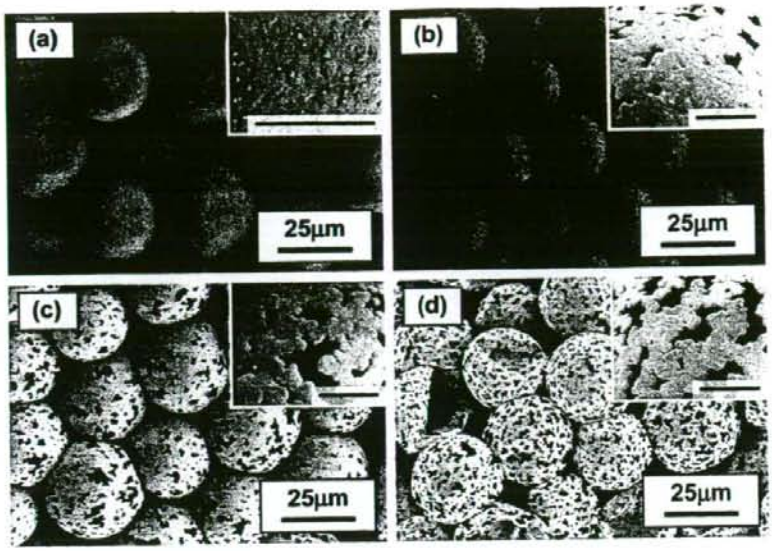


**Figure 10.** SEM images of the microcapsules prepared with size-controlled emulsion droplets containing different concentrations of SPAN85. Preparation conditions: $C_{EMA,aq,0}$ was 1.67 wt %; $C_{S,org,0}$ was (a) 0.43, (b) 0.50, (c) 0.60, and (d) 0.90 wt %; and the average droplet diameter was 32.3 $\mu$m. The insets are magnified views with the scale bars corresponding to 5 $\mu$m.

containing SPAN85 within this concentration range. The experimental conditions and microencapsulation protocol are listed in Table 3 and shown in Figure 1b, respectively. The droplets were prepared by SPG membrane emulsification. The average droplet diameter was 32 $\mu$m. Microcapsules enclosing the emulsion droplets containing 0.43, 0.50, 0.60, and 0.90 wt % SPAN85 are shown in Figure 10. As we expected, macroholes were formed in the shells of all of the microcapsules prepared in this investigation. In addition, the hole size increased with increasing SPAN85 concentration (i.e., the size of the macroholes could be controlled by adjusting the SPAN85 concentration). The holes in the shells of microcapsules prepared with 0.43 wt % SPAN85 were very small. From this result, we concluded that the critical concentration of SPAN85 for hole formation is 0.43 wt %. From the theoretical calculation, the corresponding $\theta_{EMA}$ was estimated to be 0.90, which agrees with the critical $\theta_{EMA}$ estimated for Solsperse17000.

Another parameter for controlling $\theta_{EMA}$ is the concentration of poly(E-MA) in continuous phase III. We examined the effect of $C_{EMA,aq,0}$ on the surface holes with the other conditions kept constant. As shown in Figure 5b, when the emulsion droplet diameter is 20 $\mu$m and the concentration of SPAN85 is 0.5 wt %, the concentration range of poly(E-MA) that provides a $\theta_{EMA}$ value of 0.8−0.9 is 0.8−2.0 wt %. We prepared microcapsules according to this calculated result. The experimental conditions and microencapsulation protocol are listed in Table 3 and shown in Figure 1b, respectively. The average diameter of the emulsion droplets used was 18.3 $\mu$m. The prepared microcapsules are shown in Figure 11. As expected, the microcapsules prepared with 1.67 wt % poly(E-MA) had macroholes, and those prepared with 3.33 wt % had no holes. In addition, when $C_{EMA,aq,0}$ was 2.0 wt % (i.e., $\theta_{EMA}$ was the critical value of 0.90), very small holes were formed in the microcapsule shells.

We also investigated the effect of the concentration of melamine−formaldehyde prepolymer in continuous phase III, which would not affect $\theta_{EMA}$, on the surface holes with other conditions kept constant. The experimental conditions and prepared microcapsules are shown in the Supporting Information (Figure S2). We found that the morphology of the microcapsule shell (e.g., the size and density of the macroholes) was hardly affected by the prepolymer concentration. This result agrees with our theoretical considerations.

We conclude from these experimental and theoretical investigations that cross-linked polymelamine microcapsules with macroholes in their shells can be prepared by controlling the fraction of the emulsion droplet surface covered by poly(E-MA) in the presence of an additional oil-soluble surfactant. The formation of macroholes in the microcapsule shells seems to be related to the oil-soluble surfactant adsorbed on the droplet surface, and these surfactant molecules seem to self-assemble at the surface during shell formation. We again note that, in our model, we assumed that poly(E-MA) and the oil-soluble surfactant do not interact with each other in the interfacial region and that they adsorb at the O/W interface independently. However, they might be in association equilibrium with each other, and the associated complex might adsorb on some sites in the interface. It may be possible to obtain a rigorous relationship between the ratio of the hole area of the surface of the microcapsules and the ratio of components adsorbed on the interface by applying a competitive adsorption isotherm model derived for the multicomponent system in which the adsorption of an associated complex is also considered. Although the association between polymer and surfactant has been investigated in previous studies, most of them discussed the interactions between polymer and surfactant coexisting in the same phase.[28] More knowledge about the interaction between polymer and surfactant in the oil/water interfacial region would be useful.

**Application of Microcapsules with Macroholes in Their Shells.** *Release of Core Material from Holey Microcapsules.* As an example of the applications of microcapsules with macroholes in their shells, we examined the controlled release of encapsulated material. One would expect the release rate to increase with the

(28) (a) Jones, M. N. *J. Colloid Interface Sci.* **1967**, *23*, 36–42. (b) Folmer, B. M.; Kronberg, B. *Langmuir* **2000**, *16*, 5987–5992. (c) Touhami, Y.; Rana, D.; Neale, G. H.; Hornof, V. *Colloid Polym. Sci.* **2001**, *279*, 297–300. (d) Goddard, E. D. *J. Colloid Interface Sci.* **2002**, *256*, 228–235.

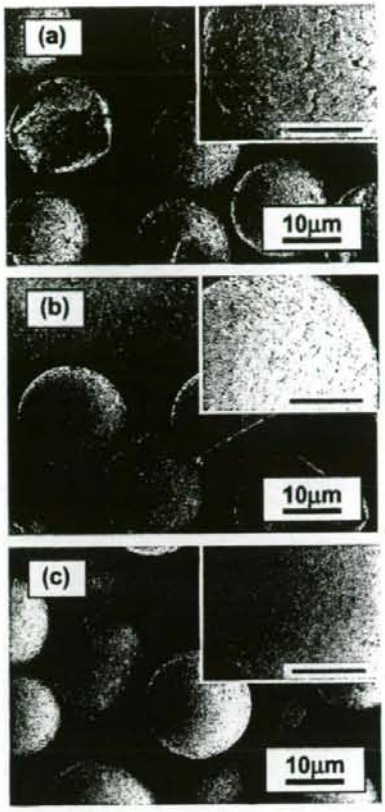


**Figure 11.** SEM images of microcapsules prepared with different concentrations of poly(E-MA). Preparation conditions: $C_{EMA,aq,0}$ was (a) 1.67, (b) 2.0, and (c) 3.33 wt % and $C_{S,org,0}$ was 0.5 wt %. SPAN85 was used as an oil-soluble surfactant. The emulsion droplets were size-controlled ones prepared by SPG membrane emulsification. The average droplet diameter was 18.3 $\mu$m. The insets are magnified views with the scale bars corresponding to 5 $\mu$m.

size of the macroholes. We investigated the release behavior by using holey microcapsules prepared from emulsion droplets containing a small amount of blue dye (oil blue N) and 0, 0.43, 0.5, and 0.6 wt % SPAN85. The average diameter of the emulsion droplets was 32.3 $\mu$m. Microcapsules prepared without SPAN85 had smooth, complete shells whereas those prepared with SPAN85 had holey shells. For each SPAN85 concentration, the holes were nearly the same size as those in the microcapsules prepared with the same SPAN85 concentration in Figure 10. The release behavior was evaluated by measuring the concentration of oil blue N that was eluted from the microcapsule into the elutriant (ethanol). The results are shown in Figure 12a, where $C_{dye}$ denotes the concentration of oil blue N in the elutriant. It is clearly shown in Figure 12a that the release rate becomes as high as the concentration of SPAN85 in the encapsulated emulsion droplet increase. The release behavior of hole-free microcapsules ([SPAN85] = 0 wt %) is obviously different from that of holey ones. Hardly any oil blue N was released in the first 300 s. However, once release began, it was almost completed by 1200 s. The reason that such a specific release profile was observed was clearly revealed in an SEM photograph of the hole-free microcapsules after the controlled release experiment. As shown in Figure 12b, almost all hole-free microcapsules were crushed

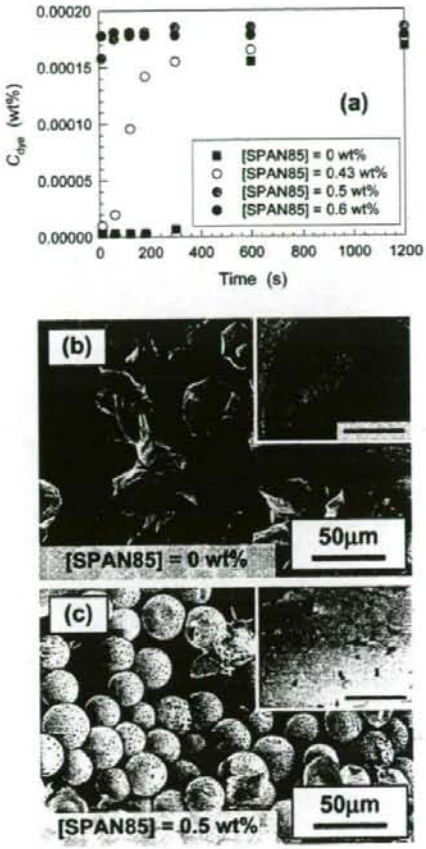


**Figure 12.** Controlled release behavior of microcapsules with and without holes in their shells. (a) Time course of the concentration of oil blue N released from the microcapsules. (b, c) SEM images of the microcapsules after elution; average droplet diameter was 32.3 $\mu$m, and SPAN85 concentration in the microencapsulated Isopar G was (b) 0 and (c) 0.5 wt %. The insets are magnified views with the scale bars corresponding to 5 $\mu$m.

and had slight cracks in their shells. In the early stage, ethanol entered the microcapsule through the shells. Because the solvent of the core material (Isopar G) could not penetrate the shells, the hole-free microcapsules burst after 300 s as a result of ethanol penetration into the cores and the formation of cracks in the shells. The materials encapsulated in the microcapsules were then eluted through the cracks. At that time, ethanol could not flow into the microcapsules because of the pressure difference inside and outside the microcapsules. Therefore, the hole-free microcapsules were crushed. However, the microcapsules with holes were not crushed during the elution of the core material. An example of an SEM photograph of holey microcapsules after the controlled release experiment is shown in Figure 12c. Because the inflow of ethanol and the outflow of the core material through the holes occurred simultaneously, the holey microcapsules were hardly crushed. In addition, as shown in Figure 12c, the holey microcapsules were not crushed after filtration and drying with ethanol. Dried holey microcapsules

(29) Daiguji, H.; Makuta, T.; Kinoshita, H.; Oyabu, T.; Takemura, F. *J. Phys. Chem. B* **2007**, *111*, 8879–8884.

contain air, so we can use them as templates for monodisperse size-controlled microcapsules encapsulating gaseous cores, which could be fabricated if we could successfully close the holes in the dried microcapsules. Microcapsule-encapsulating gaseous cores will be useful for many applications, including the weight reduction of materials and thermal and acoustical insulation (e.g., diagnostic ultrasound contrast agents).[29] The fabrication of microcapsules encapsulating gaseous cores is a topic for future work.

*Fabrication of Microcapsules Encapsulating Aqueous Solution.* As shown in Figure 12a, the core material in the holey microcapsules was quickly removed and replaced by ethanol. We expected that this ethanol substituting for the original core material could also be removed easily and replaced by water. That is, we thought it would be easy to obtain holey microcapsules containing an aqueous solution. If the holes in the shells of the microcapsules containing aqueous solution can be successfully closed, then microcapsules encapsulating aqueous solution can be fabricated. We thought that the holes could be closed by additional microencapsulation of the holey microcapsules containing aqueous solution as the cores. To demonstrate the feasibility of fabricating microcapsules with double-layer shells, we added reactive water-soluble dye to the continuous phase used for the additional microencapsulating process. We chose to use reactive blue 160 as a reactive water-soluble dye because it has both triazine and sec-amine units and thus can react with melamine−formaldehyde prepolymer. Therefore, the shells of the microcapsules synthesized in aqueous solution containing reactive blue 160 become blue. We can easily recognize the formation of the additional shells on the holey microcapsules. In addition, we used emulsion droplets including a small amount of oil-soluble red dye (oil red O) to confirm the complete removal of the oily core from the holey microcapsules. The additional microencapsulation was performed according to the process shown in Figure 1a and the conditions shown in Table 1. As continuous phase I, we used an aqueous solution with 2.5 wt % poly(E-MA) and 0.1 wt % Reactive Blue 160 dissolved in it. Instead of the disperse phase, we used a suspension of holey microcapsules prepared from emulsion droplets with 0.5 wt % SPAN85 and then washed with ethanol and distilled water to replace the core material with water. The mass fraction of the microcapsules in the suspension was about 75%. The prepared microcapsules were separated from the continuous phase and washed with distilled water. They were filtered and dried to enable us to observe the morphology by FE-SEM. The holey microcapsules and the resultant double-layer microcapsules encapsulating the aqueous solution are shown in Figure 13. Figure 13a is an SEM photograph of the holey microcapsules, which are the original starting blocks for the double-layer microcapsules encapsulating the aqueous solution. As shown in the upper inset, submicrometer-sized macroholes were formed in the shell of each microcapsule. The original holy microcapsules with the core material replaced by water were microencapsulated again. An SEM photograph of the obtained double-layer microcapsules is shown in Figure 13b. It is clear that the macroholes were successfully closed. The upper inset in Figure 13b is an SEM photograph of the crosssection of the microcapsule. This image confirms that the microcapsule had a single core. The lower insert in Figure 13b is a photograph of a dried sample. This image clearly shows that the microcapsule is blue. That is, the additional shell was formed on the surface of the original microcapsule and closed the holes. Figure 13c shows the suspensions of microcapsules. The microcapsules in the left test tube are the original ones encapsulating Isopar G with oil red O dissolved in it. Because the density of Isopar G ($7.49 \times 10^5$ g/m$^3$) was less than that of water, the microcapsules floated. The microcapsules with their core material replaced by water are in the middle test tube. The color of these microcapsules is white instead of red, which indicates that the oily core in the original microcapsules was completely removed. In addition, the microcapsules in the middle tube sank in water, which means that the oily core had been replaced by water. The double-layer microcapsules with closed holes are in the right test tube. They also sank in water (i.e., the aqueous solution was encapsulated in the microcapsules). This confirms that a holey microcapsule can be used as a template for fabricating a microcapsule encapsulating hydrophilic compounds. It should be noted that macroholes slightly larger than those shown in Figure 13a were not completely closed and that nanosized holes remained in the microcapsule shells after the additional microencapsulation. A microcapsule that contains an aqueous solution and has a shell with tiny holes is potentially applicable as a bioreactor. The

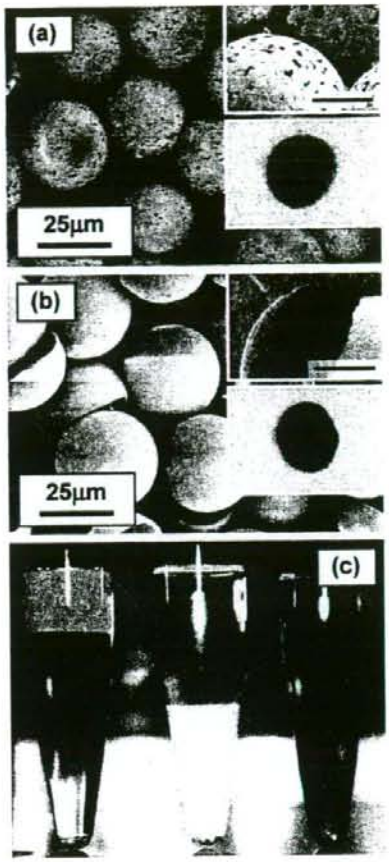


**Figure 13.** Microcapsules encapsulating aqueous solution after additional microencapsulation of the holey microcapsules. (a) Original microcapsules encapsulating aqueous solution; holey microcapsules were prepared with emulsion droplets having an average diameter of 32.3 $\mu$m and containing 0.5 wt % of SPAN85 and a small amount of a red dye (oil red O). (b) Microcapsules encapsulating an aqueous solution. The upper insets in panels a and b are magnified views with the scale bars corresponding to 10 $\mu$m, and the lower ones are photographs of filtered samples. (c) Suspensions of the microcapsules in distilled water. (Left) original microcapsules shown in panel a, (middle) holey microcapsules with their core material replaced by water, and (right) microcapsules encapsulating the aqueous solution shown in panel b.

macroholes in the shell of the original microcapsule should allow free diffusion of a living cell or a bacillus into the microcapsule. Then, the macroholes could be closed by the additional microencapsulation. The nanosized holes remaining after the additional microencapsulation should prevent a living cell or bacillus from leaving the microcapsule and should also allow free diffusion of gases, nutrients, and wastes. With the aim of making a bioreactor, we are planning to perform further investigations into the fabrication of microcapsules containing an aqueous solution suspending biocatalysts and having shells with nanosized holes.

## Conclusions

We described the preparation of cross-linked polymelamine microcapsules with macroholes in their shells by a phase-separation method. The macroholes were formed by utilizing the competitive adsorption of a water-soluble polymeric surfactant and an oil-soluble surfactant on the surface of an emulsion droplet used as the capsule core. Theoretical calculations based on the competitive adsorption of surfactants on the surface of an emulsion droplet were used to explain the formation of macroholes in microcapsule shells. The theoretical calculations revealed that the fraction of the droplet surface covered by poly(E-MA) depended on the concentrations of poly(E-MA) and the oil-soluble surfactant and the diameter of the emulsion droplet. By comparing the properties of prepared microcapsules with the calculated results, we found that for poly(E-MA) the critical degree of surface coverage needed to fabricate holey microcapsules was about 0.90. By controlling the concentrations of poly(E-MA) and the oil-soluble surfactant and the droplet diameter according to the calculated results, we successfully prepared only microcapsules with macroholes. The size and quantity of macroholes could be controlled by adjusting the fraction of the droplet surface covered by poly(E-MA). We conclude from the experimental and theoretical investigations that the macroholes formed in microcapsule shells resulted from the adsorption and self-assembly of the oil-soluble surfactant on the droplet surface during the formation of the shells of cross-linked polymelamine microcapsules.

The applications of microcapsules with macroholes in their shells were also investigated. Controlling the hole size allowed us to control the release rate of the core material. Microcapsules with macroholes have the potential to be used in controlled release applications. We also found that they were not crushed when the core material was being replaced. Microcapsules containing an aqueous solution were fabricated by using the microcapsules with macroholes as templates. The macroholes in the shells were successfully closed by an additional microencapsulation. Thus, microcapsules with macroholes in their shells can also be used as templates for fabricating microcapsules containing hydrophilic compounds, which could be used as bioreactors.

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**Supporting Information Available:** Detailed schematic explanation of SPG membrane emulsification followed by the microencapsulation process and microcapsules prepared with different concentrations of melamine–formaldehyde prepolymer with the other conditions constant. This material is available free of charge via the Internet at http://pubs.acs.org.

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ORIGINAL CONTRIBUTION

# Effects of dispersion stabilizer and reaction solvent on forming monodisperse polystyrene microspheres by dispersion polymerization

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**Abstract** We used poly(aspartic acid) (PAsp) synthesized by ion exchange with sodium polyaspartate (PAspNa) as a dispersion stabilizer. PAsp improved the dispersion stability and the solubility in the medium for dispersion polymerization. The effects of the stabilizer hydrophobicity on particle formation, conversion, particle diameter, and its distribution of polystyrene microspheres were investigated by using both biodegradable polymers as a dispersion stabilizer. According to these results, we concluded that the polymerization rate of the styrene with PAsp was higher than that of styrene with PAspNa. That is why, smaller and more monodisperse microspheres were prepared with PAsp, compared to those with PAspNa.

**Keywords** Poly(aspartic acid) · Dispersion polymerization · Monodisperse particle · Biodegradable polymer · Polystyrene

## Introduction

Dispersion polymerization is one of the major techniques for the preparation of monodisperse polymer microspheres [1–6]. In dispersion polymerization, monodisperse microspheres are formed in the presence of appropriate polymer dispersion stabilizer such as poly(*N*-vinylpyrrolidone), polyacrylic acid (PAA), and polyethylenimine [7, 8]. However, all commercial dispersion stabilizers used in dispersion polymerization are so far not biodegradable. In general, these water-soluble polymers are difficult to recover from polymer solution. Therefore, these nonbiodegradable dispersion stabilizers cause contamination of polymer microsphere surface and waste-water treatment of polluted water. Furthermore, these are made from fossil fuels. When we thought about sustainable society, cutting our dependence on fossil fuels is essential.

Sodium polyaspartate (PAspNa) synthesized by polycondensation of L-aspartic acid (L-Asp) is one of the typical hydrophilic biodegradable polymers [9, 10]. Since PAspNa is made from plant-derived materials and has biocompatibility, this polymer can be used in medical field, cosmetics, and foods. Thus, we studied dispersion polymerization with PAspNa as a dispersion stabilizer and synthesized monodisperse polystyrene microspheres with PAspNa as a dispersion stabilizer. The particle diameter ranged between 0.25 and 2.3 μm, and the particle size distribution was about 7.7%.

In this work, we synthesized poly(aspartic acid) (PAsp) by ion exchange with PAspNa. Although PAsp is also a typical hydrophilic biodegradable polymer, the solubility in water is lower than PAspNa due to weak-acid polyelectrolyte. We investigated the effect of the stabilizer hydrophobicity on the particle formation, conversion, particle size, and the distribution of polystyrene particles.

## Experimental

### Materials

All chemicals were purchased from Wako Pure Chemical Industry. Styrene (99%, with $3.0 \times 10^{-3}$% 4-tert-butylpyrocatechol stabilizer) was distilled under reduced pressure in a nitrogen atmosphere to remove the inhibitor. 2,2′-Azobis

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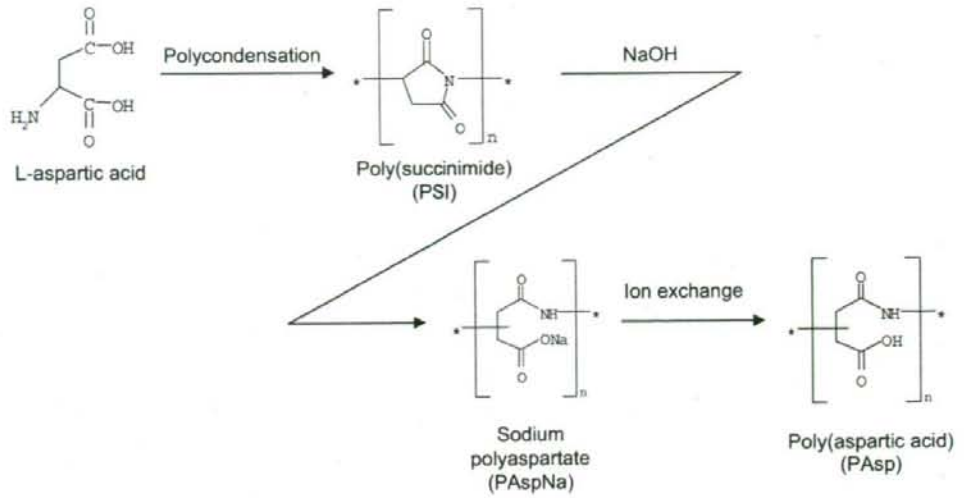


**Scheme 1** Synthesis of PSI, PAspNa and PAsp

(isobutyronitrile) (AIBN) was purified by recrystallization from methanol. Water used as a polymerization solvent was purified with a Millipore Milli-Q water purification system.

Synthesis of polysuccinimide

Polysuccinimide (PSI) was synthesized by polycondensation of L-Asp (46.6 g) using phosphoric acid (19.8 g) as a catalyst at 453 K for 8 h. Then, the products were purified, and the weight-average molecular weights were determined by gel permeation chromatography (GPC) as previously reported [11]. The determined molecular weight of PSI was $4.25\times10^4$. Proton nuclear magnetic resonance ($^1$H NMR) spectra were measured with a JEOL FT NMR System JMN-AL300 (Scheme 1).

Synthesis of PAspNa

PAspNa was prepared by hydrolysis of PSI as already reported [11]. The molecular weight of PAspNa was $6.0\times10^4$, calculated from the molecular weight of PSI. The chemical structure of PAspNa was confirmed by Fourier transform infrared spectroscopy (FT-IR; KBr method). $^1$H NMR spectra were measured with a JEOL FT NMR System JMN-AL300.

Synthesis of PAsp

PAspNa was dissolved in purified water, and an ion-exchange resin (Lewatit MonoPlus S100H) was added into the solution. After 24 h, the solution was concentrated under reduced pressure at 313 K [12]. The remaining solution was added into acetone to precipitate PAsp and which was recovered by filtration. The conversion from PAspNa to PAsp was confirmed by FT-IR (KBr method). The ion-exchange efficiency was calculated from natrium ion concentration. The natrium ion concentration was measured with an ion meter (DKK-TOA, IM-55G).

Microsphere preparation

Polystyrene microspheres were prepared by dispersion polymerization under the conditions listed in Table 1. Prepared microspheres were observed with a Hitachi S-3500N scanning electron microscope (SEM), and the average diameter and coefficient of variation (CV) were analyzed from the SEM images by WinRoof (Mitani, Ver.3.53). The average diameter and CV were defined by counting at least 200 individual microspheres from SEM image. The CV was calculated from the average diameter and standard deviation.

**Table 1** Recipe for the preparation of monodisperse polystyrene microspheres

| Ingredients | Units | Values |
|---|---|---|
| Styrene | g | 1.35 |
| AIBN | g | 0.107 |
| Dispersion stabilizer | g | 0.15 |
| Aqueous ethanol solution | Ml | 45 |

343 K; 6 h; $N_2$; in flask with stirring rate, 360 rpm
*AIBN* 2,2′-Azobisisobutyronitrile

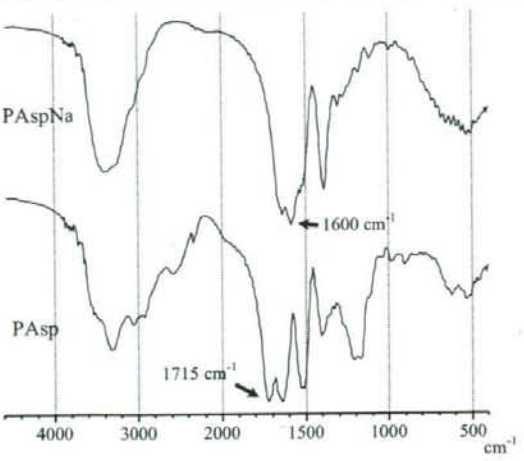


**Fig. 1** FT-IR spectra of PAspNa and PAsp

Measurements of time course of conversion, particle diameter and its distribution, and particle numbers

A small amount of polymerization solvent with PAspNa or PAsp was withdrawn at different polymerization intervals. The samples were dissolved in methanol with a small amount of 4-tert-butylcatechol to terminate the polymerization. The concentrations of residual styrene monomer dissolved in methanol solution were determined by HPLC to estimate the conversion. The samples were also measured with the SEM to determine the particle diameters of the prepared microspheres at different polymerization intervals. Particle numbers were calculated from the conversion and particle diameters.

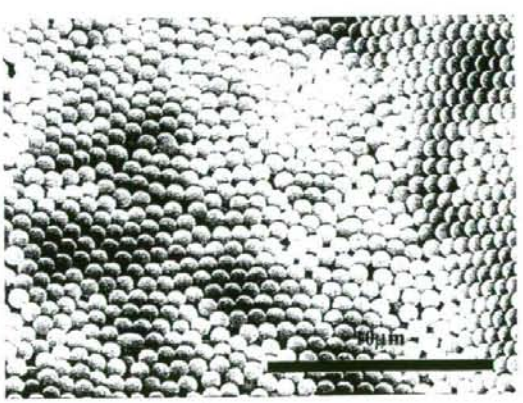


**Fig. 2** SEM image of polymeric microspheres synthesized with PAsp. Volume fraction of EtOH=60 vol.%

## Results and discussion

Characterization of synthesized PSI, PAspNa, and PAsp

The chemical structures of synthesized PSI and PAspNa were characterized by $^1$H NMR as previously reported [11, 13].

The residues of synthesized PAspNa and PAsp were determined by FT-IR [12, 14, 15]. Figure 1 shows FT-IR spectra of PAspNa and PAsp. Attention was directed to the frequency of the carboxylate group band in order to investigate the preparing of the PAsp. The spectrum of PAspNa shows a large absorbance at 1,610 $cm^{-1}$ assigned to the carboxylate anion ($-COO^-$). Meanwhile, the carboxylate anion band disappeared in the spectrum of PAsp. At

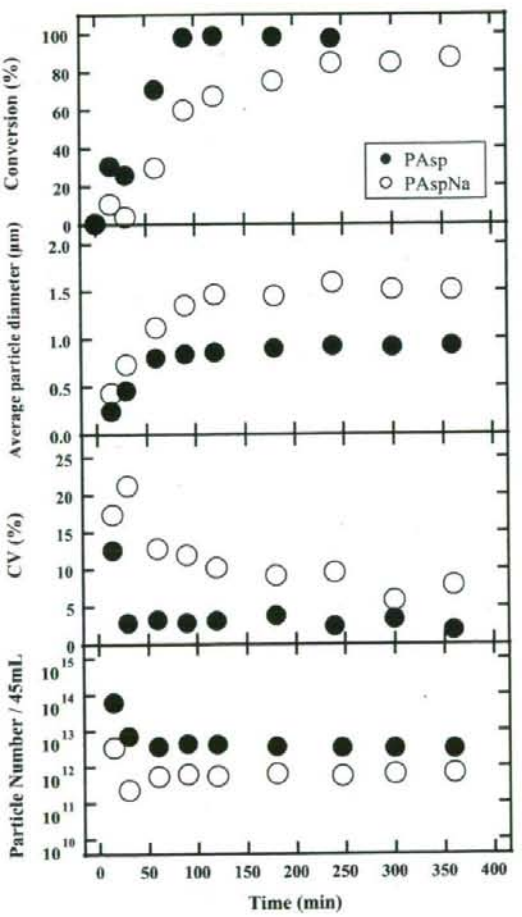


**Fig. 3** Time course of conversion, particle diameter, CV, and particle number. Concentration of dispersion stabilizer = 3.33 mg/mL. Volume fraction of EtOH=60 vol%

the same time, a new band appeared at 1,720 $cm^{-1}$. This was assigned to the carboxylate group (–COOH).

In addition, the ion-exchange efficiency was calculated from the natrium ion concentration. It was measured before and after ion exchange of PAspNa solution. As a result, the natrium ion concentration after an ion-exchange process was very low. It was considered that almost all natrium ions of PAspNa were exchanged into hydrogen ions.

Thus, it was concluded that PAsp was successfully prepared from PAspNa by ion-exchange resin.

## Kinetics of dispersion polymerization of styrene with PAspNa or PAsp

Figure 2 shows the SEM image of the polystyrene microspheres prepared with PAsp in an EtOH/water mixture with 60 vol.% EtOH. The particle diameter was approximately 0.91 μm. The CV was approximately 3.1%. The monodisperse polystyrene microspheres were clearly observed. This result indicates that PAsp acts as a dispersion stabilizer.

Figure 3 shows the time courses of styrene conversion, particle diameter, CV, and particle numbers of polystyrene microsphere in the dispersion polymerization using PAspNa or PAsp. The concentration of dispersion stabilizer was 3.4 mg/ml.

The final conversion with PAspNa is 87%. Meanwhile, the final conversion with PAsp came up to about 100%. Furthermore, the polymerization rate of styrene with PAsp was higher than that with PAspNa. The polymerization mechanism observed is similar to that in our previous studies concerning dispersion polymerization [16, 17].

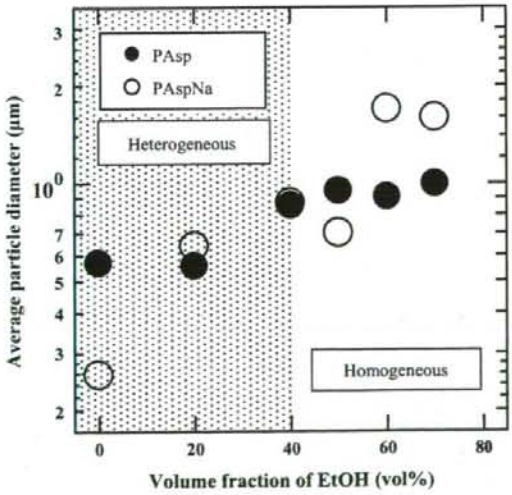


**Fig. 4** Effect of EtOH volume fraction on particle diameter. Concentration of dispersion stabilizer = 3.33 mg/mL

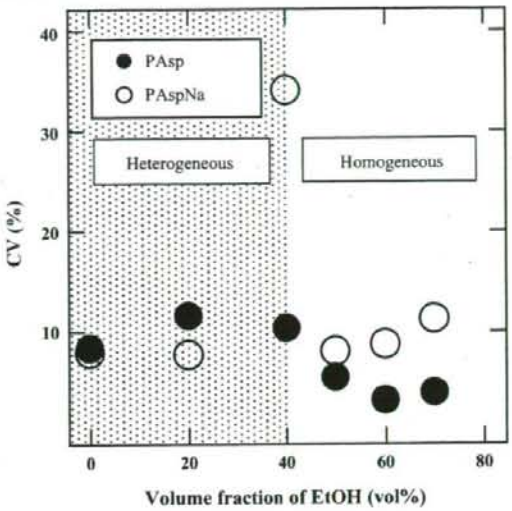


**Fig. 5** Effect of EtOH volume fraction on CV. Concentration of dispersion stabilizer = 3.33 mg/mL

The diameter of particle prepared with PAsp was smaller than that prepared with PAspNa. In addition, the growth rate of microspheres with PAsp was faster than that with PAspNa.

The CVs obtained with PAsp and PAspNa decreased with time, and the CV obtained with PAsp was smaller than that with PAspNa.

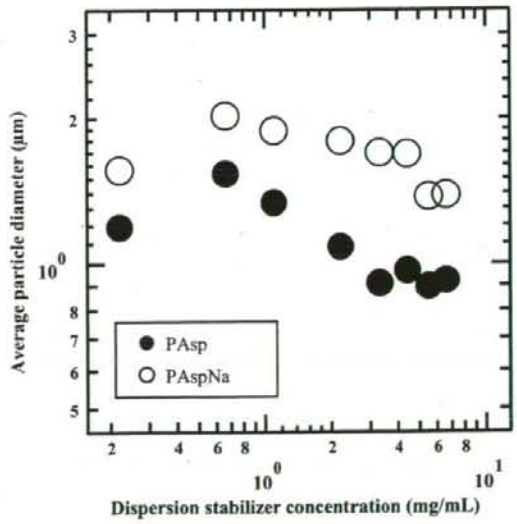


**Fig. 6** Effect of dispersion stabilizer concentration on particle diameter. Volume fraction of EtOH = 60 vol%

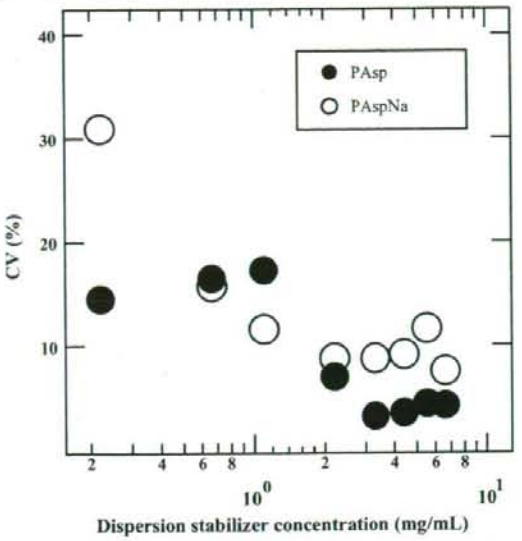


**Fig. 7** Effect of dispersion stabilizer concentration on CV. Volume fraction of EtOH = 60 vol%

Both of the particle numbers decreased with time until the particle diameter became constant.

These results show that the number of particles synthesized with PAsp was higher than that synthesized with PAspNa. This is because the increase of hydrophobicity of dispersion stabilizer provided more loci for polymerization in the reaction solution. Since PAsp was more hydrophobic than PAspNa, the affinity of PAsp to EtOH was higher than that to PAspNa. These results also lead to the fact that higher polymerization rate used PAsp as a dispersion stabilizer.

## Effect of EtOH volume fraction on average diameter and CV

Figure 4 shows the effect of the EtOH volume fraction in a polymerization medium on the average diameter when using PAspNa or PAsp. The EtOH volume fraction was between 0 and 70 vol.% with 3.4 mg/ml PAspNa or PAsp. The feed styrene monomer was completely dissolved in the EtOH/water solvent in which the EtOH content was above 40 vol.%, and the solution was homogeneous before polymerization.

The particle diameter obtained with PAspNa or PAsp increased with increasing EtOH volume fraction. There are two reasons for this result. One is that the amount of dissolved styrene was increased during EtOH volume fraction between 0 and 40 vol.%. Another reason is the critical chain length of styrene in precipitation increased with increasing EtOH volume fraction. The increasing critical chain length of styrene caused the reduction of the number of particle nuclei.

However, at higher EtOH volume fraction, microspheres synthesized with PAsp were smaller than those with PAspNa. This result concerned with the length of polymer chains in the reaction solvent. The solubility of PAsp into water was decreased as compared with that of PAspNa. In fact, however, PAsp dissolved in reaction solvent well. At the same time, PAsp had an affinity to organic solvents such as methanol and EtOH. Therefore, it was thought that PAsp solvated under condition of more spread own polymer chain at higher EtOH volume fraction. Thus, PAsp preserved the dispersion stability at higher EtOH volume fraction.

Figure 5 shows the effect of EtOH volume fraction in a polymerization medium on CV with PAspNa or PAsp. Polystyrene microspheres up to CV 10% were synthesized with PAspNa or PAsp under most conditions. At above 40 vol.% of EtOH volume fraction, the CV of prepared microspheres with PAsp was lower than those with PAsp. For the reason given above, PAsp preserved the higher dispersion stability under EtOH-rich conditions. Furthermore, the higher CV with PAspNa at 40 vol.% of EtOH was avoided using PAsp. Bimodal distribution of the particle diameter was observed when we prepared microspheres with PAsp at EtOH volume fraction of 40 vol%. We are not sure yet of the reason for the mechanism of the formation.

**Table 2** The conditions for preparing monodisperse microspheres in EtOH/water-mixed solution with PAA, PAspNa, or PAsp as a dispersion stabilizer

| Ingredients | PAA ($M_w$, $2.0\times10^5$) (non-biodegradable) | PAspNa ($M_w$, $6.0\times10^4$) (biodegradable) | PAsp ($M_w$, $4.25\times10^4$) (biodegradable) |
|---|---|---|---|
| Ethanol volume fraction in polymerization solvent (vol.%) | 70 | 60 | 60 |
| Concentration of dispersion stabilizer (mg/ml) | 10.0 | 3.33 | 3.33 |
| Concentration of monomer (mmol/ml) | 1.93 | $2.89\times10^{-1}$ | $2.89\times10^{-1}$ |
| Average diameter (μm) | 1.80 | 1.69 | 0.91 |
| CV (%) | 5.8 | 7.7 | 1.7 |

Effect of dispersion stabilizer concentration on average diameter and CV

Figure 6 shows the effect of dispersion stabilizer concentration on particle diameter. In this study, the volume fraction of EtOH was fixed at 60 vol.%. The dispersion stabilizer concentration was varied from $2.2\times10^{-1}$ to 6.7 mg/ml. This figure shows that the particle diameter decreased from approximately 2.0 to 1.4 μm by increasing the concentration of PAspNa. On the other hand, by changing the concentration of PAsp, the particle diameter decreased from approximately 1.5 to 0.9 μm. This is because the high initial stabilizer concentration causes the increasing amounts of stabilizer related to form particle nuclei in the initial stage of polymerization. Another reason is the enhancement of stabilizer absorbed on the nuclei and protected against aggregation processes in polymerization. In addition, smaller microspheres were prepared with PAsp as compared with PAspNa. This result indicated the PAsp worked as a dispersion stabilizer more effectively than PAspNa at 60 vol.% of EtOH.

Figure 7 shows the effect of dispersion stabilizer concentration on CV. The volume fraction of EtOH was 60 vol.%. The CV decreased with increasing dispersion stabilizer concentration. The monodisperse microspheres were prepared at the concentration more than 2.2 mg/ml. Thus, Figs. 6 and 7 indicate that we can control the particle diameter of monodisperse microspheres in the case of applying appropriate dispersion stabilizer to a reaction solution.

The effect of the relation between dispersion stabilizer and reaction solvent on forming monodisperse microspheres

Table 2 shows the conditions for preparing monodisperse microspheres in EtOH/water-mixed solution with several dispersion stabilizers [1]. The monodisperse microspheres with similar size were prepared using PAA or PAspNa as previously reported. PAspNa is biodegradable, and the concentration for preparing monodisperse microspheres is about one third of that of PAA.

In this study, we applied PAsp as a dispersion stabilizer to dispersion polymerization. Monodisperse microspheres were prepared at higher EtOH volume fraction, and the CV obtained was lower than that with PAspNa. Furthermore, smaller microspheres than that with PAspNa were prepared at higher EtOH volume fraction. However, at less than 40 vol.% of EtOH volume fraction, it was difficult to prepare monodisperse microspheres with PAsp. This suggested that, monodisperse microspheres were obtained when using the dispersion stabilizer having an affinity to a reaction solvent.

## Conclusion

We synthesized PAsp by ion exchange of PAspNa. We applied PAsp as a dispersion stabilizer to dispersion polymerization. The monodisperse microspheres were obtained with PAsp as a dispersion stabilizer. It suggested that PAsp acts as a dispersion stabilizer for styrene polymerization. In addition, the polymerization rate of styrene with PAsp was higher than that with PAspNa. The particle diameter with PAsp increased as the EtOH volume fraction increased. At high EtOH volume fraction, microspheres prepared with PAsp were smaller than those with PAspNa. Consequently, we obtained monodisperse microspheres by using PAsp at higher EtOH volume fraction.

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SHORT COMMUNICATION

# Synthesis of polyaspartate macromonomer having a vinyl end group and application to dispersion copolymerization of styrene

**Keisuke Tomita · Tsutomu Ono**

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**Abstract** Sodium polyaspartate (PAspNa) macromonomer with an acryloyl end group was synthesized for dispersion polymerization. At first, a poly(succinimide) (PSI) derivative with a hydroxyphthalimide end group was synthesized by polycondensation of L-aspartic acid and 4-hydroxyphthalic acid. Then, the PSI derivative was end-capped with an acryloyl group by a reaction with acrlyloyl chloride. Finally, a PAspNa derivative with a vinyl end group was synthesized by a hydrolysis of succinimide units by sodium hydroxide. The synthesized macromonomer was applied as a polymerizable stabilizer in dispersion copolymerization of styrene in a mixture of ethanol and water. The PAspNa macromonomer acted as an effective stabilizer and gave sub-micron-sized polymeric particles in dispersion polymerization in polar medium.

**Keywords** Macromonomer · Polyaspartate · Particle · Dispersion polymerization

## Introduction

Particles having a hydrophobic core and a hydrophilic layer are called hairy particles. A layer of concentrated polymer chains is formed on these particle surfaces and provides high stability and functionality. Hairy particles with well-designed functionality are promising for the applications such as affinity particles, drug delivery carriers, and catalyst. Therefore, the design of functionality of the hairy chains is a key factor for the particle usage in these applications.

Generally, there are two methods to prepare hairy particles from existing core particles: (1) "grafting onto" method [1–3], where end-functionalized polymer chains are coupled with core particles, and (2) "grafting from" method [4–11], where hairy chains are grown from the surface of core particles modified with initiators. Especially, surface-initiated living radical polymerization has been used in recent years by a lot of researchers to prepare hairy particles because this method produces hairy chains with controlled chain length on the particle surface. However, multistep reactions are required to produce the core particles modified with initiators.

Heterogeneous polymerization with macromonomer is an alternative for preparing hairy particles. Heterogeneous polymerization, such as emulsion polymerization and dispersion polymerization, is a one-pot polymerization method to obtain polymeric particles in nano- to micro-scale, and the particle diameter can be controlled by changing the reaction parameters. Polymeric particles prepared by dispersion copolymerization [12–27] or emulsion copolymerization [26–34] with macromonomer have high stability and functionality derived from anchoring of hydrophilic polymer chains on the particle surface. Almost all studies used the macromonomer based on poly(ethylene oxide) (PEO) in the heterogeneous polymerization [13, 17, 20–22, 24–26, 29, 31–34] because of the high solubility in various solvents. However, the functionalization of PEO chains is difficult owing to chemical stability of the ethylene oxide units. Thus, the heterogeneous polymerization with PEO macromonomer is not suitable to design the functionality of hairy chains.

Sodium polyaspartate (PAspNa), a hydrophilic biodegradable polymer, can easily accept the introduction of

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functional groups into the side chains. PAspNa is derived from poly(succinimide) (PSI), product from the polycondensation of L-aspartic acid, by hydrolysis with sodium hydroxide [35]. PSI reacts with various amine compounds without any coupling agent. Therefore, PAspNa derivatives with various functional pendant groups are easily designed [35–38].

In this study, we synthesized PAspNa macromonomer with an acryloyl end group and applied to prepare functionalized hairy particles. The outline of the synthesis route is shown in Scheme 1. At first, a PSI derivative with a hydroxyphthalimide end group (**1**) was synthesized by polycondensation of L-aspartic acid and 4-hydroxyphthalic acid. Then, **1** was end-capped with an acryloyl group to obtain a PSI derivative with a vinyl end group (**2**). Finally, PAspNa macromonomer (**3**) was synthesized by a hydrolysis of **2**. The synthesized PAspNa macromonomer was used as a polymerizable stabilizer in dispersion copolymerization of styrene to prepare the polymeric particles. A PAspNa derivative without vinyl end group was also synthesized in order to compare the results obtained between macromonomer and nonpolymerizable stabilizer.

## Experimental

### Materials

All materials were obtained from Wako Pure Chemical Industries. *N,N*-Dimethylformamide (DMF) was dehydrated by adding dried molecular sieves. Styrene was purified by distillation under reduced pressure. 2,2′-Azobisisobutyronitrile (AIBN) was purified by recrystallization from ethanol. Other materials were used without further purification. Water was purified by a Millipore Milli-Q purification system.

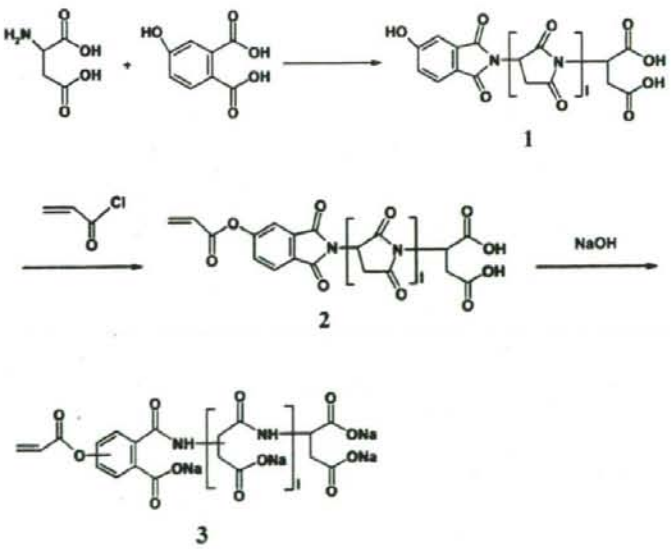
### Synthesis of PSI derivative with a hydroxyphthalimide end group (1)

A typical procedure for the synthesis of **1** is as follows: 23.4 g of L-aspartic acid (Asp), 1.19 g of 4-hydroxyphthalic acid (HPA), 9.94 g of phosphoric acid, and 1.81 g of water were mixed in a flask. The flask was placed in a rotary evaporator and heated at 453 K for 7 h under reduced pressure. The product was dissolved in 300 ml of DMF, and the solution was poured into 2 l of methanol. The precipitate was washed three times with methanol and three times with water. The resultant polymer was dried in a vacuum at 313 K to obtain the **1** (15.0 g, 83%).

### Synthesis of PSI derivative with a vinyl end group (2)

**1** (3.0 g) was dissolved in 30 ml of dry DMF, and more than ten excess of acryloyl chloride (AC) and more than ten excess of triethylamine (TEA) were added to the solution. The mixture was stirred at room temperature for 24 h and then poured into 300 ml of methanol. The precipitate was washed six times with methanol and three times with water. The resultant polymer was dried in a vacuum at room temperature to obtain the **2** (2.68 g, 88%).

**Scheme 1** Synthesis of PAspNa derivative with a vinyl end group



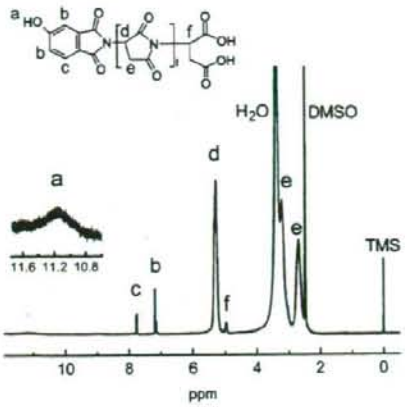



**Fig. 1** $^1$H NMR spectrum of **1** in DMSO-$d_6$

Synthesis of PAspNa derivative with a vinyl end group (**3**)

**2** (2.5 g) was dispersed in 100 ml of water, and 1 N sodium hydroxide (NaOH) solution was added dropwise so as not to exceed pH 10 in the solution. The solution was then neutralized by 1 N hydrochloric acid solution and concentrated under reduced pressure. The solution was recrystallized from methanol. The precipitate was dried in a vacuum to obtain the **3** (3.48 g, 99%).

Synthesis of a PAspNa derivative without a vinyl end group (**3′**)

**3′** was synthesized from **1** and 1 N NaOH solution using the same method for **3** (3.53 g, 99%).

Dispersion copolymerization

Dispersion copolymerization was carried out in a reactor equipped with a reflux condenser and a magnetic stirrer and placed in an oil bath equipped with a temperature control. A typical procedure for dispersion copolymerization of styrene with **3** is presented below: 0.107 g of AIBN and 1.34 g of styrene were dissolved in ethanol 27 ml, and it was added into 18 ml of aqueous solution containing of 0.10 g of **3**. The mixture was polymerized in the reactor at 343 K for 6 h under nitrogen atmosphere. The resultant particles were refined by centrifugating washes with water three times.

Measurements and characterization

$^1$H NMR spectra were measured using a NMR spectrometer (JEOL AL300 SC-NMR). Weight-average molecular weight ($M_w$) and molecular weight distribution ($M_w/M_n$) of a PSI derivative were determined by a gel permeation chromatography (TOSOH HLC-8120 GPC system) using polystyrene standards with DMF as an eluant. The flow rate and the column temperature were 0.6 ml/min and 313 K, respectively. The particle diameter and the diameter distribution were determined by scanning electron microscopy (SEM, Hitachi S-4700). The number-average particle diameter was obtained by counting 200 particles in SEM photographs. Coefficient of variation of the particle diameter was calculated using the following equation:

$$\mathrm{CV}(\%) = \frac{\text{Standard derivation } (\mu\text{m})}{\text{Number} - \text{average particle diameter } (\mu\text{m})} \times 100$$

Styrene monomer conversion was calculated from the unreacted styrene monomer concentration, which is measured by high-performance liquid chromatography. Small amount of resultant latex was added to methanol with 4-*t*-butylpyrocatechol to terminate polymerization. These solutions were centrifuged at 30,000 rpm for 15 min to remove the particles. Unreacted styrene concentration in the supernatant was measured by high-performance liquid

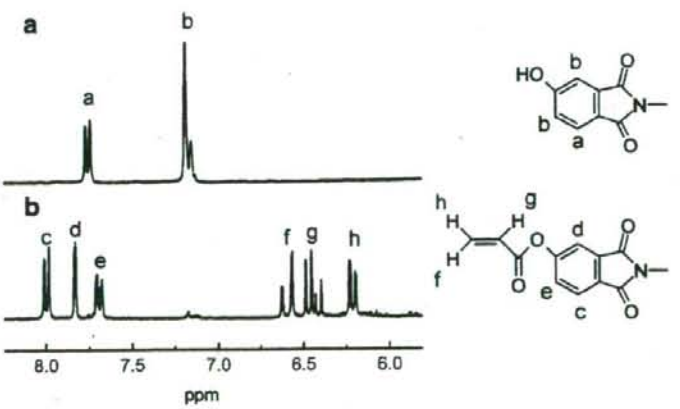
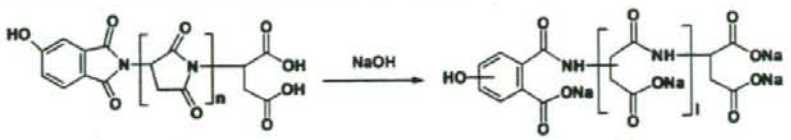



**Fig. 2** $^1$H NMR spectra **1** (**a**) and **2** (**b**) in DMSO-$d_6$

**Scheme 2** Synthesis of PaspNa derivative without a vinyl end group




chromatography (SHIMADZU Prominence HPLC system) with a UV–VIS detector (SPD-M20A, $\lambda$=254 nm) with the mixture of methanol/water=7:3 (vol/vol) as an eluant. The column was a TSK-Gel ODS-80Ts QA (150×4.6 mm, TOSOH). The flow rate and the column temperature were 0.8 ml/min and 313 K, respectively.

## Results and discussion

A PSI derivative with a hydroxyphthalimide end group (**1**) was synthesized by the bulk polycondensation of Asp and HPA in the presence of phosphoric acid. The polymer with $M_w$ of 7,700 was obtained, and the distribution was broad ($M_w/M_n$=4.2). Figure 1 shows the $^1$H NMR spectrum of **1** in deuterated dimethyl sulfoxide (DMSO-$d_6$). The peak at 4.9 ppm is assigned to a methine proton of the succinic acid end group, and the peaks at 7.2 and 7.8 ppm are assigned to aromatic protons of the phthalimide end group. The end functionality of **1** was estimated from the integration value ratio at 4.9 and 7.8 ppm. The number-average molecular weight ($M_n$) was calculated by the integration values of a peak at 4.9 and 5.3 ppm for a methine proton of the succinimide unit. The hydroxyphthalimide end functionality ($f_{OH}$) and $M_n$ estimated by $^1$H NMR spectrum were 98% and 3,400, respectively. A PSI derivative with a vinyl end group (**2**) was synthesized by the reaction of hydroxyphthalimide end group of **1** and AC in the presence of TEA. Expanded region of the $^1$H NMR spectra of **1** and **2** are shown in Fig. 2. This figure shows that the peaks at 7.2 ppm disappeared as well as three peaks at 6.2~6.7 ppm and three peaks at 7.7~8.0 ppm in $^1$H NMR spectrum of **2**. These peaks were assigned to the vinyl and aromatic protons of the acryloxyphthalimide group, respectively. Vinyl end functionality ($f_{vinyl}$) of **2** was calculated by follow equation:

$$f_{vinyl} = \frac{I_{6.3}/3}{I_{6.3}/3 + I_{7.2}/2} \times f_{OH}$$

where $I_i$ is the integration value of the peak at $i$ ppm in $^1$H NMR spectrum of **2**. **2** with 91% of $f_{vinyl}$ was obtained by the reaction with a large excess of AC. Hydrolysis of succinimide units in **2** by NaOH solution produced PAspNa macromonomer with a vinyl end group (**3**). The hydrolysis of succinimide units was confirmed by disappearance of the peak at 5.3 ppm and appearance of the peaks at 4.5 and 4.7 ppm for methine protons of an aspartic acid unit in $^1$H NMR spectrum in deuterium oxide (data not shown). A PAspNa derivative without a vinyl end group (**3′**) was synthesized by the hydrolysis of **1** (Scheme 2).

Dispersion (co)polymerization using **3** or **3′** as a dispersion stabilizer in a mixture of ethanol and water were carried out. By dispersion polymerization using **3′**, polymer colloid was obtained in 78% conversion; however, much coagulum was also formed. On the other hand, polymer colloid with no coagulum was obtained in 88% conversion by dispersion copolymerization with **3**. Figure 3 shows the SEM images of the particles prepared using macromonomer **3** and nonpolymerizable stabilizer **3′**. Particle diameter of the particles prepared using **3′** and **3** were 2.33 and 0.407 μm, respectively. Macromonomers are chemically anchored on the particle surface during dispersion poly-

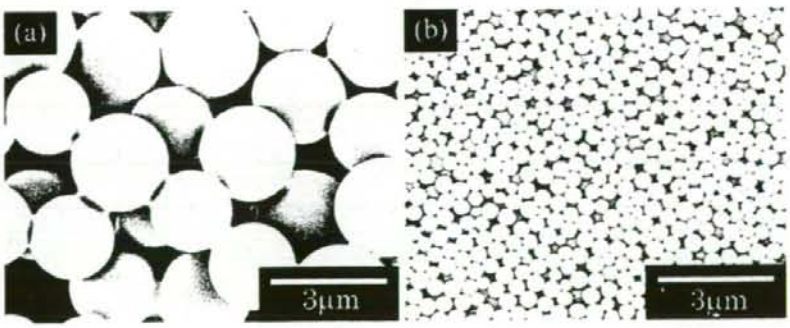



**Fig. 3** SEM images of the particles prepared by dispersion (co)polymerization of styrene with PAspNa derivatives: **a** nonpolymerizable stabilizer (**3′**); **b** macromonomer (**3**). [**3** or **3′**]=2.22 g/l

merization and provide high dispersion stability [14]. Thus, the particles prepared using **3** were much smaller than those prepared using **3′**. The comparison results indicated that the PAspNa macromonomer is an effective stabilizer in dispersion polymerization in polar medium.

## Conclusion

A PSI derivative with a hydroxyphthalimide end group was synthesized by bulk polycondensation of Asp and HPA in the presence of phosphoric acid. This polymer was reacted with AC in the presence of TEA and hydrolyzed by NaOH solution to obtain PAspNa macromonomer with an acryloyl end group. Sub-micron-sized polymeric particles were obtained by dispersion copolymerization of styrene and PAspNa macromonomer in a mixture of ethanol and water. These particles obtained were smaller than those prepared using a PAspNa derivative without polymerizable group as a dispersion stabilizer.

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# Preparation of Monodisperse Polylactide Microspheres by Dispersion Polymerization Using a Polymeric Stabilizer with Hydroxy Groups

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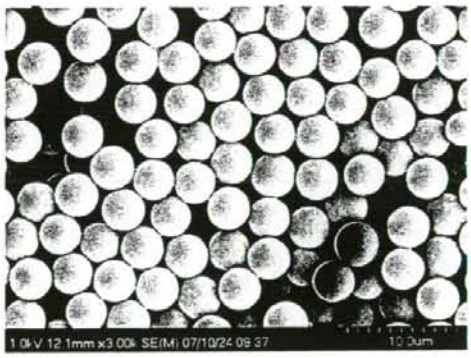
Monodisperse poly(D,L-lactide) (PDLLA) microspheres have been prepared by dispersion polymerization of D,L-lactide with a synthetic polymeric stabilizer. The polymerization is carried out in xylene/heptane (1:2, v/v) at 368 K for 3 h with poly[(dodecyl methacrylate)-*co*-(2-hydroxyethyl methacrylate)] (P(DMA-*co*-HEMA)). P(DMA-*co*-HEMA) has hydroxy groups as an initiation group for pseudoanionic dispersion polymerization. The particle diameter and the coefficient of variation concerning the diameter distribution of the obtained PDLLA microspheres are 3.9 μm and 4.3%, respectively. In addition, from the results of dynamic light scattering measurements, it is found that P(DMA-*co*-HEMA) and the PDLLA-grafted copolymer form a micellar structure in solution.




## Introduction

Biodegradable polymeric microspheres have been developed for the chemical industry, e.g., for coatings, inks, adhesives, and controlled drug delivery systems.[1–4] Polylactide (PLA), a typical biodegradable polymer, shows good mechanical properties, biodegradability, and biocompatibility to use in those areas.[5–7] PLA microspheres are mainly prepared by solvent evaporation and spray drying techniques.[8–11] However, these techniques have drawbacks such as the requirement of a multi-step preparation process and polydispersity of particle diameter. Alternatively, dispersion polymerization is well known to provide monodisperse polymeric microspheres that range from submicrometer to 15 μm. The dispersion polymerization is carried out in a reaction medium that dissolves monomer, but does not dissolve the polymer. The polymerization proceeds in the reaction medium until a critical molecular weight for the solubility of the polymer chain, and then the precipitated primary particles are stabilized by a polymeric stabilizer. Slomkowski et al. reported that poly(D,L-lactide) (PDLLA) (or poly(L,L-lactide, PLLA) microspheres with a narrow size distribution, and a coefficient of variation (CV) of 11.5%, were prepared by dispersion polymerization of D,L (or L,L)-lactide in 1,4-dioxane/heptane (1:4, v/v) using poly(dodecyl acrylate)-*graft*-poly(ε-caprolactone) as a polymeric stabilizer.[12–14] We also reported that PDLLA microspheres with a narrow

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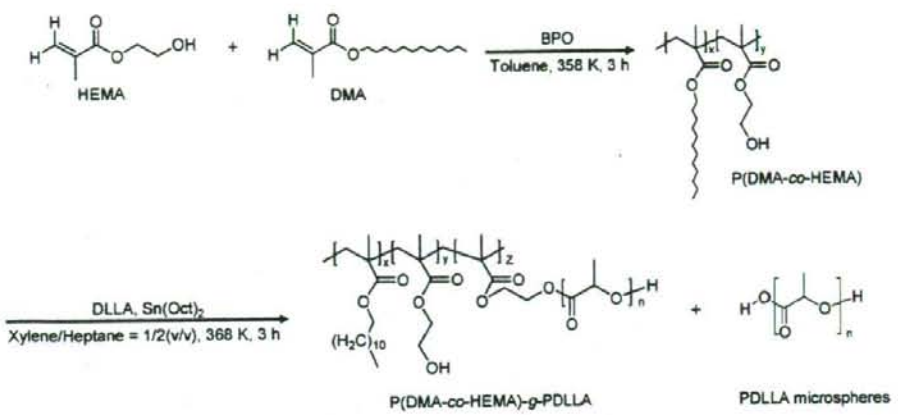


■ *Scheme 1.* Synthesis of P(DMA-*co*-HEMA) and preparation of the PDLLA microspheres.

diameter distribution, and a CV of 14.1%, were prepared by dispersion polymerization of D,L-lactide in xylene/heptane (1:2, v/v) using the copolymer grafted with PLLA, poly(dodecyl methacrylate)-*graft*-poly(L,L-lactide), as a polymeric stabilizer.[15] However, monodisperse PDLLA microspheres (less than 10% of CV) have not been prepared.

In this study, we designed a new polymeric stabilizer to prepare monodisperse PDLLA microspheres by dispersion polymerization of D,L-lactide. The stabilizer plays a critical role in the primary particle formation stage. A block or graft copolymer that contains both soluble and insoluble polymer segments for a solvent has been used as a stabilizer.[16,17] Winnik et al. showed the existence of regular micelles comprised of several hundred diblock copolymer molecules and the micelle clusters corresponded to the aggregation of tens of micelles in the aqueous solution by dynamic light scattering measurements.[18] The equilibrium state between polymeric micelles and unimers affects the adsorption rate of copolymeric stabilizer on the surface of primary particles. Thus, the mechanism of particle formation in the dispersion polymerization is complex and poorly understood when using a diblock copolymer as a dispersion stabilizer, and it is difficult to prepare monodisperse PDLLA microspheres. On the other hand, a graft copolymer is produced in situ when a precursor polymer that contains active sites for the chain transfer of radicals, such as hydroxypropyl cellulose, poly(acrylic acid), and poly(vinyl pyrrolidone) (PVP), is employed as a stabilizer in the dispersion polymerization of styrene.[19–21] Since these polymeric stabilizers are able to ignore the formation of micelles in the solution in an initial stage, it is promising to prepare monodisperse polystyrene microspheres.

Our idea is motivated by the case of the graft copolymeric stabilizer produced in situ, and thus we synthesized poly[(dodecyl methacrylate)-*co*-(2-hydroxyethyl methacrylate)] (P(DMA-*co*-HEMA)) to prepare monodisperse PDLLA microspheres (Scheme 1). P(DMA-*co*-HEMA) has hydroxy groups as an initiation group for pseudoanionic dispersion polymerization, which leads to the formation of the copolymer grafted with PDLLA, P(DMA-*co*-HEMA)-*g*-PDLLA. This paper describes the preparation of monodisperse PDLLA microspheres by dispersion polymerization of D,L-lactide using P(DMA-*co*-HEMA) as a polymeric stabilizer.

## Experimental Part

### Materials

D,L-Lactide (Purac) was purified by recrystallization from toluene. 2-Hydroxyethyl methacrylate (HEMA), dodecyl methacrylate (DMA) (Wako Pure Chemical Industries, Ltd.), and tin(II) 2-ethylhexanoate ($Sn(Oct)_2$, Aldrich)[22] were purified by distillation under reduced pressure. Xylene and heptane (dehydrated grade, Wako Pure Chemical Industries, Ltd.) were stored in a glove box filled with argon gas. Other reagents (Wako Pure Chemical Industries Ltd.) were used as received.

### Measurements

Gel permeation chromatography (HLC 8120, Tosoh, GPC) was performed on the basis of polystyrene standards with tetrahydrofuran (THF) as an eluent to determine the weight-averaged molecular weight ($\overline{M}_w$) and polydispersity index ($\overline{M}_w/\overline{M}_n$) of the synthesized polymer. $^1$H NMR (AL300 SC-NMR, JEOL) spectroscopy was conducted using $CDCl_3$ that contained tetramethylsilane (TMS, 1%, v/v) as an internal standard to determine the molecular structure of the synthesized polymer. Scanning electron micro-

scopy (S-4700, Hitachi, SEM) was performed to determine the particle diameter ($d_p$) and the diameter distribution (CV) of the prepared PDLLA microspheres. Dynamic light scattering measurement (FPAR-1000, Otsuka Electronics Co., DLS) was carried out at 293 K to determine the hydrodynamic diameter ($R_h$) of the micelles composed of the copolymeric stabilizers in xylene/heptane (1:2, v/v).

### P(DMA-*co*-HEMA) Synthesis

P(DMA-*co*-HEMA) was synthesized according to Scheme 1. DMA (31.18 mmol), HEMA (0.62 mmol), and 24 mL of dehydrated toluene as a solvent were placed into a round-bottom reactor. After nitrogen was admitted to remove oxygen, the reactor was immersed in an oil bath at 358 K. Benzoyl peroxide (BPO, 1.25 mmol) dissolved in dehydrated toluene was added to initiate the polymerization. The polymerization was conducted for 3 h. After cooling, the reaction mixture was poured into excess methanol to remove the remaining DMA. After the purification, the obtained polymer was dried under reduced pressure at 313 K.

### PDLLA Microspheres Preparation

PDLLA microspheres were prepared according to Scheme 1. D,L-Lactide (3.47 mmol) was added to 17 mL of a solution of P(DMA-*co*-HEMA) dissolved in dehydrated xylene/heptane (1:2, v/v). The solution was stirred at 120 rpm with a magnetic stirrer. $Sn(Oct)_2$ (0.12 mmol) dissolved in a solution of dehydrated xylene and heptane (3 mL, 1:2, v/v) as a catalyst was prepared. The solution was added by a syringe and the polymerization was conducted at 368 K for 3 h. After the polymerization, the reaction solution was poured into excess cold heptane. The solution was centrifuged for 3 min at 6 000 rpm, and the microspheres were redispersed into excess heptane. The solution was filtered to collect the obtained microspheres.

## Results and Discussion

### P(DMA-*co*-HEMA) Synthesis

P(DMA-*co*-HEMA) ($\overline{M}_w = 40\,000$, $\overline{M}_w/\overline{M}_n = 2.48$) was successfully obtained by free radical copolymerization of DMA and HEMA using BPO as an initiator. The $^1$H NMR spectrum of P(DMA-*co*-HEMA) is shown in Figure 1a. The spectrum shows peaks at 3.9 ppm ($COOCH_2$ for DMA unit) and 4.1 ppm ($COOCH_2$ for HEMA unit). Furthermore, the peaks at around 5.6 and 6.1 ppm (double bond for DMA and HEMA) were not detected in the spectrum. Therefore, P(DMA-*co*-HEMA) was identified. The number of HEMA units in P(DMA-*co*-HEMA) ($N_{HEMA} = 2.0$) was calculated from the $^1$H NMR spectrum using the integration ratios that correspond to

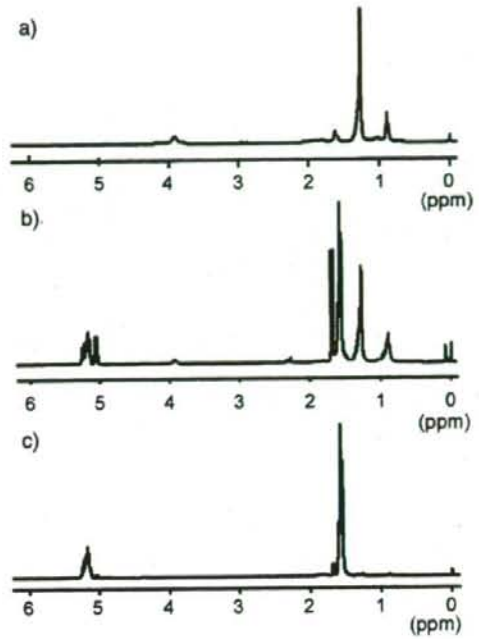


*Figure 1.* $^1$H NMR spectra of a) P(DMA-*co*-HEMA), b) PDMA-*g*-PDLLA, and c) PDLLA microspheres.

around 3.9 ppm ($COOCH_2$ for DMA unit) and 4.1 ppm ($COOCH_2$ for HEMA unit).[23]

### PDLLA Microspheres Preparation

Figure 2 shows SEM images of PDLLA microspheres prepared by dispersion polymerization with different concentrations of P(DMA-*co*-HEMA). By observing the spherical particles, it was clear that P(DMA-*co*-HEMA) played a critical role as a dispersion stabilizer. In addition, from Figure 1b, the $^1$H NMR spectrum of the polymeric

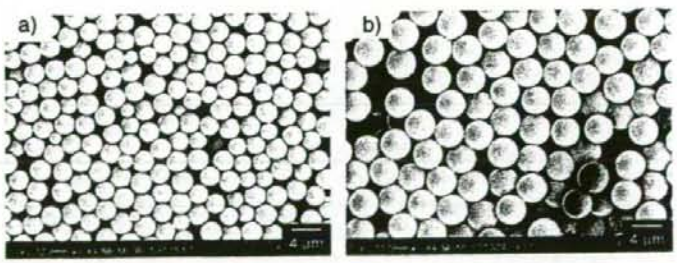


*Figure 2.* SEM images of monodisperse PDLLA microspheres prepared with different concentrations of P(DMA-*co*-HEMA): a) $[P(DMA\text{-}co\text{-}HEMA)] = 250\ mmol \cdot L^{-1}$ ($d_p$ = 2.7 μm, CV = 8.3%), b) $[P(DMA\text{-}co\text{-}HEMA)] = 63\ mmol \cdot L^{-1}$ ($d_p$ = 3.9 μm, CV = 4.3%).


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stabilizer after polymerization showed peaks for both DMA and PLA ($CH_3$; 1.7 ppm, CH; 5.2 ppm). Furthermore, the peak at 4.1 ppm ($COOCH_2$ for HEMA unit) was not detected. Therefore, the graft copolymer, PDMA-*g*-PDLLA, was produced in situ by polymerization of D,L-lactide from all the hydroxy groups in P(DMA-*co*-HEMA). According to the $^1$H NMR spectrum of the purified PDLLA microspheres, only two peaks are observed at 1.7 and 5.2 ppm ($CH_3$ and CH for PLA), and the peaks of PDMA-*g*-PDLLA were not detected (Figure 1c). In the dispersion polymerization of styrene or methyl methacrylate using a conventional stabilizer, PVP, the existence of a graft copolymer on the resultant particle surface has been confirmed by FT-IR and X-ray photoelectron spectroscopy.[24,25] Therefore, it was suggested that the adsorption of the graft copolymeric stabilizer on the particle surface was low in the case of the dispersion polymerization of D,L-lactide using P(DMA-*co*-HEMA) as a dispersion stabilizer. The particle diameter of the PDLLA microspheres decreased with increasing P(DMA-*co*-HEMA) concentration. This tendency of particle size control has been reported by other researchers.[24,25] In addition, monodisperse PDLLA microspheres ($d_p = 3.9$ μm, $CV = 4.3\%$) were obtained when using 63 mmol·L$^{-1}$ P(DMA-*co*-HEMA).

The hydrodynamic diameter ($R_h$) of P(DMA-*co*-HEMA) was estimated by DLS measurement to investigate the presence of P(DMA-*co*-HEMA) micelles and the micelles comprised of the graft copolymers derived from P(DMA-*co*-HEMA) in xylene/heptane (1:2, v/v). The results of the DLS measurements are shown in Figure 3. P(DMA-*co*-HEMA) exhibited a bimodal size distribution that consists of the unimers and the polymeric micelles (formed by self-aggregation) (Figure 3a). The average diameters of the unimers and the polymeric micelles were about 1.3 and 8.8 nm, respectively. In addition, it was found that most of P(DMA-*co*-HEMA) were in a unimer state in the solution. Thus, the presence of P(DMA-*co*-HEMA) unimers during the initial stage would be a contributing factor to prepare monodisperse PDLLA microspheres. By producing graft copolymers in situ, the single size distribution based on the polymeric micelles appeared (Figure 3b). The average diameter was about 43.8 nm, which was almost same as that obtained with dextran grafted with PLLA.[26] This result indicates that the equilibrium state between the polymeric micelles and unimers in the solution influences the particle diameter and the distribution of PDLLA microspheres. Moreover, it was proposed that the molecular structure of P(DMA-*co*-HEMA) strongly affected the equilibrium state, which was important to prepare monodisperse PDLLA microspheres.

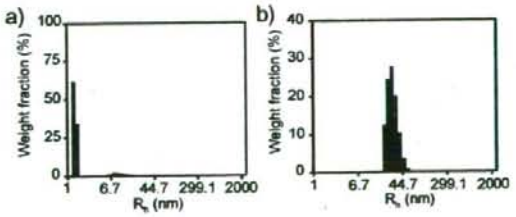


*Figure 3.* Size distributions of aggregates of the polymeric stabilizer in xylene/heptane (1:2, v/v): a) before the polymerization, b) after the polymerization, [P(DMA-*co*-HEMA)] = 250 mmol·L$^{-1}$.

## Conclusion

We synthesized P(DMA-*co*-HEMA) that contained hydroxy groups and used it as a stabilizer in the dispersion polymerization of D,L-lactide. From the results, PDLLA microspheres in the size range of 2.7–3.9 μm were obtained using P(DMA-*co*-HEMA). In particular, when using 63 mmol·L$^{-1}$ P(DMA-*co*-HEMA), monodisperse PDLLA microspheres ($d_p = 3.9$ μm, $CV = 4.3\%$) were obtained. In addition, most of P(DMA-*co*-HEMA) showed a unimer state in the solution. It produced a graft copolymer in situ during the polymerization and formed polymeric micelles (by self-aggregation). Therefore, it is supposed that the molecular structure of P(DMA-*co*-HEMA) affects the equilibrium state between the polymeric micelles and unimers in the solution, which would be a contributing factor to prepare monodisperse PDLLA microspheres.

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