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ORIGINAL CONTRIBUTION

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Synthesis of temperature-sensitive submicron-size composite polymer particles

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Abstract Temperature-sensitive composite polymer particles were prepared by seeded emulsion copolymerization of dimethylaminoethyl methacrylate and ethylene glycol dimethacrylate with 0.17 μm-sized monodispersed polystyrene seed particles. The adsorption and desorption behaviors of low molecular weight cationic emulsifier as well as albumin were examined to determine the variation of surface properties as a function of temperature below and above 35 °C.

Key words Composite particle – temperature-sensitive – biomolecules – adsorption – surface

Introduction

Temperature-sensitive polymer particles are considered to be useful as a carrier for biomolecules in the biomedical field. Recently poly-N-isopropylacrylamide (poly-NIPAM) is getting more attention because it has such an attractive temperature-sensitive property [1–5]. This property is due to the reversible formation and cleavage of the hydrogen bonds between the amide group and surrounding water molecules with changing temperature. Kawaguchi et al. reported that such a property is suitable for applying poly-NIPAM particle as an adsorbent in the biomedical field [6].

In a previous article [7], we found that polydimethylaminoethyl methacrylate (PDM) in an aqueous medium also exhibited a similar temperature-sensitive property as poly-NIPAM and utilized it as a temperature-sensitive flocculent.

In this article, it will be tried to produce temperature-sensitive composite polymer particles based on PDM by seeded emulsion copolymerization of dimethylaminoethyl methacrylate (DM) and ethylene glycol dimethacrylate (EGDM) with submicron-sized monodispersed polystyrene (PS) seed particles. EGDM was used as a cross-linking agent to prevent the solubility of PDM.

Experimental

Materials

Styrene (S) was distilled under reduced pressure in a nitrogen atmosphere. DM and EGDM were of reagent grade and used as received. 2,2′-azobis(2-amidinopropane) hydrochloride (AIBA), trimethyl stearyl ammonium chloride (TSAC) and polyoxyethylene sorbitan monooleate (Tween 80) were also of reagent grade. Albumin from eggs (Wako Pure Chemicals Co.) was preserved in the refrigerator and used as received. Deionized water was distilled with a Pyrex distillator. Other chemicals used were of analytical grade.

Preparation of PS seed particles

Emulsion polymerization of S was carried out in the presence of nonionic emulsifier at 60 °C for 24 h under a nitrogen atmosphere at a stirring rate of 100 rpm in a round bottomed four necked flask under the conditions listed in Table 1.

Seeded emulsion copolymerization of DM and EGDM

Seeded emulsion copolymerization of DM and EGDM was carried out in the presence of PS seed particles at 60 °C under the conditions presented in Table 1. The conversion was about 98%. The produced composite polymer particles were washed repeatedly by serum replacement with deionized water to remove any traces of ionized salt and/or emulsifier. The solid content of the redispersed particles was adjusted in the range of 3 to 5 g/l. The conductance of the purified composite emulsion was found to be under 10 μS/cm.

The hydrodynamic diameters of particles at 40 and 25 °C were measured by dynamic light scattering (DLS).

Measurement of relative viscosity

1% PDM aqueous solution was prepared and its pH value was adjusted to 4 and 9 with HCl and KOH aqueous solution, respectively. The falling time of each aqueous solution was measured separately at different temperatures, using Ostwald viscometer. The falling time of deionized water was also measured at the respective temperatures, using the same viscometer. The relative viscosity at a particular temperature was then calculated by dividing the falling time of aqueous solution with that of water.

Adsorption of cationic emulsifier

10 ml of purified emulsion (solid content, 3–5 g/l) was mixed with 10 ml of cationic TSAC emulsifier aqueous solution. The concentration of the emulsifier in the mixture was below the critical micelle concentration. The conductance of the mixture, whose pH was adjusted to 9 with KOH aqueous solution, was 156 μS/cm at 25 °C. In comparison with it, a TSAC emulsifier aqueous solution whose pH and conductance were, respectively, 9 and 155 μS/cm at 25 °C was prepared. In order to examine the adsorption and desorption behaviors of the emulsifier onto the composite polymer particles at 40 and 25 °C, the conductance was measured alternatively at 40 and 25 °C. Before the measurement, the mixture was kept at the respective temperatures for 1 h. The same measurement was carried out at pH 4.

The amount of emulsifier adsorbed at pH 9 was calculated by subtracting the emulsifier concentration in the medium from the initial concentration. The emulsifier concentration in the medium was obtained using two calibration curves, representing the relationship between the concentration and conductance, for the emulsifier aqueous solution at 40 and 25 °C, assuming that the adsorbed emulsifier molecules do not contribute to the conductance.

Table 1 Preparation of polystyrene seed and PS/P(DM-EGDM) composite emulsion by emulsion polymerizations[a]

Ingredients		PS	PS/P(DM-EGDM)
PS emulsion[b]	(g)	–	66.22
Styrene	(g)	64	–
DM	(g)	–	8.33
EGDM	(g)	–	0.44
Tween 80	(g)	0.160	–
AIBA	(g)	0.256	2.00
Water	(g)	300	500
Polymn. time	(h)	24	8

[a] 60 °C, N_2 atmosphere, 100 rpm

[b] Polymer solid 150 g/l

Abbreviations: PS, polystyrene; DM, dimethylaminoethyl methacrylate; EGDM; ethylene glycoldimethacrylate; Tween 80, polyoxyethylene sorbitan monooleate; AIBA, 2,2′-azobis-(2-amidinopropane) hydrochloride

Adsorption of albumin

10 ml of purified emulsion (solid content 3–5 g/l) was mixed with 10 ml of albumin aqueous solution. The pH value of the mixture was immediately adjusted to 4.9 with 0.2 M acetate buffer. The mixture was allowed to stand at 40 °C for 3 h, and then centrifuged at 10,000–15,000 g, 40 °C for 10 min. In order to remove completely wafting particles, the supernatant was centrifuged twice at 25,000 g, 40 °C. Albumin concentration in the medium was measured by ultraviolet spectrophotometry at 280 nm. The same measurement was carried out at 25 °C, by keeping the pH at 4.9. The amount of albumin adsorbed was calculated by subtracting the albumin concentration in the medium from that of the initial concentration.

Results and discussion

Figure 1 shows the variations of relative viscosity of PDM aqueous solution with temperature at pH values of 4 and 9. At pH 9, the viscosity decreased sharply around 35 °C. This seems to be due to some conformational change that occurred around 35 °C. At 38 °C, the clear transparent solution became suddenly turbid. The temperature, at which the solution become turbid, increased gradually with a decrease in the concentration of PDM [7]. These

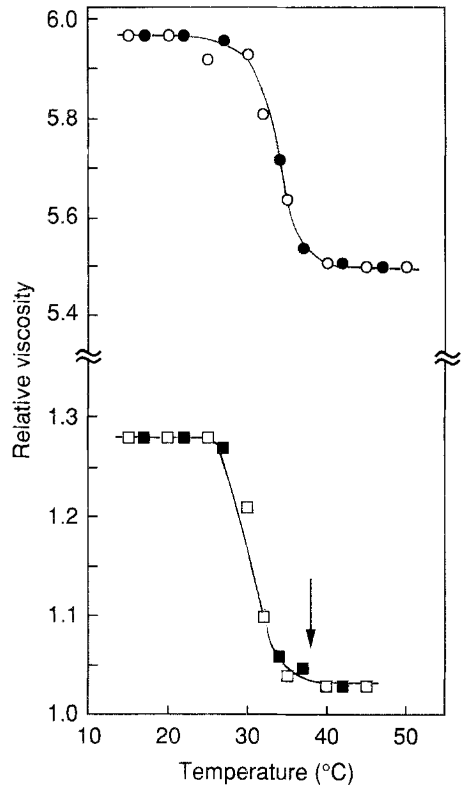

Fig. 1 Variations of relative viscosities of 20 g/l poly(dimethylaminoethyl methacrylate) at pH values of 4 (○, ●) and 9 (□, ■) with temperature: □, ○, ascending; and ■, ●, descending. Arrow indicates the temperature at which the aqueous solution became turbid

behaviors as the function of temperature were reversible. This indicates that the PDM aqueous solution has the lower critical solution temperature (LCST). That is, below 35 °C PDM molecules dissolve in water, whereas above 35 °C they aggregate, resulting in particles. At pH 4, at which the amine groups in PDM molecule are ionized, the viscosity decreased similarly. This suggests that the conformation of PDM molecule becomes similarly more compact with an increase in the temperature around 35 °C regardless of pH. However, at pH 4 the absolute value was much higher than that at pH 9 and the solution did not become turbid. This seems to be based on that they do not aggregate each other because of strong ionic repulsion at pH 4.

Figure 2 shows the transmission electron micrographs of PS seed and PS/P(DM-EGDM) composite particles. Both PS and the composite particles were monodispersed and their diameters were, respectively, 0.17 and 0.18 μm. Their coefficients of variation were, respectively, 2.76% and 2.38%. No P(DM-EGDM) copolymer particle was byproduced. In addition, no soluble PDM existed in the medium after the copolymerization, which was acknowledged from the result that the absorbance of the supernatant obtained from non-washed composite emulsion was almost zero. These indicate that the seeded emulsion copolymerization of DM and EGDM was carried out in PS seed particles.

Figure 3 shows the variations of hydrodynamic diameters of the PS seed and purified composite particles, measured by DLS, with temperature at pH values of 4 and 9. The diameters of the composite particles at pH 4 were slightly higher than those at pH 9 but still the differences were not so high as it was presumed from the measurement of relative viscosities at pH values of 4 and 9. It is understandable that the crosslinking derived from EGDM component should affect greatly the expanding/shrinking of the shell layer consisting DM-EGDM copolymer at the temperature below/above the LCST, but we have no clear idea about the reason why the difference between hydrodynamic diameters at pH 4 and 9 was so small. Though we have no any data, it may be based on that the diameter of the expanding particle which seems to have socalled "hairy layer" was measured by DLS. In the case of PS/P(DM-EGDM) composite particles, a sharp drop in the diameter was observed around 35 °C at both pH values of 4 and 9. The identical behavior at pH 4 and 9 may be due to the same reason as already discussed in Fig. 1. That is, regardless of pH value, the conformation of cross-linked PDM shell layer is drastically changed below and above 35 °C. Whereas in the case of PS seed particles, no such

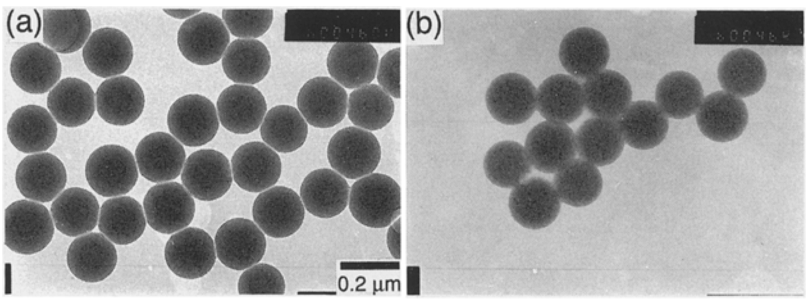

Fig. 2 Transmission electron micrographs of PS seed (a) and PS/P(DM-EGDM) composite (b) particles

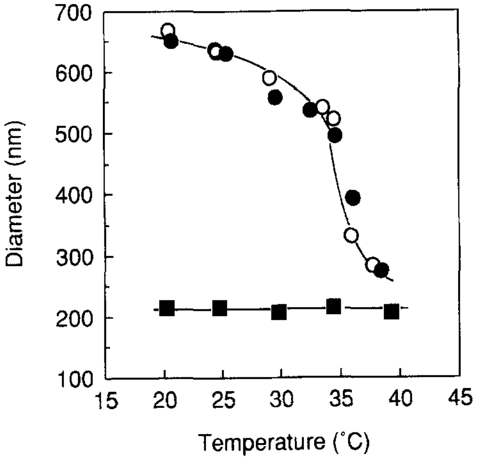

Fig. 3 Variations of hydrodynamic diameters of PS (■) and PS/P(DM-EGDM) composite particles (○, ●) with temperature at pH values of 4 (○) and 9 (■, ●)

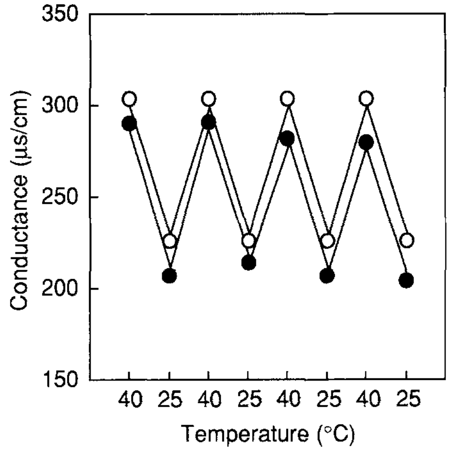

Fig. 5 Conductances of trimethyl stearyl ammonium chloride emulsifier aqueous solution (○) and of the emulsifier-present PS/P(DM-EGDM) composite emulsion (●) measured alternatively at 40 and 25 °C: pH 4: particles, 3.04 g/l; emulsifier, 1.51 g/l

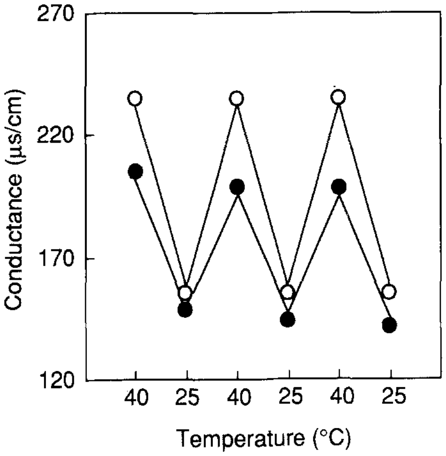

Fig. 4 Conductances of trimethyl stearyl ammonium chloride emulsifier aqueous solution (○) and of the emulsifier-present PS/P(DM-EGDM) composite emulsion (●) measured alternatively at 40 and 25 °C: pH 9: particles, 3.04 g/l; emulsifier, 1.51 g/l

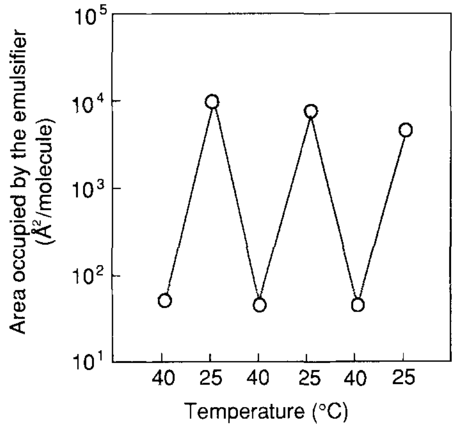

Fig. 6 Variations of the areas occupied by trimethyl stearyl ammonium chloride on PS/P(DM-EGDM) composite particles measured alternatively at 40 and 25 °C: pH 9: particles, 3.04 g/l; emulsifier, 1.51 g/l

phenomenon was observed. These results suggest that above the LCST (about 35 °C), the shell layer of the composite particles is hydrophobic and shrink, and below the LCST it is hydrophillic and swell. In order to clarify this point, the adsorption and desorption behaviors of cationic trimethyl stearyl ammonium chloride emulsifier onto PS/P(DM-EGDM) composite particles below and above the LCST were examined.

Figures 4 and 5 show the conductances of the emulsifier-present PS/P(DM-EGDM) composite emulsion and the emulsifier aqueous solution of the same concentration, respectively, at pH 9 and 4. As seen in Fig. 4, at pH 9, the differences of the conductances between the emulsifier-present PS/P(DM-EGDM) composite emulsion and the emulsifier aqueous solution at 40 °C were always higher than those at 25 °C. The relationship between the areas occupied (As) by the emulsifier, which was calculated from the above successive conductance measurements, at 40 and 25 °C and the total surface area, is shown in Fig. 6. The total surface area was obtained from the solid content of the emulsion and the hydrodynamic diameters at 40 and 25 °C. The As value was always much lower at 40 °C than at 25 °C. In other words, the concentration of the emulsifier on the composite particle surface was much higher at 40 °C than at 25 °C. The adsorption and desorption were almost reversible among the consecutive measurements.

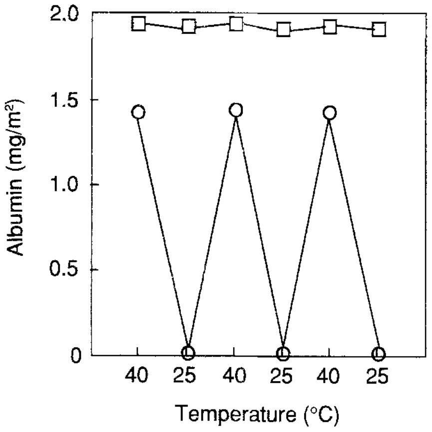

Fig. 7 Variations of the amount of albumin adsorbed on PS seed (□) and PS/P(DM-EGDM) composite (○) particles at pH 4.9 under the constant concentration of albumin against the total particle surface area, measured alternatively at 40 and 25 °C: PS system: particles, 5.09 g/l; albumin, 0.42 g/l; PS/P(DM-EGDM) system: particles, 5.07 g/l; albumin, 0.37 g/l

As will be seen in Fig. 5, at pH 4, the differences in the conductances at 40 °C between the emulsifier-present PS/P(DM-EGDM) composite emulsion and the emulsifier aqueous solution of the same concentration were small and almost similar to those at 25 °C. This means that only a small amount of the emulsifier was adsorbed onto the composite particles even at 40 °C. This may be due to the strong ionic repulsion between the cationic emulsifier and the particle surface at which protonation of the amine groups takes place at the low pH value.

Figure 7 shows the adsorption and desorption behaviors of egg albumin as an example of macromolecular adsorbent onto the PS seed and composite particles. The experiment was carried out at the isoelectric point of the albumin to eliminate the effect of ionic interaction between the particles and albumin. In the case of PS seed particles, the amount of adsorption was always higher than that for the composite particles at 40 °C and the desorption by descending the temperature from 40 to 25 °C was negligible. This is possibly because the surface hydrophobicity of the composite particles at 40 °C is somewhat lower than that of PS seed particles. On the other hand, in the case of the composite particles the concentration of albumin adsorbed (about 1.4 mg/m^2) at 40 °C was higher than that (about 0 mg/m^2) at 25 °C. The adsorption and desorption were almost reversible among the consecutive measurements.

From these results, it is clear that PS/P(DM-EGDM) composite particles had the temperature-sensitive surface property.

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