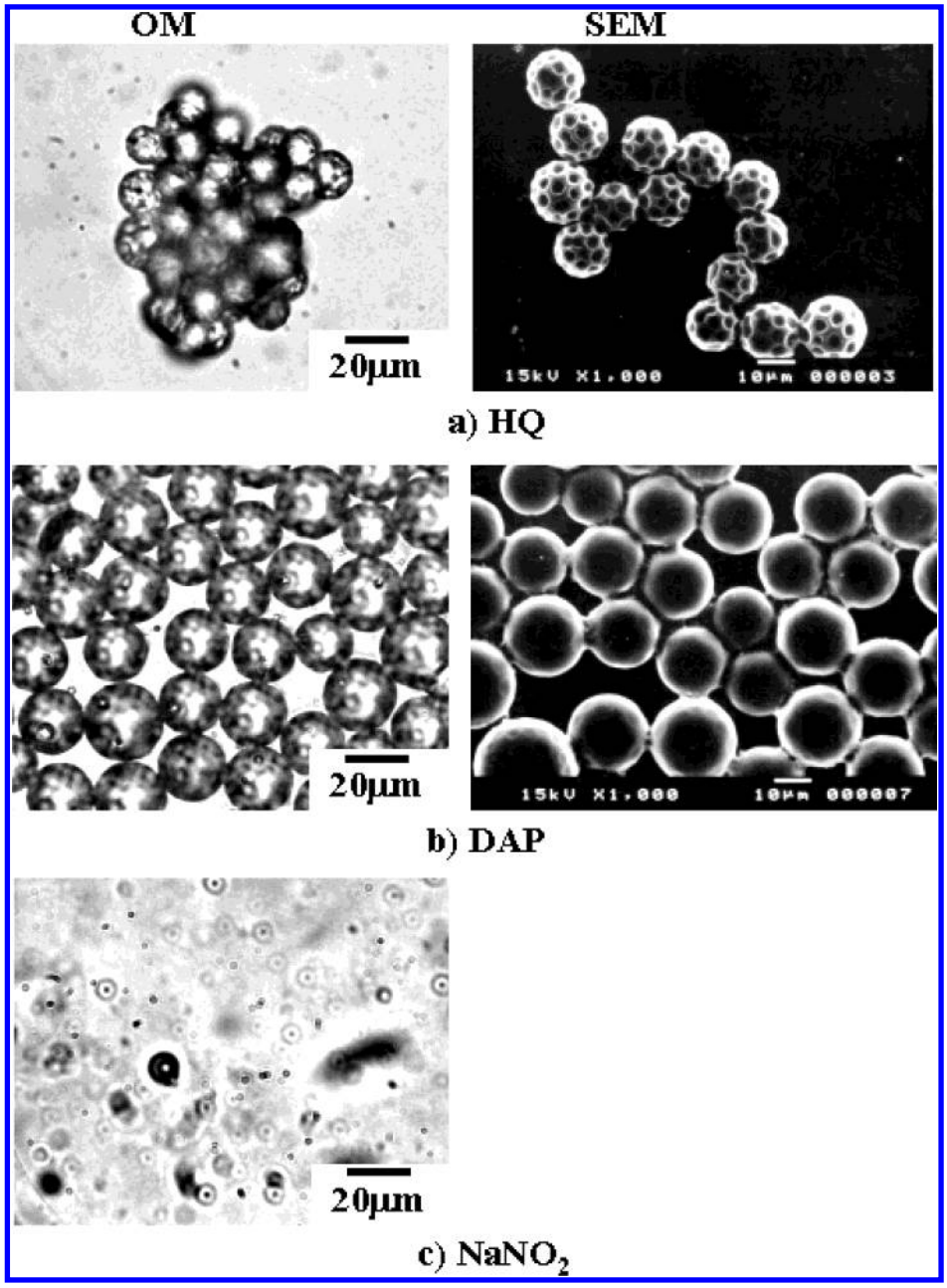

Figure 2. OM and SEM photographs of composite particles showing effect of water-soluble inhibitor. Inhibitor: (a) HQ; (b)DAP; (c) $NaNO_2$. Left side, OM; right side, SEM.

Table 3. Effect of PVP Amount on the Polymerization Results of Composite Particles[a]

run no.	PVP amount/g	d_e/μm	CV of d_e/%	d_p/μm	CV of d_p/%	particle yield/%	coagulum/g	total conversion/%
19	2.0	22.1	7.0	21.6	8.0	48.8	42.4	91.2
18	3.0	21.1	10.4	20.9	9.1	80.8	17.2	98.0
13	4.0	21.7	10.2	20.7	8.6	82.4	1.4	83.8
21	5.0	21.4	10.5	21.4	10.7	90.2	8.7	98.9
16	6.0	20.8	9.2	20.8	9.2	68.2	24.0	92.1
17	7.0	22.0	8.2	20.9	14.9	18.9	58.8	77.8

[a] d_e = number-average diameter of droplet before polymerization. d_p = number-average diameter of polymer particle after polymerization. Other conditions: KPS = 0; DVB or EGDMA = 0; MBAAm = 0; water-soluble inhibitor DAP = 0.1 (g). Refer to Table 1.

fectively when DAP was used, the coagulum usually still formed. Therefore, the effect of other parameters was investigated in the following experiments.

Effect of PVP Stabilizer on the Preparation of the Composite Particle. It was found that the addition amount of stabilizer PVP showed an apparent effect on the stability of composite particles after polymerization. As shown in Table 3, PVP was varied from 2 to 7 g in 225 g of water. The polymerization results are summarized in Table 3, the effect of PVP on particle yield and coagulum is shown in Figure 3, and the SEM photographs of obtained particles are shown in Figure 4. From Figure 4, it was evident that the formation of coagulum was prevented effectively when PVP increased to 4 g. However, when PVP was increased further, the amount of coagulum increased again, prob-

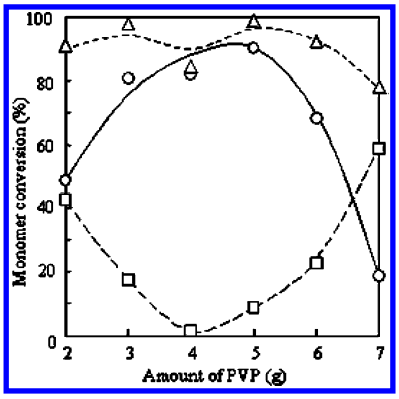

Figure 3. Effect of PVP amount on the particle yield and formation of coagulum: (○) particle yield; (□) coagulum; (△) total monomer conversion.

ably due to the bridging effect of too much free PVP. It can also be known from Figure 4 that the particles coagulated easily when the addition amount of PVP was 2 or 7 g. Therefore, it can be concluded that the suitable addition amount of PVP was 4−5 g.

Effect of Cross-Linking Agent on the Preparation of Composite Particles. It is interesting to know how the cross-linking agent affects the particle yield and the morphology of hydrophilic−hydrophobic composite particles. The cross-linking agent will increase the cross-linking rate,[13] so that the increased viscosity and the network inside the particle can affect the morphology and retard the monomer diffusion into the outer aqueous phase.

DVB, EGDMA, and the water-soluble cross-linking agent MBAAm were used to investigate their effects on the morphology and particle yield. The results are shown in Table 4. Here, water-soluble initiator was also added to increase the monomer conversion of AAm. It was found that the particle yield increased and coagulum decreased with the increase of cross-linking agent,

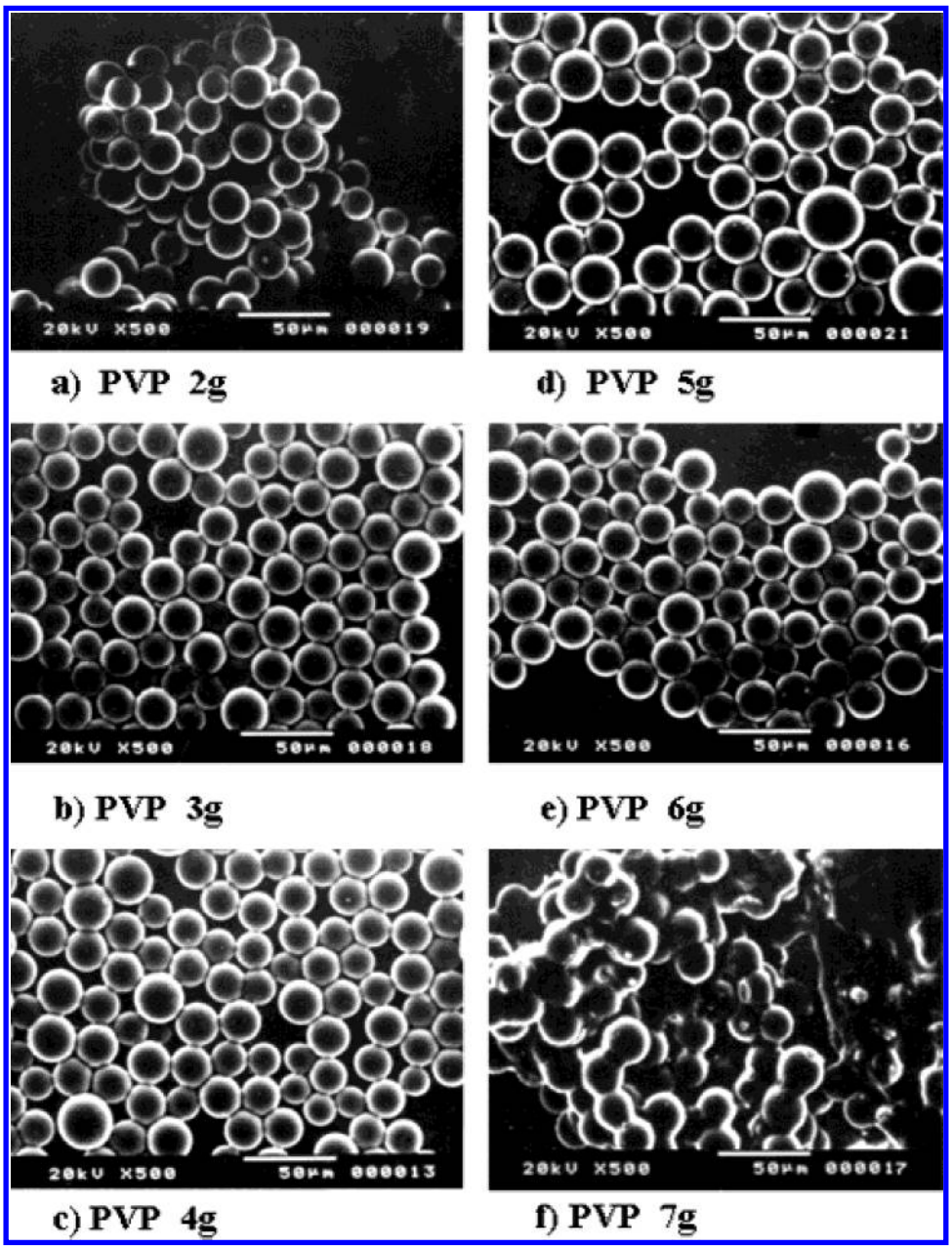

Figure 4. SEM photographs of composite particles showing effect of PVP amounts. PVP amount in 225 g of water (g): (a) 2, (b) 3, (c) 4, (d) 5, (e) 6, and (f) 7.

Table 4. Effect of Cross-Linking on the Polymerization Results of Composite Particles[a]

	cross-linking agent/wt %							
run no.	DVB[b]	EGDMA[b]	MBAAm[c]	d_p/μm	CV/%	particle yield/%	coagulum/%	total monomer conv/%
24	0	0	0	21.8	9.6	76.8	6.3	83.1
34	0.5	0	0	21.5	8.3	82.4	5.4	87.8
28	2.0	0	0	21.3	9.7	95.3	3.6	98.9
29	6.0	0	0	21.2	9.0	98.4	1.6	100.0
25	10.0	0	0	21.3	9.7	98.8	1.2	100.0
38	0	2.0	0	21.7	8.9	96.2	3.8	100.0
37	0	6.0	0	22.0	8.7	99.0	1.0	100.0
36	0	10.0	0	21.6	9.3	100.0	0	100.0
32	0	0	10.0	20.6	9.9	67.5	2.6	70.1
35	10.0	0	10.0	21.4	9.6	82.4	1.9	84.3
39	0	10.0	10.0	21.7	9.6	96.7	0.9	97.6

[a] Other conditions: KPS = 0.1 (g); DAP = 0.1 (g); PVP = 4 (g). Refer to Table 1. d_p = number-average diameter of polymer particle after polymerization. Oil-soluble cross-linking agent. [b] Based on the amount of styrene. [c] Based on the amount of AAm. [d] Based on the total solid content.

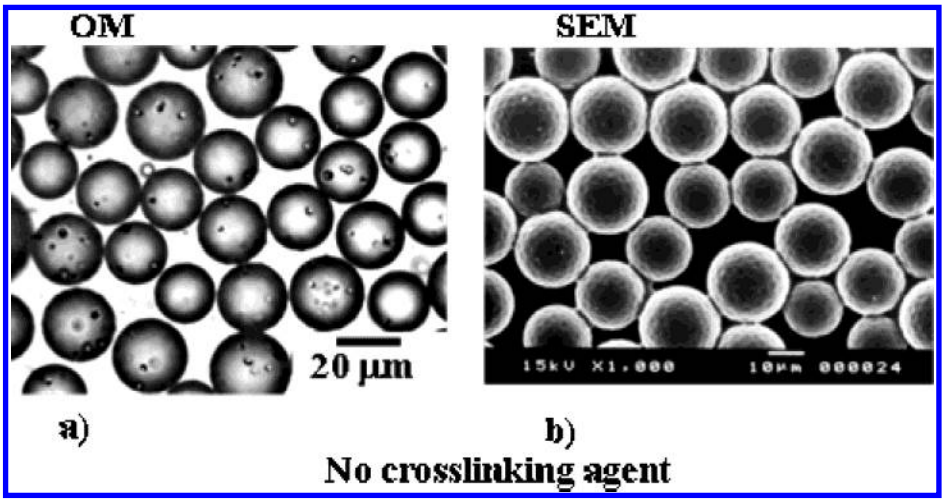

Figure 5. OM and SEM photographs of composite particle without addition of cross-linking agent: (a) OM; (b) SEM.

when DVB or EGDMA was used. For example, when the cross-linking agent was not used, the particle yield and total monomer conversion were 76.8 and 83.1 wt %, respectively, while they attained 95.3% and 98.9% with adding 2.0 wt % DVB and attained 96.2% and 100% after adding 2.0 wt % EGDMA. On the other hand, MBAAm did not show a positive effect on the monomer conversion, unless it was used together with DVB or EGDMA. This result suggested that the addition of MBAAm did not contribute to the improvement of polymerization rate of the ST. ST monomer is still easy to diffuse into the outer aqueous phase.

Figure 5 shows the OM and SEM photographs of uncross-linked PST-AAm particles (run 24, Table 4). The OM photograph (a) depicts that some tiny spherical domains of PAAm were formed. The SEM photograph (b) shows that no PAAm tiny domain exists, and the surface of the particles is golf ball-like, being covered with tiny dents. This surface feature implied that some of the PAAm domains have localized on the surface of composite particles; they were washed away from the particle, and tiny dents were formed. Because PAAm was hydrophilic, it preferred to move toward the surface during the polymerization. When the PST matrix was not cross-linked, AAm monomer and PAAm microdomains move to the surface more easily. The 100% monomer conversion in run 24 will yield a total polymer amount composed of 86.3% PST and 13.7% PAAm (calculated from Table 4). The observed 83.1% monomer conversion may indicate that the PAAm was substantially lost during the precipitation−washing cycles with methanol. Furthermore, dissolving PAAm chains in the outer aqueous phase accelerated the formation of coagulum by bridging during the polymerization.

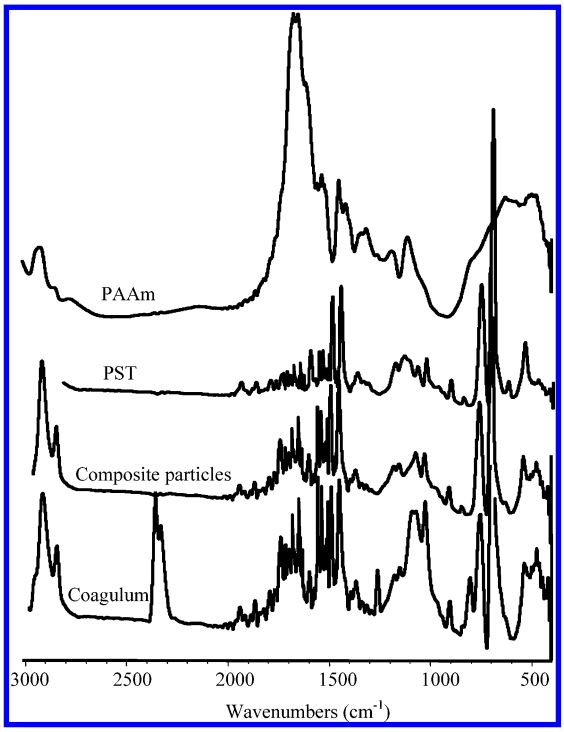

Figure 6. IR spectra of PAAm, PST, and PAAm−PST composite particle (run 24, ST:AAm = 6:1 g/g).

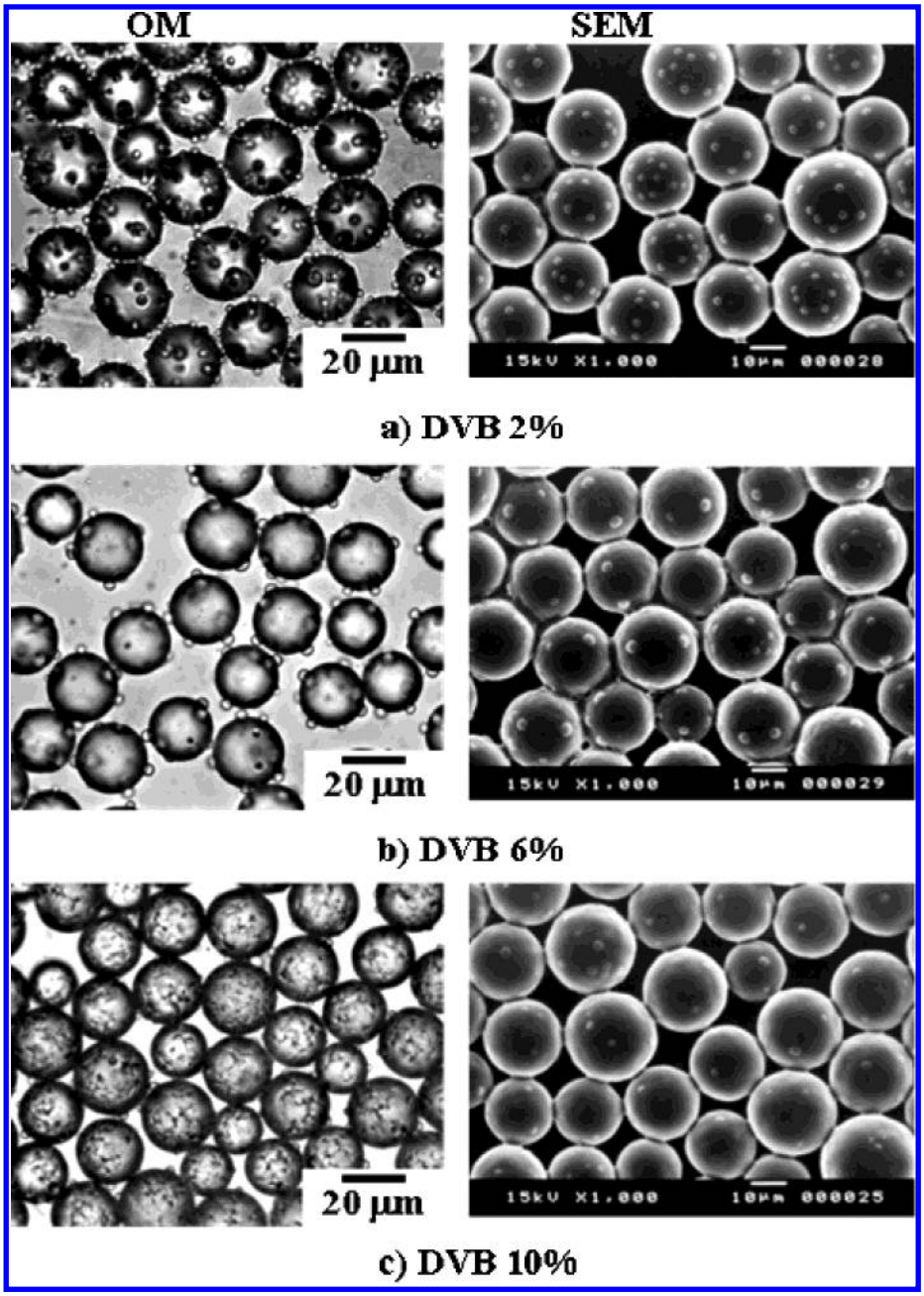

Figure 7. OM and SEM photographs of composite particles showing effect of DVB amounts. DVB amount (wt % in oil phase): (a) 2, (b) 6, and (c) 10. Left side, OM; right side, SEM.

To confirm the above assumption, the IR measurement was carried out for the particle after washing with methanol and coagulum. The results are shown in Figure 6. PAAm homopolymer shows a characteristic absorbance of the $-CONH_2$ stretch vibration at 1680 cm^{-1}. The PST−PAAm particles show a weaker absorbance at 1680 cm^{-1} than the coagulum. This result implied that a large part of PAAm moved to the surface or the outer aqueous phase, or AAm diffused into the aqueous phase to polymerize there, which worked as a bridging agent.

To restrain movement of PAAm to the surface and eventually into the aqueous phase, cross-linking of PST phase was attempted. The OM and SEM photographs of cross-linked PST−AAm composite particles with an increasing amount of DVB in the oil phase are shown in Figure 7. By comparing the OM and SEM photographs, it was known that some of tiny PAAm microdomains localized on the surface of the composite particles, and the number of PAAm domains on the particle surface decreased as DVB amount increased, implying the PAAm domains entrapped inside a particle decreased. Furthermore, it can be known that the PAAm microdomains decreased and the particle became spherical with a smooth surface when the DVB amount increased to 10 wt %, while an OM photograph showed clear phase separation inside the particle. This phenomenon confirmed again that the PAAm was retained inside the particles by the cross-linking network of PST matrix. In some of previous studies, we found that the polymerization rate and monomer conversion of PST particles could be increased by adding DVB when styrene droplet was polymerized.[13,18] Therefore, because of rapid formation of the PST network, the movement of PAAm and AAm through the PST matrix was gradually suppressed, and more PAAm domains re-

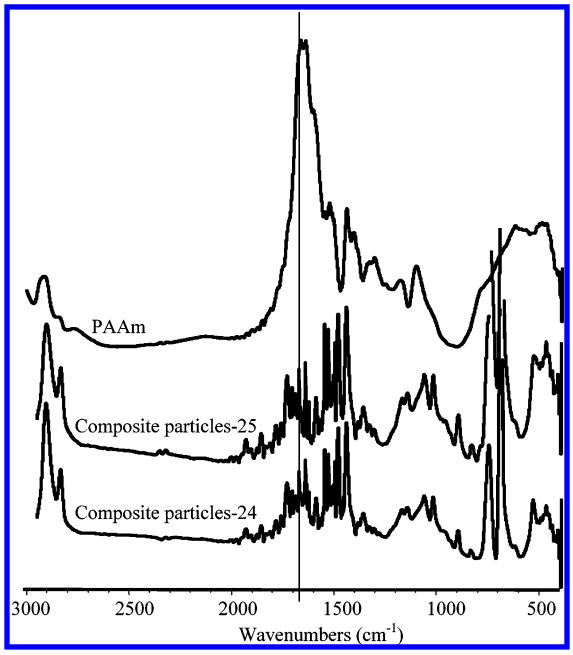

Figure 8. IR spectra of PST−PAAm composite particles showing the effect of DVB.

Table 5. Number of PAAm Microdomains Exposed on the Surface of Polymer Particle

run no.	content of cross-linking agent[a]/wt %	no. of PAAm domains	
		DVB	EGDMA
24	2	40	16
28	6	8	10
25	10	4	0

[a] wt % of cross-linking agent based on the styrene in the oil phase.

mained inside. The IR measurement was carried out to confirm the effect of cross-linking, and the results are shown in Figure 8. Comparing the cases with and without using DVB, it was evident that the absorbance at 1680 cm^{-1} of the $-CONH_2$ stretch vibration increased largely after DVB was added.

A total of 30−50 polymer particles were randomly selected from magnified SEM photographs, and the number of PAAm microdomains exposed on the surface was counted. The number was doubled except the domains exactly located on the circumference. The average number of PAAm microdomains is shown in Table 5 as a function of DVB content. The number of PAAm domains on the particle surface decreased dramatically after DVB content increased from 2 to 6 wt %.

The inside of composite particles with and without addition of DVB was observed by TEM microscopy after the particle was sliced to ultrathin films and treated by phosphotungstic acid, which stains PAAm domains selectively. The TEM photographs are shown in Figure 9. It was clear that almost no PAAm domain was observed when no cross-linking agent was used, while PAAm domains distributed inside the particle and on the particle even after the particle was washed when the DVB cross-linking agent was added. TEM observation confirmed that PAAm microdomains preferred to move the particle surface and outer aqueous phase if the PST phase was not cross-linked. However, even when the cross-linking agent was added, a part of PAAm domains were observed outside the particles as shown in Figure 9b; this was because some PAAm domains escaped out of the particles when the ultrathin film was stained by immersing it in a phosphotungstic acid solution.

The effect of EGDMA on morphology of PST−PAAM composite particles was also investigated. As shown in Table 4, an increasing amount of EGDMA promoted a nearly complete monomer conversion while suppressing the formation of coagulum. The OM and SEM photographs are shown in Figure 10. A similar result was observed; that is, the number of PAAm domains exposed on the particle surface decreased with the increase of EGDMA content. The average number of PAAm domains exposed on the surface of polymer particle is shown in Table 5. It was found that the number of PAAm domains exposed on the surface was smaller compared with the particles prepared by the corresponding amount of DVB. The main reason is that only 55% of the DVB reagent was effective for cross-linking.

Because the monomer conversion was very high when the particle was cross-linked by DVB and EGDMA, it can been considered that the PAAm domains almost can be included completely inside a particle except those on the surface. The total number of PAAm domains (N_{PAAm}) can be calculated from the feed volumes of PST (V_{PST}) and PAAm (V_{PAAm}), and diameters of PAAm small domains (d) and large spheres (D) as follows, by assuming d and D as 2 and 20 μm, respectively.

$$N_{PAAm} = (D^3/d^3)[V_{PAAm}/(V_{PST} + V_{PAAm})] \approx (D^3/d^3) \times [W_{PAAm}/(W_{PST} + W_{PAAm})] = (20/2)^3(2/14.6) = 137$$

where W_{PST} and W_{PAAm} are the feed weights of ST and AAm, respectively.

The effect of water-soluble cross-linking agent MBAAm on morphology of the composite particles was investi-

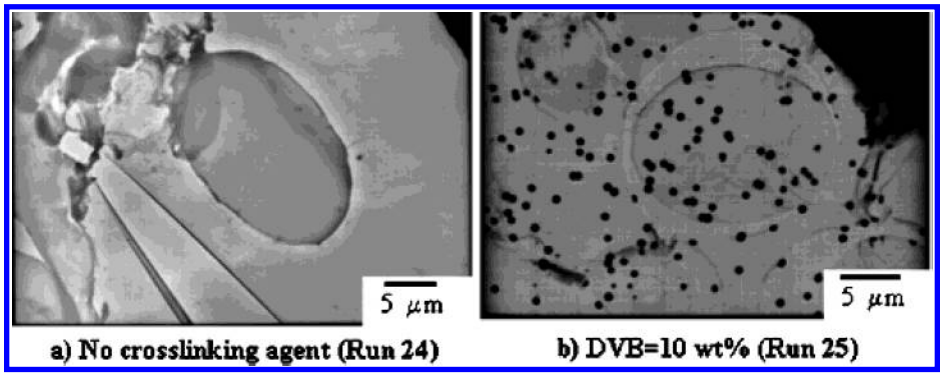

Figure 9. TEM photographs of microtomed PST−PAAm composite particles after washing with methanol (dark area: PAAm microdomains stained with phosphotungstic acid): (a) without addition of cross-linking agent; (b) with addition of 10 wt % DVB.

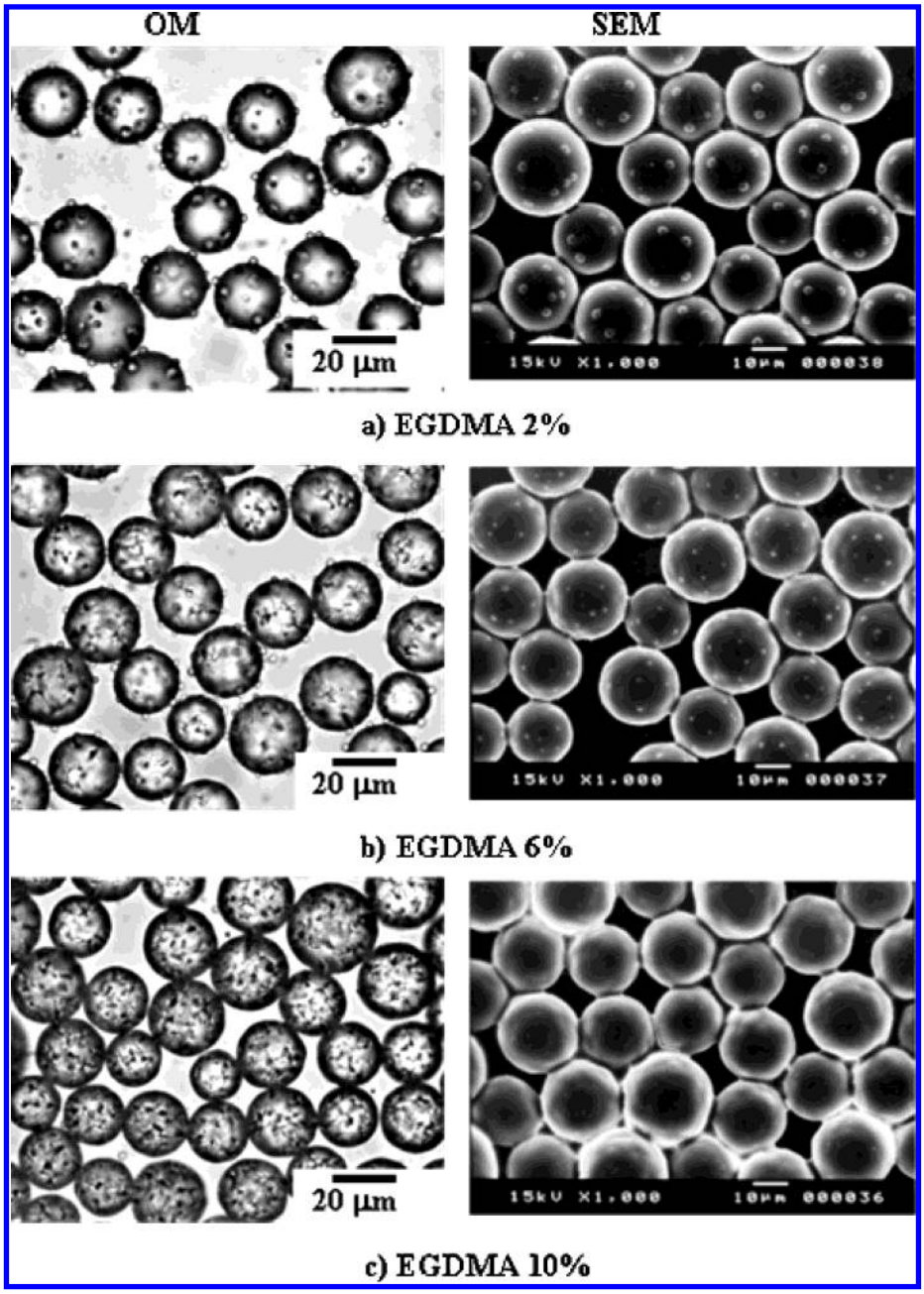

Figure 10. OM and SEM photographs of composite particles showing effects of EGDMA amounts. EGDMA amount (wt % in oil phase): (a) 2, (b) 6, and (c) 10. Left side, OM; right side, SEM.

gated. Figure 11a shows OM and SEM photographs of the composite particles. The OM image was similar to that shown in Figure 5a; however, the SEM image depicted that the dents on the surface were larger and deeper. This is understandable considering that the cross-linked PAAm domain behaves as a hydrogel and absorbs a substantial amount of water during the polymerization. The PAAm domains on the surface grew larger by the swelling with water. The SEM photographs of Figure 11b,c indicate that very few numbers of PAAm domains remained on the surface of polymer particles. In particular, when 10 wt % EGDMA was added in the oil phase (Figure 11c), almost no PAAm domain was found in the particles. The deep dents are no more to be seen.

The effect of cross-linking on morphology is shown in Figure 12 schematically. When no cross-linking agent was used, PAAm inner aqueous phase preferred to move to the particle surface and outer aqueous phase during polymerization; the dents would be formed after PAAm domains on the particle surface was washed away during purification. When a lower amount of cross-linking agent was added in the ST phase, the PAAm microdomains on the surface would not be washed away and were retained on the surface to form alternately hydrophobic−hydrophilic arranged surface. When a higher amount of cross-linking agent was added, more PAAm microdomains were restrained inside a particle, and the number of PAAm microdomains on the particle surface decreased.

From above results, it is clear that the number of the PAAm domains can be controlled by the amount of cross-linking agent added in the oil phase. As the amides can be easily hydrolyzed to carboxylic acids and are degradable to amines by Hofmann degradation reaction,[22] the PST−AAm cross-linked particle has

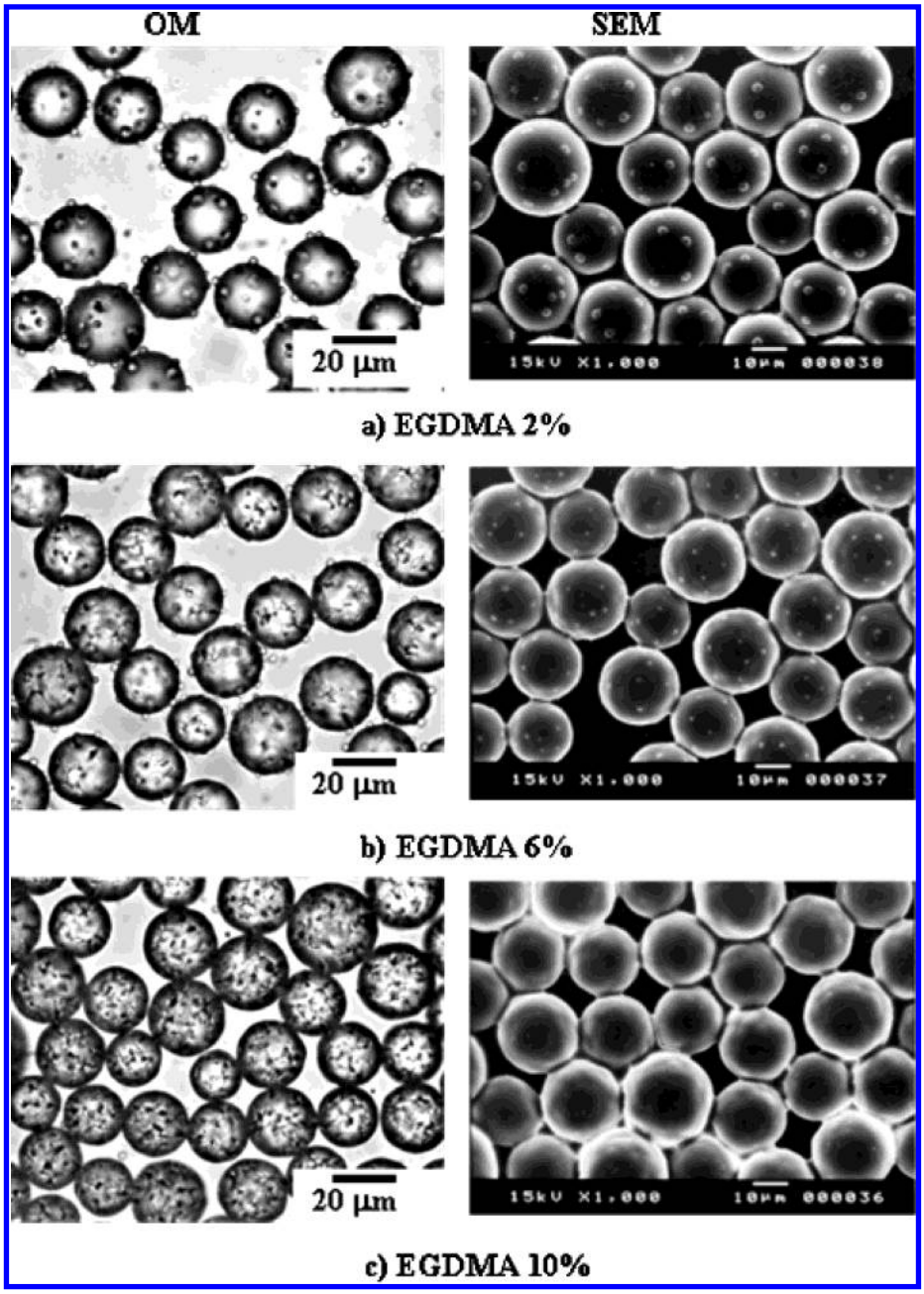

Figure 11. OM and SEM photographs of composite particles showing effects of DVB, EGDMA, and MBAAm amounts. Cross-linking amount (wt % in inner water phase or oil phase): (a) MBAAm 10 wt %; (b) DVB 10 wt %, MBAAm 10 wt %; (c) EGDMA 10 wt %, MBAAm 10 wt %. Left side, OM; right side, SEM.

potential applications for adsorption or immobilization media of active species such as proteins and cells. Okubo et al.[23,24] prepared composite microspheres with the similar surface properties by seeded polymerization and used in immobilization of enzyme, immunoactivity, and protein separation. By using such composite microspheres in the separation of bovine fibrinogen (BFb) and bovine serum albumin (BSA), the selective adsorption of BFb was observed for the composite microspheres due to the difference of steric structure of proteins. However, the homopolymer microspheres such as PST, poly(ethyl methacrylate), and poly(methyl methacrylate) did not show such selective adsorption behavior.

Okano et al.[25,26] found that the alternately arranged hydrophobic−hydrophilic domains on the film surface can prevent the adhesion of blood platelets, so that can prevent thrombosis when it was exposed to blood. On the other hand, the blood platelets adhered on the surface composed of hydrophilic poly(2-hydroxyethyl methacrylate) (PHEMA) homopolymer or hydrophobic PST homopolymer. They found that this was because of the different arrangement on the surface of antigen in the blood. Even when the surface is composed of poly-(2-hydroxyethyl methacrylate) (PHEMA) homopolymer, the hydrophilic Fab of antigen will adsorb on the surface and allow the hydrophobic Fc to expose the surface, and a large area of hydrophobic surface will induce the adhesion of platelets. The alternately arranged hydrophobic−hydrophilic surface can prevent the contact of large hydrophobic area with the platelets, therefore suppressing the adhesion of platelets on the surface.

This study also provides a basic knowledge to prepare other composite particles or microcapsules with a narrow size distribution by combining the SPG emulsification technique and the W/O/W double emulsion process. For example, the particle will show a weak adhesive

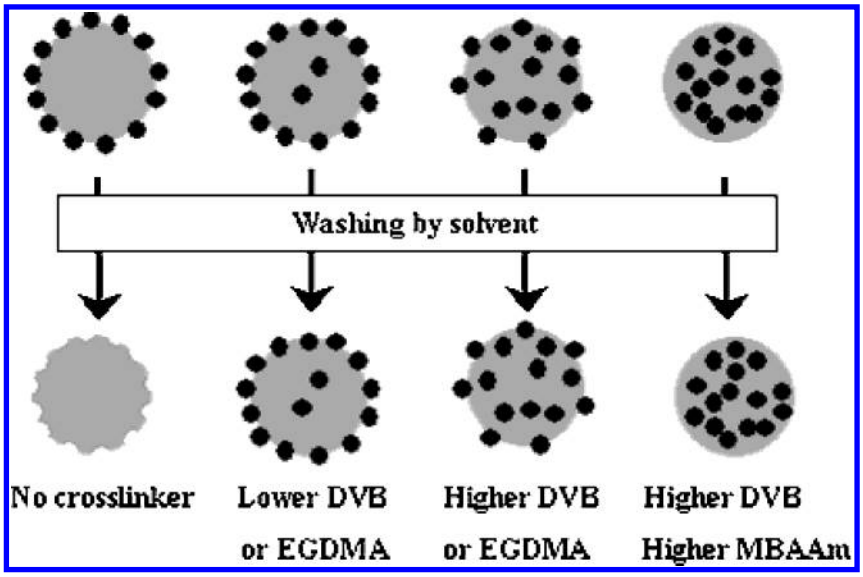

Figure 12. Scheme showing effect of cross-linking agent on particle morphology.

force if the adhesive polymer was used instead of PAAm. Such weak adhesive particles with uniform size can be used in application of Post-it and can be used as the spacer in a liquid crystal panel. Furthermore, uniform microcapsules containing hydrophilic drugs can be prepared by using drug aqueous solution as inner water phase instead of AAm aqueous solution. Also, PAAm−PSt composite particles with PSt hydrophobic small domains dispersed in a PAAm large sphere also can be prepared by preparing O/W/O emulsions with St phase as the inner oil phase.

Conclusion

The uniform-sized hydrophilic−hydrophobic composite particles with tiny spherical PAAm-rich microdomains exposed on the surface as well as buried inside were prepared from a W/O/W emulsion and subsequent suspension polymerization. The W/O emulsion was prepared using an ultrasonic mixer, and then it was pressed through the pores of a SPG membrane (pore size: 5.25 μm) into the aqueous phase to obtain uniform W/O/W emulsion, with styrene as an oil phase and AAm aqueous solution as an inner aqueous phase. The W/O phase was quite stable by increasing the oil phase viscosity with a small amount of polystyrene dissolved in styrene, so that the SPG emulsification was able to be carried out to obtain W/O/W emulsion with uniform droplet size. After polymerizing W/O/W emulsion, the PST−PAAm composite particles with the diameter around 20 μm and coefficient of variation of around 10% were obtained, when DAP was used as a water-soluble inhibitor in outer aqueous phase and an adequate amount of PVP stabilizer was added there. The numbers of the PAAm microdomains on the surface changed depending on the amount of the cross-linking agent added in the oil phase. The cross-linking of PST matrix was effective to reduce the number of PAAm domains on the surface and confined them inside the particle.

As amides can be easily hydrolyzed to carboxylic acid or degraded to amines, these PST−PAAm particles can be used as adsorption or immobilization media of biologically active substances such like proteins and cells.

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