# Synthesis of Hemispherical Poly(2-hydroxylethyl methacrylate-*co*-methyl methacrylate)/Poly(styrene-*co*-glycidyl methacrylate) Composite Particles with Heterobifunctional Groups by Soap-Free Seeded Emulsion Polymerization

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ABSTRACT: Poly(2-hydroxylethyl methacrylate-*co*-methyl methacrylate)/poly(styrene-*co*-glycidyl methacrylate) (P(HEMA−MMA))/P(St−GMA)) composite polymer particles were prepared by soap-free seeded emulsion polymerization. The P(HEMA−MMA) seed latexes cross-linked by 0, 10, and 20 wt % ethylene glycol dimethacrylate (EGDMA) were prepared by soap-free emulsion polymerization with different HEMA/MMA monomer composition at 80 °C. 2,2′-Azobis(2-amidinopropane) dihydrochloride (V-50) was used as an initiator in the two stages. The morphologies of composite particles were investigated by using seed particles with various HEMA content and cross-link degree, and the effects of solvent addition, preswelling of seed particles by monomers and solvent, and the weight ratio of secondary monomer to seed particles (M/P) on the particle morphology were discussed. Un-cross-linked P(HEMA−MMA) seed latex showed the property of gel particles capable of being swollen by water, which led to the instability of seed particles and a specific morphology variation. In principle, the addition of solvent favors to form thermodynamically stable morphology, but the preswelling process by monomers and toluene did not affect the particle morphology, possibly because the equilibrium swelling might be already attained during the nitrogen purge and heating intervals under lower M/P ratio. Various spherical and anomalous particle morphologies were obtained by controlling polymerization conditions; that is, sandwich-like, hemispherical, and microphase-separated spherical particles were formed when the seed particles with lower HEMA content and lower cross-link degree were employed under smaller M/P ratio. On the other hand, dumbbell, popcorn, and bunch of grapes like anomalous particles were formed under some specific conditions. When the cross-linker, divinylbenzene (DVB), was charged into the seed stage, the anomalous particles were changed to spherical particles due to the reduction of polymer chain mobility.

## Introduction

Polymer latexes are currently enjoying numerous applications in many fields including biochemistry, colloid science, and medicine.[1] The integration of nanotechnology with biology and medicine is expected to produce major advances in molecular diagnostics, therapeutics, molecular biology, and bioengineering. Polymeric biomaterials are widely used in both medical and pharmaceutical application. Binding of biological molecules such as proteins to latex particles can be accomplished by physical adsorption or covalent coupling. To achieve covalent coupling of biological molecules to the surface of the polymer latex, specific functional groups are used, such as oxirane,[2] acetal,[3,4] carboxylate,[5,6] aldehyde,[7] chloromethyl,[8,9] and hydroxyl groups.[10] Because of the monofunctional surface of these polymer particles, however, the utility was limited to some applications such as purification of biological molecules. Namely, the surface reactivity of conventional particles can be hardly controlled to give multifunctional anisotropic polymer particles. The objective of the present work is the synthesis of hemispherical nanosphere with different functional groups on either side, which can be stage-modified by chemical and biological molecules to conjugate with chemical and biological nanosystems.

Poly(2-hydroxyethyl methacrylate), PHEMA, is a nontoxic and biocompatible synthetic polymer with adequate mechanical strength for most biotechnological applications. Another advantage is the presence of hydroxyl groups that act as attachment sites for bioactive species after activation or conversion to other functional groups. It was found that copolymer particles produced by soap-free emulsion copolymerization of styrene and HEMA had an anomalous shape with uneven surface,[11] whose unevenness was increased with the HEMA content. Such anomalous particles were not formed by coagulation among particles but by growing with polymerization. The spherical particle could be obtained when seeded emulsion polymerization of styrene was carried out with dropwise addition of the monomer in the presence of PHEMA seed particles.

We reported in the previous paper that the polymer particle with poly(glycidyl methacrylate) (PGMA) shell surface on which cisplatin-DNA was immobilized is a very powerful tool for affinity purification of proteins.[12] The PGMA chains on the particle surface make its environments rather hydrophilic, which significantly reduces nonspecific adsorption of proteins. The oxirane groups of GMA can directly bind to amino groups of biological molecules or can be converted to another functional groups by reactions with bifunctional linkers.

Composite latex particles with different morphologies are usually prepared by seeded emulsion polymerization under different conditions, where a secondary monomer is polymerized in the presence of seed latex particles.

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By controlling the polymerization process variables, seeded emulsion polymerization can produce structured latexes exhibiting a wide variety of particle morphologies such as core−shell, hemispherical, or inverted core−shell particles in which second-stage polymer is incorporated at the center of the particle and the seed polymer is located on the periphery of the composite particle. The morphology of two-stage latex particle is controlled by two major factors concomitantly acting in the system:[13−17] thermodynamic factors that determine the equilibrium morphology of the final composite latex particles and kinetic factors that determine the ease with which the thermodynamically favored equilibrium morphology can be achieved. On the other hand, some composite polymer particles produced by seeded emulsion polymerization, which consist of two kinds of homopolymers, have confetti-like, raspberry-like,[15] and void[18] morphologies. The mechanism of producing various morphologies is based on the heterogeneous structures generated by the phase separation of the different polymers in the particles during polymerization.

In this paper, the hemispherical P(HEMA−MMA)/P(St−GMA) composite particles bearing hydroxyl and oxirane groups on either side were prepared by soap-free seeded emulsion polymerization using P(HEMA−MMA) seed particles cross-linked by EGDMA. To obtain positively charged polymer particles and prevent the ring opening of oxirane group of GMA, 2,2′-azobis(2-amidinopropane)·2HCl (V-50) was used as an initiator in the two stages of polymerization.

## Experimental Section

**Materials.** Monomers: 2-hydroxyethyl methacrylate (HEMA), methyl methacrylate (MMA), glycidyl methacrylate (GMA), and styrene (St). Initiator: water-soluble 2,2′-azobis-(2-amidinopropane)·2HCl (V-50). Cross-linker: polar ethylene glycol dimethacrylate (EGDMA) and nonpolar divinylbenzene (DVB, ca. 55% *m*- and *p*- isomer). Additive solvent: toluene. Amination reagent: 28% ammonia solution. Biotinylation reagent: sulfosuccinimidyl-D-biotin (Biotin-Sulfo-OSu). All the reagents except for DVB supplied by Tokyo Kasei Industry Co. Ltd. (Tokyo, Japan) were purchased from Wako Pure Chemical Industry Co. Ltd. (Osaka, Japan). Streptavidin labeled by 10 nm colloidal gold was purchased from Sigma-Aldrich Co. (St. Louis, MO). All the monomers were distilled under reduced pressure before use. The other reagents were used without further purification. Water was distilled and deionized (DDI) by employing a Milli-Q water purification system with the conductivity of 18.3 MΩ $cm^{-1}$ and measured pH value of 6.7 (Millipore).

**Preparation of Seed Latexes.** The seed latexes were prepared by soap-free emulsion polymerization carried out in a four-necked 300 mL separator flask, equipped with a stirrer, a spiral condenser, a dropping funnel with a nitrogen inlet, and a nitrogen inlet. After the initiator solution and DDI water were charged into the reactor, the system was bubbled with nitrogen gas for 60 min under stirring at an agitation speed of 200 rpm. At the same time, the mixture of all the monomers and the cross-link agent (EGDMA) was also bubbled with nitrogen gas for 60 min after being fed in the dropping funnel. The initiator solution was then heated for 30 min up to 80 °C with a programmed heating device. The mixture of the monomers and EGDMA was added into the reactor at a rate of 7.5 g/h when the reaction temperature reached up 80 °C. After the polymerization was carried out at 80 °C for 15 h under the nitrogen blanket, the produced seed latex was transferred into a seamless cellulose tube and dialyzed under flowing tap water for 24 h, and thereafter in DDI water for 24 h at ambient temperature, to remove the remaining monomers, oligomers, and initiator.

**Preparation of Composite Nanoparticles.** The composite nanoparticles were prepared by soap-free seeded emulsion polymerization. All the ingredients except the initiator solution were fed in a four-neck reactor with an agitator, a spiral condenser, a nitrogen inlet, and a dropping funnel with a nitrogen inlet for adding the initiator solution. On using a preswelling process, the reaction mixture was bubbled with nitrogen gas for 1 h, and then swelling of the seed particles by monomers and toluene was undertaken for 24 h at ambient temperature under stirring. Then the initiator solution (about 10 g) was charged in the dropping funnel, the whole system was bubbled with nitrogen gas for 1 h before elevating the temperature, and the initiator was added into the reactor when the reaction temperature attained to 70 °C. The polymerizations were performed for 24 h at 70 °C under the nitrogen blanket, with the stirring rate being fixed at 200 rpm.

**Characterization of Seed and Composite Nanoparticles.** The conversion of the monomers to polymer particles was determined gravimetrically; namely, a known amount of latex was dried and weighed. The $\zeta$ potential of polymer particles was measured by electrophoretic light scattering (ELS-8000, OTSUKA Electronics Co., Japan). The size and morphology of particles were observed by a transmission electron microscopy (TEM) (HITACHI H-7000H). The TEM specimen was prepared as follows. One drop of dilute latex ($1 \times 10^{-4}$ g/mL) was cast on a copper mesh covered with a thin carbon film and dried at ambient temperature, and then the sample was stained with ruthenium tetraoxide ($RuO_4$) vapor in a sealed container for 2 h at room temperature. Therefore, the dark part stands for the P(St−GMA) domain, and the gray part represents the P(HEMA−MMA) domain in the TEM photos. The particles size was determined by direct measurements of about 100 particles/sample in TEM photos.

**Confirmation of Hemisphere-like Structure of Composite Nanoparticles.** Ammonia solution (28%, 5 mL) was added into 10 g of composite latex, and then the mixture was incubated at 70 °C for 24 h and washed four times with DDI water. About 10 mg of amino-modified composite particles was dispersed into 20 mL of phosphate buffer (PB, pH 7.4) containing about 2 mg of Biotin-Sulfo-OSu, which was incubated at room temperature for 20 h with continuous stirring. The biotinylated composite particle was then washed three times with DDI water. Streptavidin labeled by 10 nm colloidal gold (0.2 mL solution) was added into about 10 mg of biotinylated composite particles dispersed in about 10 mL of phosphate buffer (PB, pH 7.4) to label the composite particles. The reaction was carried out at room temperature for 24 h with continuous stirring. The composite particles labeled by 10 nm colloidal gold were observed by TEM without any treatment.

## Results and Discussion

**Preparation of P(HEMA−MMA) Seed Latexes.** The stable un-cross-linked and cross-linked P(HEMA−MMA) seed particles were prepared by soap-free emulsion polymerization at 80 °C, using 2,2′-azobis(2-amidinopropane) dihydrochloride (V-50) as an initiator. Ethylene glycol dimethacrylate (EGDMA) was used as a cross-linker to prepare cross-linked P(HEMA−MMA) seed particles. The recipes for preparation and some properties of the seed particles are shown in Table 1. To prevent the coagulation of polymer particles[19] and to obtain more uniformly composition of copolymer in the seed, the monomers were continuously fed in all runs. The use of V-50 as an initiator for the present system is due to the following two reasons. One is to maintain the pH of final latex near to the neutral value compared with that of persulfate initiator system for preventing the ring opening of oxirane of GMA in the secondary stage, and the other is to obtain the positively charged particles for future application in the field of nanobiotechnology. Since the isoelectric points of many proteins are less than pH 7, they are negatively charged at neutral pH and above neutral pH. To maintain the

**Table 1. Recipes for Preparation and Some Properties of P(HEMA−MMA) Seed Particles[a]**

| run no. | HEMA (g) | MMA (g) | EGDMA (g) | V-50 (g) | monomer conv (wt %) | $d_p$ ($\mu$m) | $\zeta$ potential (mV) |
|---|---|---|---|---|---|---|---|
| 904 | 3.0 | 12.0 | 0 | 0.45 | 100 | 0.12 | −27.62 |
| 905 | 2.7 | 10.8 | 1.5 | 0.45 | 100 | 0.11 | −24.03 |
| 908 | 1.5 | 12.0 | 1.5 | 0.45 | 97 | 0.13 | −21.23 |
| 909 | 1.2 | 10.8 | 3.0 | 0.45 | 100 | 0.12 | 8.36 |
| 910 | 0.6 | 11.4 | 3.0 | 0.45 | 99 | 0.11 | 15.30 |
| 911 | 0.7 | 12.8 | 1.5 | 0.45 | 89 | 0.12 | 10.53 |

[a] Total reaction mixtures were 300 g. Reaction temperature, reaction time, and agitation rates are 80 °C, 15 h, and 200 rpm, respectively. In all runs, monomers are fed continuously at a rate of 7.5 g/h. $d_p$ is the number-average diameter of polymer particles.

**Table 2. Recipes for Preparation of P(HEMA−MMA)/P(St−GMA) Composite Nanoparticles[a]**

| run no. | seed latex (solid) (g) | seed run no. | styrene (g) | GMA (g) | V-50 (g) | toluene (g) |
|---|---|---|---|---|---|---|
| 1901 | 40 [2] | 904 | 1 | 0.05 | 0.02 | 4 |
| 1902 | 40 [2] | 905 | 1 | 0.05 | 0.02 | |
| 1903 | 40 [2] | 905 | 1 | 0.05 | 0.02 | 4 |
| 1904 | 40 [2] | 905 | 1 | 0.05 | 0.02 | 4[b] |
| 1905 | 40 [2] | 905 | 0.5 | 0.05 | 0.02 | 4 |
| 1906 | 40 [2] | 905 | 0.5 | 0.05 | 0.02 | |
| 1907 | 40 [2] | 905 | 0.4 | 0.1 | 0.02 | 4 |
| 1908 | 40 [2] | 905 | 0.4 | 0.1 | 0.01 | 4 |
| 1909 | 40 [2] | 905 | 2 | 0.05 | 0.04 | 4 |

[a] Reaction time, temperature, and agitation rate are 24 h, 70 °C, and 200 rpm, respectively. Total reaction mixtures are 200 g. [b] Seed particles are preswelled by both monomers and toluene in run 1904.

stability of oxirane groups of produced latex beads and the stability of proteins, neutral pH condition is necessary for protein immobilization. Therefore, the use of positively charged support can improve the immobilization efficiency of enzymes due to the electrostatic interaction between negatively charged proteins and supports[20] (particles). No coagulum was formed in all runs, although the $\zeta$ potentials of final particles were lower as shown in Table 1. There is a trend that the value was changed from about −30 to +15 mV with the decrease of charged HEMA content and the increase of cross-link degree. According to the report of Ni et al.,[21] it is thought that when the hydrophilic monomer (HEMA) content is higher and the cross-linker (EGDMA) is lower, the hairy layer of polymer particles extends into water phase, and the slipping plane is also transferred to water phase, where the potential is dominated by the counterions with minus charge. Consequently, the $\zeta$ potentials become minus. On the other hand, when the content of hydrophilic monomer (HEMA) is decreased and the content of cross-linker (EGDMA) is increased, the polymer particles become rigid and the hair layers shrink. Therefore, the $\zeta$ potential is gradually changed to plus due to the charge of fragments of V-50 in the copolymer chain end.

**Preparation of P(HEMA/MMA)/P(St/GMA) Composite Particles.** *Use of Un-Cross-Linked P(HEMA−MMA) Seed Particles.* At first, the P(HEMA−MMA)/P(St−GMA) composite nanoparticles was prepared from un-cross-linked P(HEMA−MMA) seed particles (run 904 in Table 1) according to the recipe in Table 2 (run 1901). The TEM photographs of resulting composite particles are shown in Figure 1, where Figure 1a shows the TEM sample prepared just after polymerization and Figure 1b shows the sample after storage for 1 week. Though both of the composite particles were spherical, the particle morphology was changed from the hemisphere-like structure (Figure 1a) to the microphase-separated structure (Figure 1b) when the composite particles was held at room temperature for 1 week. This morphology change might be due to the gelatinous property of un-cross-linked P(HEMA−MMA) seed, which can be swollen by water. Indeed, un-cross-linked P(HEMA−MMA) seed particles coagulated by agitation after standing for 1 week at ambient temperature and consequently could not be used as seed particles (data not shown). Since P(HEMA−MMA) is swollen by water for the storage period and the viscosity inside the polymer particles were lowered, the secondary polymer P(St−GMA) can probably be distributed into the HEMA-rich domains to form the small domains as shown in Figure 1b. The instability of un-cross-linked P(HEMA−MMA) seed particles is thought to contribute to the perturbation of hair layer by the agitation, which may lead to the instability of $\zeta$ potential of particles.

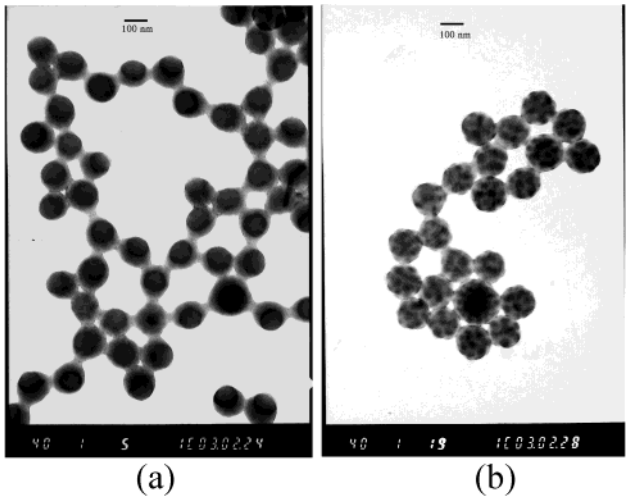


**Figure 1.** TEM photographs of P(HEMA/MMA)/P(St−GMA) composite particles (run 1901) prepared by using un-cross-linked P(HEMA/MMA) seed particles under HEMA/MMA = 20/80. Second-stage seeded polymerization is done under M/P = 1/2 in the presence of toluene. (a) Immediately after polymerization; (b) after stored at ambient temperature for 1 week.

*Use of P(HEMA−MMA) Seed Particles Cross-Linked by EGDMA.* To obtain stable morphology of final composite particles, P(HEMA−MMA) seed particles cross-linked by 10 wt % EGDMA with 20/80 weight ratio of HEMA/MMA (run 905 in Table 1) were used according to the recipes in Table 2. At first, the effects of solvent addition and preswelling of seed particles on the composite particle morphology were investigated. The TEM photographs of resulting composite particles are exhibited in Figure 2. Figure 2a shows the composite particles (run 1902) for which no solvent was charged in the seeded polymerization stage, and Figure 2b shows the composite particles (run 1903) for which toluene was added to swell the seed. It is clear that the morphology was changed from the microphase-separated structure (Figure 2a) to the hemispherical structure (Figure 2b) when toluene was added. As the addition of a solvent lowers the viscosity in the polymer particles and enhance the mobility of the polymer chain, one can easily obtain the thermodynamically favorable particle morphology.[22] Figure 2c shows the composite particles (run 1904) prepared from the seed particles preswelled by both the monomers and toluene. Comparing parts c and b of Figure 2, however, no clear morphology difference was observed. As reported in previous paper,[13] equilib-

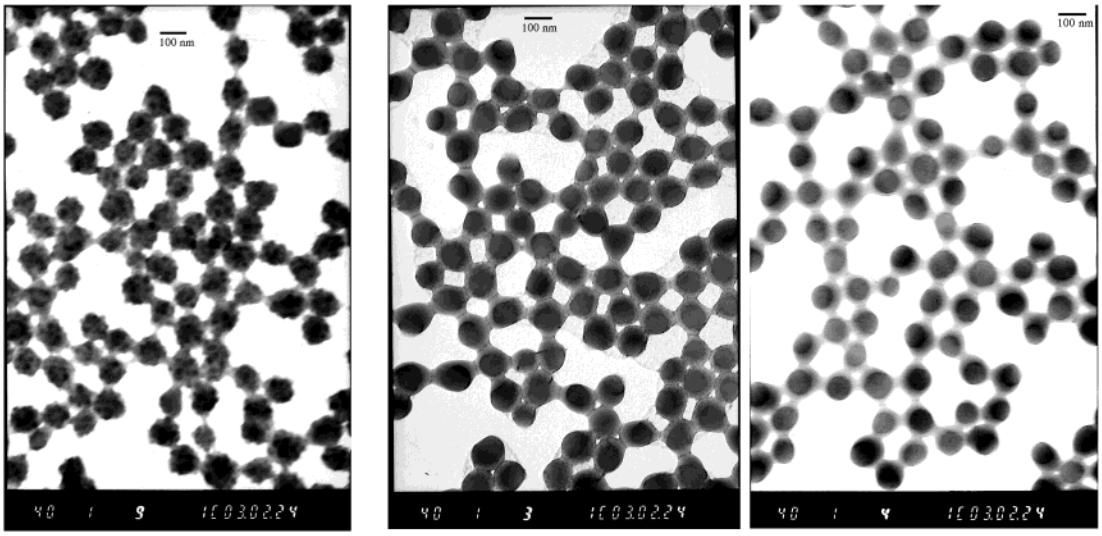


**Figure 2.** TEM photographs of P(HEMA/MMA)/P(St−GMA) composite particles prepared by using P(HEMA/MMA) seed particles cross-linked by 10 wt % EGDMA under HEMA/MMA = 20/80. Second-stage seeded polymerization is done under M/P = 1/2 (a) without toluene, (b) with toluene, and (c) using seed particles preswelled by both monomers and toluene.

rium swelling must be achieved during the nitrogen purge (1 h) and heating (30 min) when the M/P ratio was small. Since the M/P ratio is small (1/2) in this case (run 1904 in Table 2), the equilibrium swelling must be rapidly achieved during the nitrogen purge and heating, and thus no morphology differences occur between parts b and c of Figure 2.

To obtain heterobifunctional particles with high directionality, the smaller P(St−GMA) domain is preferable on the particles surface. For this reason, we prepared the P(HEMA−MMA)/P(St−GMA) composite under lower M/P ratio (runs 1905−1908 in Table 2). The TEM photographs of resulting composite particles of runs 1905, 1906, 1907, and 1908 are shown in parts a, b, c, and d of Figure 3, respectively. Comparing run 1905 with run 1903, only the amount of styrene monomer was reduced from 1 to 0.5 g in run 1905. The same hemisphere-like structures were observed in Figure 2b and Figure 3a. Interestingly, when toluene was not used (run 1906), the thermodynamically unfavorable particle morphology (sandwich-like) was also formed (Figure 3b). In runs 1907 and 1908, on the other hand, the content of GMA in the secondary monomers was elevated to increase the oxirane group at the surface of final composite particles. Figure 3c shows the TEM photograph of resulting composite particles as the ratio of GMA/St composition was increased from 1/10 (run 1905) to 1/4 (run 1907). The secondary smaller particles (Figure 3c) are clearly observed on the surface of the main particles, whose size is much larger than that of the corresponding seed particles and that of the other runs using the same weight ratio of M/P (Figure 3a,b). The formation of the larger particles (Figure 3c) may be due to the coagulation between the seed particles and the secondary small particles. To prevent the formation of the secondary small particles in the seeded polymerization stage, the amount of the initiator (V-50) was reduced from 0.02 to 0.01 g (run 1908). The TEM photograph of final particles is shown in Figure 3d, where no secondary small particles exist and the contrast of particle images became obscure. Comparing Figure 3d with Figure 3a, the increase in styrene content (a hydrophobic monomer) is preferable to the phase separation between P(HEMA−MMA) and P(St−GMA) because the difference in hydrophilicity between these two domains becomes more prominent.

The P(HEMA−MMA)/P(St−GMA) composite particles was also prepared by employing the higher M/P ratio to investigate the morphology change (run 1909 in Table 2). The TEM photograph of resulting composite particles is shown in Figure 4. It was found that dumbbell and popcorn-like anomalous particles without contrast were formed when the M/P ratio was increased from 1/2 to 1. Comparing the size between the dumbbell and popcorn-like anomalous particles and the seed particle, it can be seen that such anomalous particles were not formed by coagulation among particles. The formation of anomalous particles is probably due to the higher hydrophilic HEMA monomer and the cross-link of the seed polymer. It was found that copolymer particles produced by soap-free emulsion copolymerization of styrene and HEMA had an anomalous shape with uneven surface,[11] whose unevenness became more prominent with the HEMA content. Furthermore, the use of cross-linked seed particles can promote the phase separation between the seed polymer and the secondary polymer in the seed emulsion polymerization system, and hence the anomalous particles are formed easily. In this case, the increase in the M/P ratio leads the viscosity of polymer particles to decrease and enhances the mobility of P(HEMA−MMA) and P(St−GMA) chains in the polymer particles, especially the mobility of P(St−GMA) chains, because it was not cross-linked. Consequently, the phase separation was increased to generate the anomalous particles. On the other hand, the weight ratio of styrene to GMA was also increased (40/1) in run 1909. Homopolymerization of styrene on the surface of the seed particles could be the cause of the anomalous staining because a more rich layer of PS is situated on the surface of the final composite particles. Though the reason for uniform stain of anomalous particles is not clear, it may be due to the dye agent ($RuO_4$) used for staining HEMA and St. Since the formation of the anomalous particles should be accompanied by the

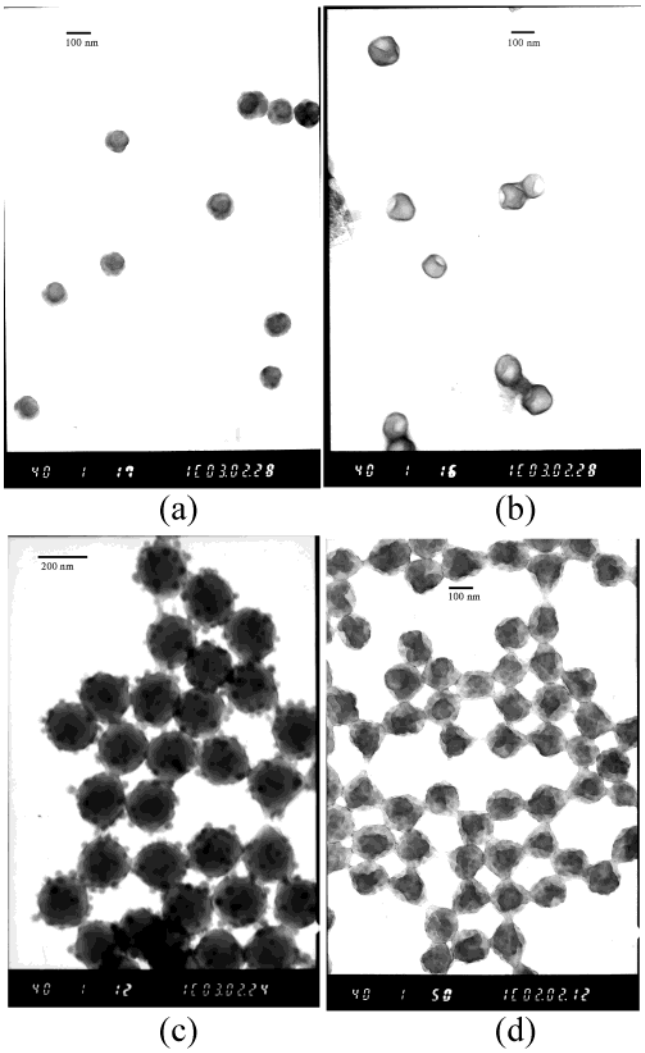


**Figure 3.** TEM photographs of P(HEMA/MMA)/P(St−GMA) composite particles prepared by using P(HEMA/MMA) seed cross-linked by 10 wt % EGDMA under HEMA/MMA = 20/80. Second-stage seeded polymerization is done under M/P = 1/4. (a) GMA/St = 1/10, with toluene; (b) GMA/St = 1/10, V-50 (0.2 g), without toluene; (c) GMA/St = 1/4, V-50 (0.2 g), with toluene; (d) GMA/St = 1/4, V-50 (0.1 g), with toluene.

phase separation between P(HEMA−MMA) and P(St−GMA), it is not likely that the uniform stain of the anomalous particles was caused by uniform composition.

**Effects of HEMA Content in the Seed Polymer.** The P(HEMA−MMA)/P(St−GMA) composite particles were produced by use of the seed particles with lower HEMA content (runs 908 and 911) to gain a better contrast between P(HEMA−MMA) and P(St−GMA) and investigate its effect on the composite particle morphology. The cross-link degree of the seed particle was fixed at 10 wt %. Two series runs using seed particles with 11/89 or 5.2/94.8 HEMA/MMA composition were carried out with the different M/P ratio. The recipes are shown in Table 3, and the TEM photographs of resulting composite particles produced from seed particles with 11/89 HEMA/MMA composition (run 908) are shown in Figure 5. In parts a, b, and c of Figure 5, 1/4, 1/2, and 1 of the M/P weight ratio were employed in the recipe, respectively (runs 1910, 1911, and 1912). It is clear that the resulting composite particle morphology was changed from spherical microphase-separated structure to hemisphere-like and to popcorn-like anomalous structure, as the M/P ratio was increased from 1/4 to 1/2 and 1. In

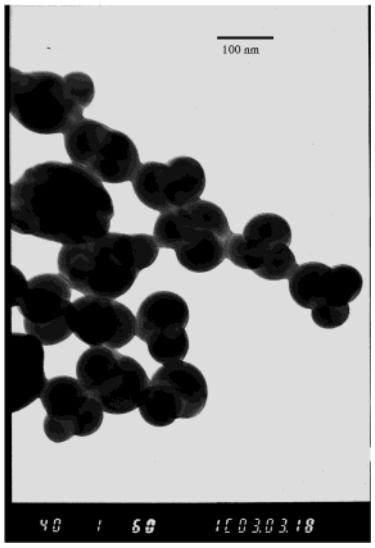


**Figure 4.** TEM photographs of P(HEMA/MMA)/P(St−GMA) composite particles prepared by using P(HEMA/MMA) seed particles cross-linked by 10 wt % EGDMA under HEMA/MMA = 20/80. Second-stage seeded polymerization is done under M/P ratio = 1.

**Table 3. Recipes for Preparation of P(HEMA−MMA)/P(St−GMA) Composite Nanoparticles**[a]

| run no. | seed latex (solid) (g) | seed run no. | styrene (g) | GMA (g) | V-50 (g) | toluene (g) |
|---|---|---|---|---|---|---|
| 1910 | 41.42 [2] | 908 | 0.5 | 0.05 | 0.01 | 4 |
| 1911 | 41.42 [2] | 908 | 1 | 0.05 | 0.02 | 4 |
| 1912 | 41.42 [2] | 908 | 2 | 0.05 | 0.04 | 4 |
| 1913 | 44.88 [2] | 911 | 1 | 0.05 | 0.02 | 4 |
| 1914 | 44.88 [2] | 911 | 2 | 0.05 | 0.04 | 4 |
| 1915 | 44.88 [2] | 911 | 8 | 0.4 | 0.16 | 4 |

[a] Reaction time, temperature, and agitation rate are 24 h, 70 °C, and 200 rpm, respectively. Total reaction mixtures are 200 g.

Figure 5b, the hemisphere-like structure was observed, while microphase-separated structure was formed when the M/P ratio was reduced to 1/4 (run 1910, Figure 5a). The decrease in the M/P ratio seems to lower viscosity in the polymer particles and reduces the mobility of P(HEMA−MMA) and P(St−GMA) chains in the polymer particles. Consequently, thermodynamically unfavorable particle morphology such as microphase-separated structure was formed. Furthermore, popcorn-like anomalous particles were obtained as the M/P ratio was increased to 1 (run 1912, Figure 5c). As mentioned above, the use of cross-liked seed particles can accelerate the phase separation between seed polymer and secondary polymer in the seed emulsion polymerization system, and the anomalous particles are easily formed. The increase of the M/P ratio leads the viscosity of polymer particles to decrease and enhances the mobility of P(HEMA−MMA) and P(St−GMA) chains in the polymer particles. Consequently, the phase separation was increased, and anomalous particle could be formed. However, the anomalous particle without contrast was also observed, even when the HEMA/MMA composition in the seed was reduced from 20/80 to 11/89.

To obtain higher contrast in the composite particles, furthermore, the P(HEMA−MMA) with 5.2/94.8 HEMA/MMA composition (run 911) was used as a seed and the M/P ratio was changed from 1/2 to 1 and 4 (runs 1913, 1914, and 1915). The TEM photographs of resulting composite particles are shown in Figure 6, from which

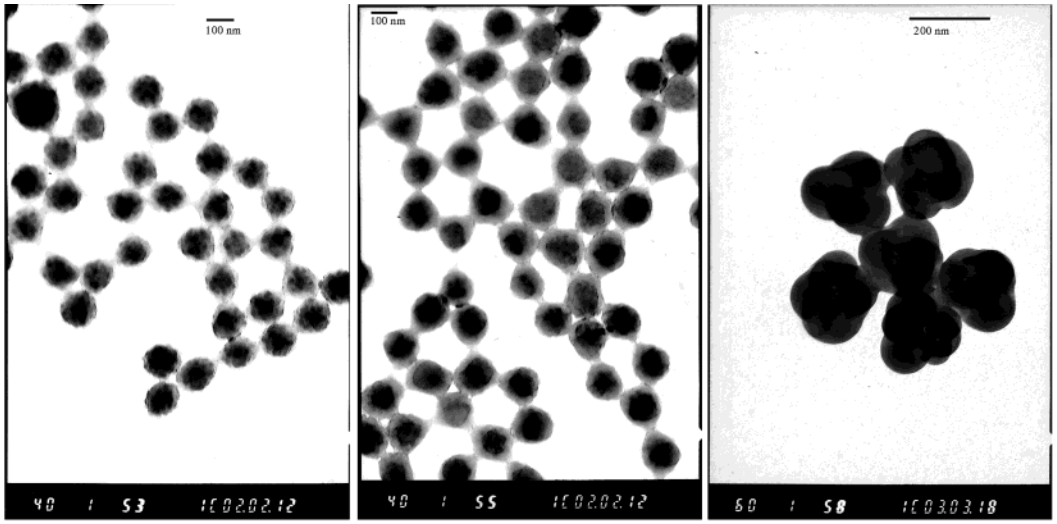


**Figure 5.** TEM photographs of P(HEMA/MMA)/P(St−GMA) composite particles prepared by using P(HEMA/MMA) seed particles cross-linked by 10 wt % EGDMA under HEMA/MMA = 11/89. Second-stage seeded polymerization is done under (a) M/P = 1/4, (b) M/P = 1/2, and (c) M/P = 1.

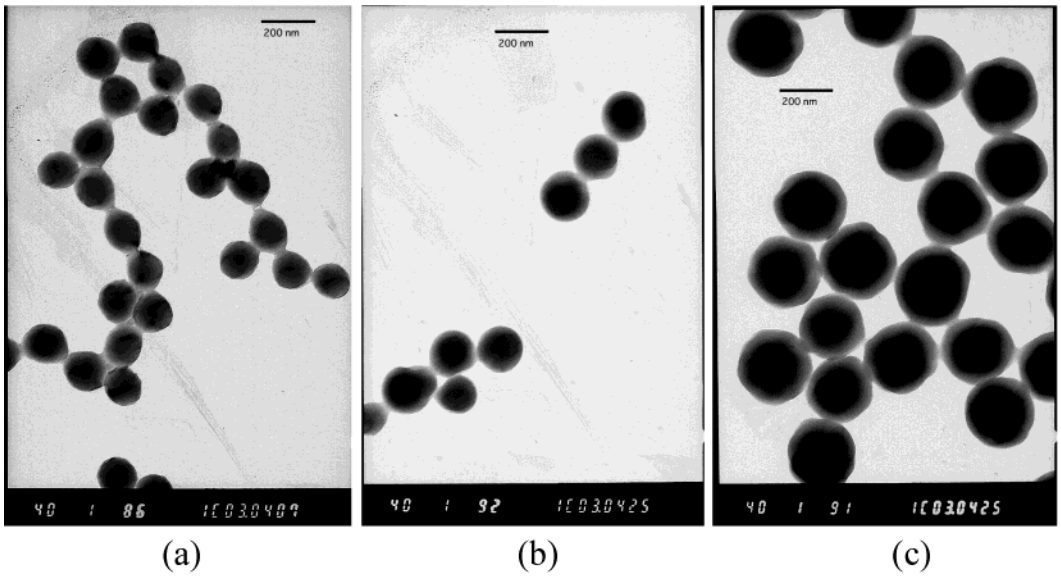


**Figure 6.** TEM photographs of P(HEMA/MMA)/P(St−GMA) composite particles prepared by using P(HEMA/MMA) seed particles cross-linked by 10 wt % EGDMA under HEMA/MMA = 5.2/94.8. Second-stage seeded polymerization is done under (a) M/P = 1/2, (b) M/P = 1, and (c) M/P = 4.

it was found that the spherical particles were obtained in all M/P ratios when P(HEMA−MMA) with 5.2/94.8 HEMA/MMA composition and 10 wt % cross-link degree was used as a seed, even if the M/P ratio was increased to 4. The reduce of HEMA content decreases the hydrophilicity of P(HEMA−MMA) chains and diminishes the phase separation between P(HEMA−MMA) and P(St−GMA), which results in the formation of spherical particle. Figure 6a shows the hemisphere-like structure when the secondary monomer concentration is lowered (M/P = 1/2). On the contrary, Figure 6b,c shows the same inverted core−shell structure when the M/P ratio was increased to 1 and 4. When M/P is increased, the viscosity in the polymer particles must be decreased even in the presence of additive solvent, leading to the increase of the mobility of polymer chains. The relatively hydrophilic polymer P(HEMA−MMA) is thought to migrate toward the particle surface, and the relatively hydrophobic polymer P(St−GMA) is supposed to migrate into the particle core. As a result, the inverted core−shell structure with P(St−GMA) core and P(HEMA−EGDMA−MMA) shell was formed to obtain thermodynamically stable state.

**Effects of Cross-Link Degree of Seed Particles.** The P(HEMA−MMA)/P(St−GMA) composite particles were also prepared by employing the seed particles with higher cross-link degree (20 wt %, run 909) to investigate its effect on the composite particle morphology. The recipes are summarized in Table 4, where the HEMA/MMA composition in the seed was fixed at 10/90. At first, M/P ratio was varied from 1/2 to 1 and 4 (runs 1916, 1917, and 1918). The TEM photographs of composite particles are shown in Figure 7. In parts a, b, and c of Figure 7, M/P ratio was 1/2, 1, and 4, respectively. It was found that anomalous particles were obtained in all runs when P(HEMA−MMA) with 20 wt % cross-link degree and 10/90 HEMA/MMA composition was used as a seed particle. On the contrary, spherical particles were formed as P(HEMA−MMA) with 10 wt % cross-link degree, and the same HEMA/MMA com-

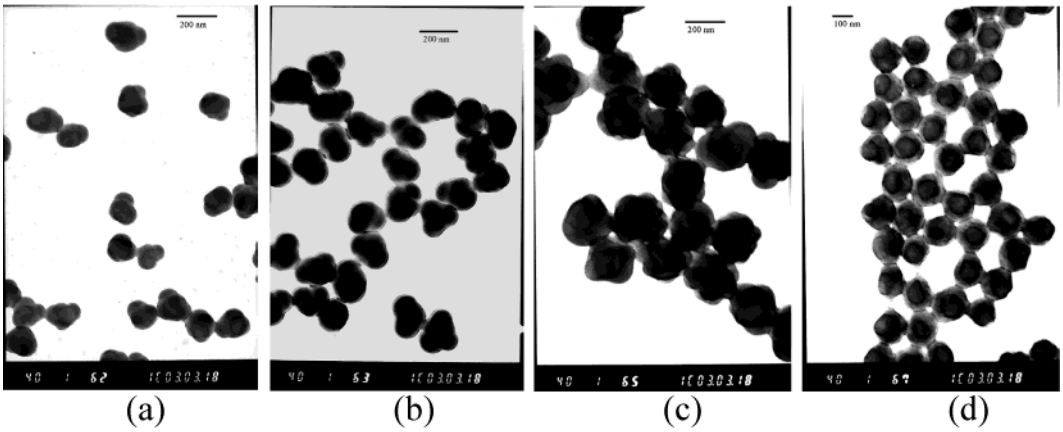


**Figure 7.** TEM photographs of P(HEMA/MMA)/P(St−GMA) composite particles prepared by using P(HEMA/MMA) seed particles cross-linked by 20 wt % EGDMA under HEMA/MMA = 10/90. Second-stage polymerization is done in the presence of DVB (0.046 wt %) under (a) M/P = 1/2, (b) M/P = 1, (c) M/P = 4, and (d) M/P = 1/2.

**Table 4. Recipes for Preparation of P(HEMA−MMA)/P(St−GMA) Composite Nanoparticles[a]**

| run no. | seed latex (solid) (g) | seed run no. | styrene (g) | GMA (g) | DVB (g) | V-50 (g) | toluene (g) |
|---|---|---|---|---|---|---|---|
| 1916 | 40 [2] | 909 | 1 | 0.05 | / | 0.02 | 4 |
| 1917 | 40 [2] | 909 | 2 | 0.05 | / | 0.04 | 4 |
| 1918 | 40 [2] | 909 | 8 | 0.1 | / | 0.16 | 4 |
| 1919 | 40 [2] | 909 | 1 | 0.05 | 0.05 | 0.02 | 4 |
| 1920 | 40 [2] | 909 | 1 | 0.05 | / | 0.02 | 4 |
| 1921 | 40 [2] | 909 | 2 | 0.05 | / | 0.04 | 4 |

[a] Reaction time, temperature, and agitation rate are 24 h, 70 °C, and 200 rpm, respectively. Total reaction mixtures are 200 g. Batch processes are used in runs 1916−1919, and monomers are fed dropwise in runs 1920 and 1921 (1 g/h).

position was used as a seed particle (run 1911 in Figure 5b). The use of higher cross-liked seed particles could promote the phase separation between seed polymer and secondary polymer to generate the anomalous particles easily. From Figure 7a−c, moreover, it can be seen that the surface unevenness becomes more prominent with increasing the M/P ratio, which is due to the increased mobility of polymer chains and the efficient separation between seed polymer and secondary polymer. To obtain spherical particles, a small amount of cross-link agent (DVB) was introduced into the seed polymerization stage (run 1919 in Table 4). The TEM photograph of resulting composite particles is shown in Figure 7d, where it is confirmed that spherical particles with hemisphere-like structure was obtained. The addition of DVB led to the formation of cross-linked secondary copolymer and, consequently, reduced the mobility of polymer chains and the degree of phase separation between seed polymer and secondary polymer.

Kamei et al. reported the production of anomalous particles in the process of emulsifier-free emulsion copolymerization of styrene and HEMA.[11] To study the effect of viscosity in the particles during the polymerization on the formation of anomalous particles, seeded emulsion polymerization of St was carried out in the presence of PHEMA seed particles by the following two methods of styrene monomer addition. Anomalous particles were formed when PHEMA was preswelled by all St monomer before seeded emulsion polymerization. In the case of dropwise system, on the other hand, the spherical particles with smooth surface were obtained. These results indicate that the anomalous particles are formed when the viscosity is low (former case) during the polymerization where phase separation occurs easily. The dropwise addition mode of monomers was also employed in this system to obtain spherical particles, whose recipes are shown in Table 4 (runs 1920 and 1921). The TEM photographs of resulting composite particles are shown in Figure 8, where anomalous particles with much unevenness surface like bunches of grapes were observed in run 1920 (Figure 8a) and run 1921 (Figure 8b). As 2 times the amount of solvent (toluene) to seed particles was used, the viscosity in the polymer particles is lower. Namely, the polymerization was carried out in the lower viscosity environment, and therefore phase separation occurs easily to form anomalous particle with a very uneven surface.

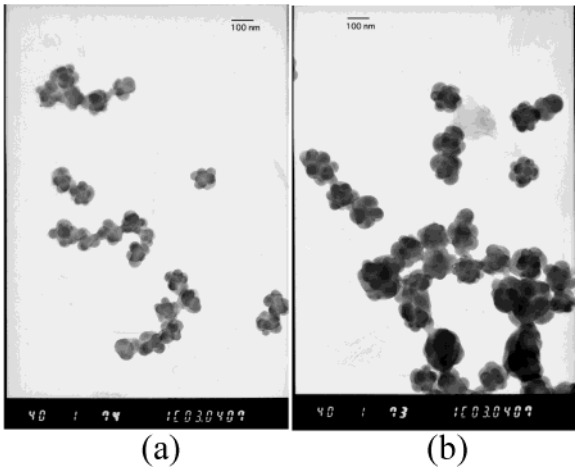


**Figure 8.** TEM photographs of P(HEMA/MMA)/P(St−GMA) composite particles prepared by using P(HEMA/MMA) seed particles cross-linked by 20 wt % EGDMA under HEMA/MMA = 10/90. Second-stage seeded polymerization is done using dropwise addition of monomers under (a) M/P = 1/2 and (b) M/P = 1.

P(HEMA−MMA) with 5/95 HEMA/MMA composition and 20 wt % cross-link degree (run 910) was also used as seed particles to prepare the P(HEMA−MMA)/P(St−GMA) composite particles. The recipes of polymerization are shown in Table 5. In runs 1922−1924, the M/P ratio was fixed at 1/2, and the effects of toluene and DVB on the composite particle morphology were investigated. The TEM photographs of final composite particles are shown in Figure 9, where Figure 9a shows a sandwich-like structure with uneven surface when toluene was not added (run 1922). On the other hand, the morphology was changed to a hemisphere-like structure with

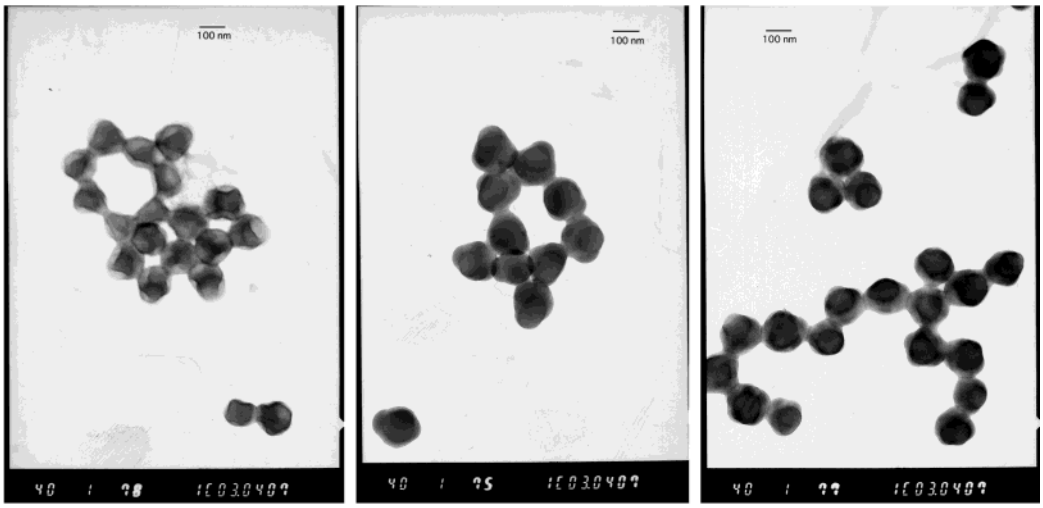


**Figure 9.** TEM photographs of P(HEMA/MMA)/P(St−GMA) composite particles prepared by using P(HEMA/MMA) seed particles cross-linked by 20 wt % EGDMA under HEMA/MMA = 5/95. Second-stage seeded polymerization is done under M/P = 1/2 (a) without toluene, (b) with toluene, and (c) with toluene in the presence of DVB (0.046 wt %).

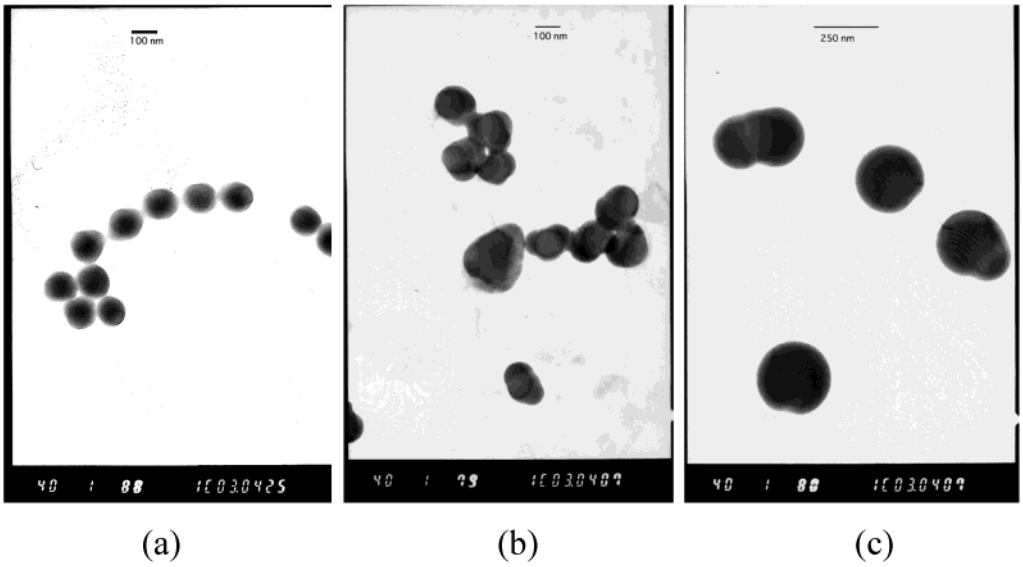


**Figure 10.** TEM photographs of P(HEMA/MMA)/P(St−GMA) composite particles prepared by using P(HEMA/MMA) seed particles cross-linked by 20 wt % EGDMA under HEMA/MMA = 5/95. Second-stage seeded polymerization is done under (a) M/P = 1/4, (b) M/P = 1, and (c) M/P = 4/1.

**Table 5. Recipes for Preparation of P(HEMA−MMA)/P(St−GMA) Composite Nanoparticles**[a]

| run no. | seed latex (solid) (g) | seed run no. | styrene (g) | GMA (g) | DVB (g) | V-50 (g) | toluene (g) |
|---|---|---|---|---|---|---|---|
| 1922 | 40.57 [2] | 910 | 1 | 0.05 | / | 0.02 | / |
| 1923 | 40.57 [2] | 910 | 1 | 0.05 | / | 0.02 | 4 |
| 1924 | 40.57 [2] | 910 | 1 | 0.05 | 0.05 | 0.02 | 4 |
| 1925 | 40.57 [2] | 910 | 0.5 | 0.05 | / | 0.01 | 4 |
| 1926 | 40.57 [2] | 910 | 2 | 0.05 | / | 0.04 | 4 |
| 1927 | 40.57 [2] | 910 | 8 | 0.4 | / | 0.16 | 4 |

[a] Reaction time, temperature, and agitation rate are 24 h, 70 °C, and 200 rpm, respectively. Total reaction mixtures are 200 g.

uneven surface (Figure 9b) when toluene was added. As mentioned above, the addition of toluene could lead to the formation of thermodynamically favorable particle morphology. Comparing Figure 9b with Figure 6a and Figure 7a, it can be seen that the increases of cross-link degree and HEMA content in the seed particles accelerate the phase separation between seed polymer and secondary polymer, and anomalous particles are readily formed. As show in Figure 9c, the unevenness of the particle surface became smooth when a small amount of DVB was added in the seed stage.

P(HEMA−MMA)/P(St−GMA) composite particles were also prepared by varying the M/P ratio (runs 1925, 1926, and 1927), using run 910 as seed particles. The TEM photographs of resulting composite particles are shown in Figure 10. The M/P ratio in parts a, b, and c of Figure 10 is 1/4, 1, and 4, respectively. Comparing Figure 10 with Figure 9b, it is obvious that the particle surface became much unevenness as the M/P ratio was increased. The composite particles were spheres when the M/P weight ratio was 1/4, while anomalous particle was formed with increasing M/P due to the lower viscosity in the polymer particles and the promoted phase separation. Comparing Figure 10 with Figure 7, it was found that unevenness of particles surface became remarkable as the P(HEMA−HEMA) particles with higher HEMA content were used as seeds. The higher

**Table 6. Relationships between Polymerization Condition and Composite Particle Morphology**

| Polymerization condition | Particle morphology or its variation |
|---|---|
| Uncross-linked P(HEMA-MMA) seed | Can be swollen by water |
| Addition of solvent in seed stage | |
| Lower HEMA content and cross-link degree in seed, lower M/P ratio in seed stage | Toluene; Lower M/P ratio; Higher M/P ratio |
| Use of seed with 5.2/94.8 HEMA/MMA composition and 10 wt % cross-link degree | Spherical particle |
| Higher HEMA content and cross-link degree in seed, higher M/P ratio in seed stage | M/P ratio increase; Anomalous particle |
| Addition of divinylbenzene in seed stage | Spherical particle |

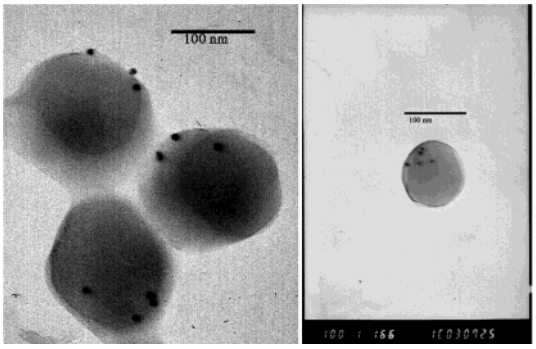


**Figure 11.** TEM photograph of P(HEMA/MMA)/P(St−GMA) composite particles of run 1924, which are labeled with 10 nm streptavidin−colloidal gold.

content of HEMA elevated the hydrophilicity of seed polymer and enhanced the phase separation.

**Confirmation of Hemisphere-like Structure of Composite Particles.** The ζ potential of hemisphere-like structure of composite particles of run 1924 was measured to be +27.29 mV, and the structure was confirmed by the labeling with 10 nm streptavidin−colloidal gold. At first, the oxirane groups of composite particles were converted to the amino groups by the addition of ammonium solution. The amino groups of amino-modified composite particles were then reacted with water-soluble biotin, sulfosuccinimidyl-D-biotin. The biotinylated composite particles were able to specifically bind with streptavidin−colloidal gold. The streptavidin−colloidal gold labeled composite particles were observed by TEM, whose photograph is shown in Figure 11. It is clear that the colloidal golds were bound only to the one side of the composite particles. The side labeled by colloidal golds is the P(St−GMA) domain, and the other side without colloidal golds is the P(HEMA−MMA) domain.

**Summary of Relationship between Polymerization Condition and Composite Particle Morphology.** The relationships between polymerization condition and composite particle morphology in present research are summarized in Table 6. The un-cross-linked P(HEMA−MMA) seed particle can be swollen by water. Prepared composite particle morphology is unstable and changed with storage time. The addition of solvent (toluene) in the seed stage favors the formation of thermodynamically stable morphology, changing microphase-separated structure and sandwich-like structure to hemisphere-like structure. The lower HEMA content and cross-link degree in seed polymer and the lower M/P ratio in seeded polymerization stage favor the formation of spherical particles. The hemisphere-like, microphase separation, and inverted core−shell structures were obtained by controlling the M/P ratio. Spherical particles should be formed when P(HEMA−MMA) with 5.2/94.8 HEMA/MMA composition and 10 wt % cross-link degree were used as a seed, even when the M/P ratio was higher. On the other hand, the higher HEMA content and cross-link degree in seed polymer and higher M/P ratio in seed stage favor the formation of anomalous particles, and the unevenness of composite particle surface increased with the M/P ratio. Anomalous particles could become spherical by adding a small amount of cross-link agent (DVB) in the seed stage.

## Conclusion

P(HEMA−MMA))/P(St−GMA)) composite polymer particles were prepared by soap-free seeded emulsion polymerization, using P(HEMA−MMA) seed particles cross-linked by EGDMA. The stable P(HEMA−MMA) seed latexes with various cross-link degree and HEMA content were prepared by soap-free emulsion polymerization, using continuous feed polymerization process to obtain stable particles. 2,2′-Azobis(2-amidinopropane) dihydrochloride (V-50) was used as an initiator in the two stages to prevent the ring opening of the oxirane group of GMA. The effects of solvent addition, preswelling of seed particles, and the M/P ratio on the morphology were discussed by using P(HEMA−MMA) seed with various HEMA content and cross-link degree. Various spherical and anomalous particle morphologies were obtained by controlling polymerization conditions. The

lower HEMA content and cross-link degree in seed particles and the lower M/P weight ratio favor the formation of hemispherical heterobifunctional particles. The hemisphere-like structure of composite particles with positive charge was confirmed by the labeling with streptavidin−colloidal gold. The biotinylated P(HEMA−MMA)/P(St−GMA) composite particle would be functionalized by the immobilization of biomolecules through the activation of hydroxyl group, and the resultant heterobifunctional particles would be applied to the field of nano-biotechnology.

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