Polymer Journal, Vol. 35, No. 12, pp 901—910 (2003)

Thermosensitive Behavior of Poly(*N*-isopropylacrylamide) Grafted Polystyrene Nanoparticles**

Ming-Qing CHEN,†† Takeshi SERIZAWA, Mei LI,* Chi WU,* and Mitsuru AKASHI†,†††

Department of Nanostructured and Advanced Materials, Graduate School of Science and Engineering,
Kagoshima University, 1–21–40 Korimoto, Kagoshima 890–0065, Japan
**Department of Chemistry, The Chinese University of Hong Kong, Shatia, N. T., Hong Kong*

(Received August 20, 2001; Accepted October 10, 2003)

ABSTRACT: We analyzed the thermosensitive behavior of monodisperse polystyrene (PSt) nanoparticles having different densities and chain lengths of grafted poly(*N*-isopropylacrylamide) (PNIPAAm) on their surfaces. The PSt nanoparticles were synthesized by free radical copolymerization of styrene monomer with PNIPAAm macromonomers in mixed ethanol/water solvents. The grafted PNIPAAm densities on the PSt nanoparticle surfaces can be controlled by varying polymerization conditions such as reaction temperature and initiator concentrations. The nanoparticle sizes and densities of grafted PNIPAAm on their surfaces significantly affected temperature-response, which was investigated by dynamic laser light scattering. The larger PSt nanoparticle sizes and higher grafted PNIPAAm densities showed a more significant decrease in hydrodynamic diameters when temperature of dispersed solutions was raised above lower critical solution temperature of the linear PNIPAAm. The molecular weight of grafted PNIPAAm on nanoparticle surfaces had no relationship with a visible decrease in hydrodynamic diameter.

KEY WORDS Poly(*N*-isopropylacrylamide) / Macromonomer / Polystyrene Nanoparticle / Thermosensitivity / Dispersion Copolymerization /

There has been a geometric increase in the number of publications involving water-soluble poly(*N*-isopropylacrylamide) (PNIPAAm), because it exhibits a remarkable phase transition in the aqueous solution when temperature is raised above the lower critical solution temperature (LCST).[1–5] For these ingenious applications, the phase transition behavior of PNIPAAm has been also described in the metal colloids[6] and protein conjugation systems.[7] Copolymers containing PNIPAAm with hydrophilic-hydrophobic segments can exhibit the similar phase transition properties in a large range of temperature.[8] The copolymers with acrylic acid units show a pH sensitive property as well as a thermosensitive one.[9] PNIPAAm-based gels also exhibit thermo-reversible swelling properties.[10] They have been exploited to control enzymatic reactions[11] and drug delivery of biomolecules such as proteins of human gamma globulin.[12] On the other hand, when PNIPAAm was grafted on the external surfaces of substrates such as polymeric films and silicons, it acts as a reversible thermovalve for the separation column.[13] The water absorption and electrostatic charge of surfaces can be controlled for a temperature stimulation.[14] Based on the thermosensitivity of PNIPAAm, it may be used as other material fields in order to develop other functionalities.

Polymeric particles that have an extremely large surface area are useful because of their potential application, not only in the technological, but also biomedical fields. In order to expand functions of particle surfaces, Okano *et al.* prepared thermo-responsive polymer nanoparticles with a core–shell micell structure, in which PNIPAAm chains show the coil-to-globule transition at their surface.[15] Makino *et al.* reported the particle synthesis with the crosslinked PNIPAAm layer by seed polymerization.[16] Lyon investigated a core–shell type PNIPAAm microgel in detail.[17] The core–shell structure was observed after dyeing by uranyl acetate. The sterically stabilized polystyrene (PSt) particles have been synthesized by Napper *et al.* using the two step continual polymerization of NIPAAm and styrene, in which the particle sizes were dependent on the temperature changes in dispersed solutions.[18] In order to examine conformation change of adsorbed PNIPAAm chains on the surfaces of surfactant-free PSt nanoparticles, we monitored the hydrodynamic ra-

---

[†] To whom all correspondence should be addressed (TEL: +81-6-6879-7356, FAX: +81-6-6879-7359, E-mail: akashi@chem.eng.osaka-u.ac.jp).

[††] Current address: School of Chemical and Material Engineering, Southern Yangtze University, Wuxi 214036, P. R. China

[†††] Present Address: Department of Molecular Chemistry, Graduate School of Engineering, Osaka University, Yamada-oka, Suida 565–0871, Japan.

[**] This paper is part XXXIV in the series of the study on "Graft Copolymers Having Hydrophobic Backbone and Hydrophilic Branches". Part XXVIII is as follows T. Serizawa, K. Taniguchi, and M. Akashi, Colloids Surf., 169, 95 (2000).

dius of the particles to reveal the coil-to-globule and globule-to-coil transition by using dynamic laser light scattering (LLS) in the heating up and cooling down processes, respectively.[19] The technique was useful for studying the phase transition of PNIPAAm.

On the other hand, monodisperse polymeric nanoparticles were also prepared by the dispersion copolymerization of hydrophilic macromonomers with hydrophobic comonomers in the presence of a radical initiator in polar solvents without any emulsifier. And, their particle sizes can be widely controlled *via* varying reaction parameters such as concentration of macromonomer, initiator and styrene, and polymerization temperature.[20–28] The hydrodynamic diameter of the nanoparticles consisting of a PSt core and grafted PNIPAAm chains on their surfaces was dependent on the temperature of the dispersion solution.[22] Thus, the grafted PNIPAAm chains showed the coil-to-globule transition at a LCST in our previous work.[22] However, thermosensitive behaviors of the PSt nanoparticles with different diameters, molecular weights, and densities of grafted PNIPAAm on their surfaces are not clear at all. Therefore, the studies of the parameters that affect to the thermosensitive properties of the nanoparticles are needed. Furthermore, the process of the particle formation including of self-assembly of grafted copolymer[29] will be understood by studying the effects of the above parameters.

In this paper, we studied thermosensitive behaviors of PSt nanoparticles that were prepared with narrow size distribution, and different densities and molecular weights of grated PNIPAAm on their surfaces by the macromonomer technique using the dynamic LLS in detail.[30]

EXPERIMENTAL

Materials

2,2′-Azobisisobutyronitrile (AIBN) (Wako Pure Chemical Ind., Ltd.) was recrystallized from ethanol and dried in a vacuum. NIPAAm monomer was supplied by Kohjin Co., Ltd., (Japan) that was recrystallized from hexane and dried in a vacuum. Styrene (Wako Pure Chemical Ind., Ltd.) was distilled under reduced pressure under nitrogen. The solvents, *N*,*N*-dimethylformamide (DMF), ethanol, and water were purified in a usual way prior to use. 2-Mercaptoethanol and tetrabutylphosphonium bromide (TBPB) (Wako Pure Chemical Ind., Ltd.) were used without further purification. *P*-Chloromethylstyrene (CMSt) was supplied by Nippon Oil and Fats Co. (Tokyo, Japan) and used without further purification. The PNIPAAm macromonomers were prepared by the two step process.[22,23] In the first step, a hydroxyl group terminated NIPAAm oligomer was prepared by the free radical oligomerization of NIPAAm using AIBN as an initiator and 2-mercaptoethanol as a chain transfer agent. In the second step, the NIPAAm oligomer was stirred with more than the ten-fold excess (to equimolecular amount of oligomer) of 50% aqueous potassium hydroxide solution in DMF at 30 °C for a half-hour. Then, the similar excess of CMSt was added into the flask. The reaction was continued for 72 h in the presence of TBPB (half-molecular amount of oligomer) as a phase transfer catalyst at 30 °C. The potassium chloride precipitate was removed by filtration. The products were purified by dialysis in distilled water for 5 d. The PNIPAAm macromonomer was obtained by lyophilization of dialyzed aqueous solution.

Copolymerization

The copolymerization of PNIPAAm macromonomers and St was carried out in ethanol/water mixtures (v/v, 7/3) under different reaction conditions, using the recipes given in Table I. In our previous work, the dispersed solution of the nanoparticles showed the slight decrease in a transmittance with an increase in temperature.[22] This behavior might be due to the fact that some free PNIPAAm macromonomers have not been removed by the only dialysis purification. Accordingly, the resulting products were first dialyzed in distilled water using a cellulose dialyzer tube (Wako Pure Chemical Ind., Ltd, the cutoff molar mass: 12000–14000) to remove unreacted macromonomers and monomers for 5 d. The polymeric nanoparticles obtained were centrifuged and collected to purify them further. The nanoparticles were then redispersed in distilled water. This procedure was repeated for three times.

Characterization Methods

The number-average molecular weight (Mn) of the macromonomer was measured by gel permeation chromatography (GPC), using poly(ethylene glycol) as a standard. The size distribution and morphology of nanoparticles were determined by means of transmission electron microscopy (TEM) (Hitachi H-7010A). The chemical components of grafted copolymers (nanoparticles) were estimated by ^{1}H NMR (400 MHz) in $CDCl_3$ solutions at room temperature. The glass transition temperature (T_g) of nanoparticles was measured using a differential scanning calorimeter (DSC) (EXSTAR6000 system). About 10 mg of nanoparticles was analyzed at a scan rate of 10 deg min^{-1} from 0 to 180 °C. The hydrodynamic diameters of PSt nanoparticles were determined using dynamic LLS (a modified

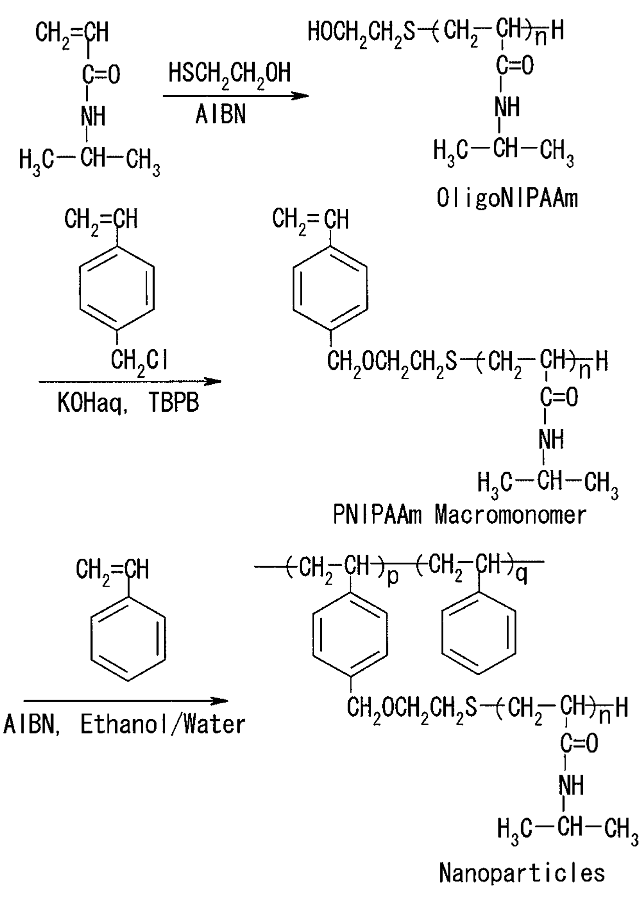
Table I. Recipes for the dispersion copolymerization[a]

Sample No.	PNIPAAm in feed (mol%)	M_n[b]	M_w/M_n[b]	AIBN[c] (mol%)	Temp.[d] (°C)
S1–1	5	3600	1.87	1.0	60
S1–2	4	3600	1.87	2.0	60
S1–3	3	3600	1.87	2.0	70
S2–1	4	2200	1.75	1.0	60
S2–2	4	3600	1.87	1.0	60
S2–3	4	5000	1.92	1.0	60
S2–4	4	6100	1.98	1.0	60
S3–1	4	3600	1.87	2.5	75
S3–2	2	3600	1.87	1.0	60
S3–3	1	3600	1.87	1.0	60
S3–4	0.5	3600	1.87	1.0	60

[a]Copolymerizations of PNIPAAm macromonomer with St (3 mmol) were carried out in ethanol/water (v/v, 7/3, 3 mL) mixed solvents for 24 h. [b]Number-average molecular weight of PNIPAAm macromonomer. [c]AIBN concentration to total monomer in feed. [d]Polymerization temperature.

research-grade ALV/SP-150 LLS spectrometer with an ALV-5000 time correlator) at an angle of 45°.

In order to study the thermosensitive change in the particle diameter in dispersion solutions, all measurements were performed at 20.0 ± 0.1 °C and 35.0 ± 0.1 °C. An individual PNIPAAm chain can reach the equilibrium state as fast as the temperature changes (a few hundred seconds).[3] In the study, the data was obtained after the dispersion solution reached the desired temperature. The average line width ($\langle\Gamma\rangle$) and relative distribution width ($\mu_2/\langle\Gamma\rangle^2 = \int G(\Gamma)(\Gamma - \langle\Gamma\rangle)^2\, d\Gamma/\langle\Gamma\rangle^2$) were obtained from the cumulants analysis of the measured time correlation function. $\langle\Gamma\rangle$ is related to the average transnational diffusion coefficient $\langle D\rangle$ by $\langle\Gamma\rangle = q2\langle D\rangle$ where $q = (4\pi n/\lambda_0)\sin(\theta/2)$ with n, λ_0, and θ being the solvent refractive index, the wavelength of light *in vacuo*, and the scattering angle, respectively. Furthermore, $\langle D\rangle$ is related to the average hydrodynamic diameter $\langle d_h\rangle$ (or simply written as d_h) by $d_h = k_B T(3\pi\eta D)$, where k_B and η are the Boltzmann constant and solvent viscosity, respectively. Our results showed that $\mu_2/\langle\Gamma\rangle^2$ were in the range 0.02–0.13, indicating that all the samples were narrowly distributed in the solutions at 20 °C, confirming the TEM results.



Scheme 1. Preparation of nanoparticles by dispersion copolymerization of PNIPAAm macromonomers with St in mixed ethanol/water (7/3, v/v) solvents.

RESULTS AND DISCUSSION

Preparation of Nanoparticles

As shown in Scheme 1, thermosensitive PSt nanoparticles can be prepared through the three main steps: (1) synthesis of the NIPAAm oligomer with a hydroxyl end group; (2) replacement of the hydroxyl end group with CMSt to produce PNIPAAm macromonomer; (3) formation of PSt nanoparticles *via* copolymerization of PNIPAAm macromonomer with St in a polar solvent such as ethanol or ethanol/water mixed solvent. In the dispersion copolymerization of hydrophilic macromonomers with hydrophobic comonomers, the mechanism involved in the nanoparticle formation indicated that hydrophilic chains cover a hydrophobic core

Table II. Effect of densities of grafted PNIPAAm on the thermosensitive properties

Sample No.	PNIPAAm[a] (mol%) × 10^3	d_h (nm) in 20 °C	d_h (nm) in 35 °C	μ_2/Γ^2 in 20 °C	μ_2/Γ^2 in 35 °C	Δd_h (%)
S1–1	3.1	680	470	0.13	0.11	31
S1–2	1.8	630	476	0.04	0.06	24
S1–3	0.8	596	560	0.06	0.08	6

[a]Grafted PNIPAAm unit (p)/ St (q) in nanoparticles as is shown in Scheme 1.

Table III. Effect of molecular weight of grafted PNIPAAm on the thermosensitive properties

Sample No.	PNIPAAm[a] (mol%) × 10^3	d_h (nm) in 20 °C	d_h (nm) in 35 °C	μ_2/Γ^2 in 20 °C	μ_2/Γ^2 in 35 °C	Δd_h (%)
S2–1	1.2	616	530	0.06	0.02	14
S2–2	1.3	598	536	0.05	0.08	10
S2–3	1.1	506	462	0.08	0.03	9
S2–4	1.2	460	414	0.13	0.12	10

[a]Grafted PNIPAAm unit (p)/ St (q) in nanoparticles as is shown in Scheme 1.

to produce the similar structures core-corona ones.[20–27] PSt particle sizes and grafted PNIPAAm densities on the PSt nanoparticle surfaces can be controlled by varying reaction parameters. The initiator concentration, polymerization temperature, macromonomer concentrations, and St concentrations could affect to the obtained nanoparticle diameters. The size distribution of PSt nanoparticles, however, became larger than 1.05 when the volume fractions of St monomer exceeded 40% in reaction solution.[24, 31] In the previous works, the small amount of water adding into the reaction solvent showed narrow particle size distribution.[23, 24] It was found that PNIPAAm macromonomer was precipitated from mixed ethanol/water solvents when the water volume fraction above 0.4. For this reason, the mixed ethanol/water solvents (7/3, v/v) were selected as polymerization solvents in this study. Three series of experiments were then designed in order to prepare different densities and chain length of grafted PNIPAAms or different particle sizes having the same chain length on nanoparticle surfaces.

In order to prepare PSt nanoparticles which have different densities of grafted PNIPAAms on their surfaces and similar diameters, the samples of S1 series were synthesized under the established concentrations and polymerization temperature using a PNIPAAm macromonomer with the number-average molecular weight of 3600 g mol^{-1} as a steric stabilizer, as shown in Table I (S1 series). The larger particles can be obtained when the lower macromonomer concentration was used, the smaller ones, however, were prepared by using the higher initiator concentration at the given concentrations of macromonomer and comonomer.[23, 24, 27] Followed by the obtained S1–1 nanoparticle condition, S1–2 sample was then prepared by both decreasing the macromonomer concentration and increasing the initiator (AIBN) concentration. Furthermore, the smaller nanoparticles were also synthesized by using a higher polymerization temperature.[23, 24, 27] Therefore, sample S1–3 was synthesized by both decreasing the macromonomer concentration and raising the polymerization temperature from 60 to 70 °C, based on preparing S1–2 condition. The characterizations of the resulting samples were shown in Table II.

In order to prepare nanoparticles with different grafted PNIPAAm chain lengths on their surfaces, we prepared the macromonomers in various molecular weights, which controlled on their oligomer molecular weights by changing the concentrations of the chain transfer agent. The number-average molecular weight of PNIPAAm macromonomers were 2200, 3600, 5000, and 6100 g mol^{-1} according to GPC analysis. We have known that the smaller particles were formed by using the higher molecular weight of macromonomer at the given concentration.[22, 24, 27] To prepare nanoparticles with similar diameters and different chain lengths on their surfaces, the lower concentration of PNIPAAm macromonomer will be used for the macromonomer having larger molecular weights. Thus, the contents of PNIPAAm segment on the nanoparticles will be different. Therefore, we did not have to vary PNIPAAm macromonomer concentrations to prepare the nanoparticles with similar diameter and different chain lengths on their surfaces. The nanoparticles of the S2 series were synthesized under the same concentration and polymerization temperature (60 °C) using the obtained macromonomers with different molecular weights as a steric stabilizer. The characterizations of the resulting samples were summarized in Table III.

In order to prepare PSt nanoparticles with different diameters and similar PNIPAAm chain lengths on their surfaces, the S3 series was synthesized by the dispersion copolymerization in which PNIPAAm macromonomer with the number-average molecular

Table IV. Effect of particle size on the thermosensitive properties

Sample No.	PNIPAAm ^{a} (mol%) $\times 10^3$	d_h (nm) in 20 °C	d_h (nm) in 35 °C	μ_2/Γ^2 in 20 °C	μ_2/Γ^2 in 35 °C	Δd_h (%)
S3–1	1.1	436	418	0.13	0.13	4
S3–2	1.3	598	536	0.05	0.08	10
S3–3	0.6	816	712	0.02	0.08	13
S3–4	0.5	1278	846	0.03	0.11	34

^{a}Grafted PNIPAAm unit (p)/ St (q) in nanoparticles as is shown in Scheme 1.

weight of 3600 g mol^{-1} was selected as a steric stabilizer. On the basis of the obtained S2–2 condition, sample S3–1 was then prepared by an increase in the AIBN concentration and polymerization temperature (75 °C) to obtain smaller nanoparticle size in diameter. In order to prepare the larger size nanoparticles (S3–3, S3–4) with a narrow size distribution, the PNIPAAm macromonomer concentrations were decreased because higher St concentration induced to form polydispersed particles.[23,24] The characterizations of resulting samples were showed in Table IV.

The yield of copolymers obtained was more than 95% in the copolymerization of PNIPAAm macromonomer with St. The composition of the copolymers was determined by NMR after purification of the products. From the results of the yield and the NMR analysis, it is considered that the St show fairly high reactivity in the copolymerization and highly random copolymers were obtained. The chemical compositions of grafted copolymers (nanoparticles) were estimated by ^{1}H NMR after the dried nanoparticles were dissolved in $CDCl_3$. The peak area ratio of the methine group $(-C\underline{\mathbf{H}}(CH_3)_2)$ in PNIPAAm side chain at 4 ppm to all the protons in the backbone $(-C\underline{\mathbf{H}}_2-C\underline{\mathbf{H}}-)$ and methyl groups $(-CH(C\underline{\mathbf{H}}_3)_2)$ in the copolymer at 1–2.4 ppm gave PNIPAAm contents in the nanoparticles. The amounts of the PNIPAAm segment were 3.1, 1.8, and 0.8×10^{-3} mol% [PNIPAAm unit (p)/St unit (q)] for sample S1–1, S1–2, and S1–3, respectively as shown in Table II. The compositions of nanoparticles were also analyzed by ^{1}H NMR for samples of S2–1 to S2–4. The smaller nanoparticles in size were prepared by using the macromonomer having higher molecular weights in the dispersion copolymerization.[22] However, the nanoparticles with the PNIPAAm with various molecular weights on their surfaces have been obtained by controlling the reaction parameters as shown in Table III. From the chemical composition analysis, the prepared PSt nanoparticles showed different amounts of PNIPAAm units for the sample S2–1 to S2–4. However, we found that the molar number of PNIPAAm macromonomer unit in the nanoparticles was almost the same. Also, the compositions of nanoparticles were analyzed by ^{1}H NMR for sample S3 series. The resulting contents of PNIPAAm unit are shown in Table IV. According to the mechanism of nucleation in the dispersion copolymerization,[20b] hydrophilic macromonomer such as PNIPAAm, PEG, and PNVIBA macromonomer aggregated on the surfaces of the nanoparticles. Also, the surface compositions have been analyzed by ESCA. No significant differences in N_{1s}/O_{1s} and O_{1s}/C_{1s} ratios were observed between nanoparticle surfaces and PNIPAAm homopolymer as reference.[22] It means that the surfaces of the nanoparticles were fully covered by grafted PNIPAAm branches. Thus, the surface compositions and the integral nanoparticles were different from the dates of ESCA and NMR. It may be considered that the nanoparticles have core–shell structures. Furthermore, cross-sectional morphology of the nanospheres by TEM[32] could be useful for thorough understanding. We will report elsewhere.

Figure 1 shows the TEM images of dried PSt nanoparticles. From these TEM images, the obtained products were spherical in form and monodispersed nanoparticles. The number (D_n), weight (D_w) average diameters and the polydispersity index (PDI) were calculated from the images using the following equations:

$$D_n = \Sigma \mathrm{d_i}/\mathrm{N} \tag{1}$$

$$D_w = \Sigma \mathrm{d_i}^4/\Sigma \mathrm{d_i}^3 \tag{2}$$

$$\mathrm{PDI} = D_w/D_n \tag{3}$$

where N is the total number of nanoparticles and d_i is the diameter of a nanoparticle in a TEM image. For example, the D_n, D_w, and PDI were 465 nm, 470 nm, and 1.01 for S1–1, respectively. The PDI was 1.02 and 1.01 for S1–2 and S1–3, respectively. The morphologies of other nanoparticles (S2 series and S3 series) were also observed. If the a PDI value is less than 1.05, the resulting particles are considered to be monodispersed.[20,21] In the study, all samples obtained were spherical in form and monodispersed nanoparticles because the values of the PDI were less than 1.05.

Effect of Grafted Densities on Thermosensitive Behavior

The PSt nanoparticles, obtained by the dispersion copolymerization with the different densities of a grafted PNIPAAm on their surfaces, were analyzed by

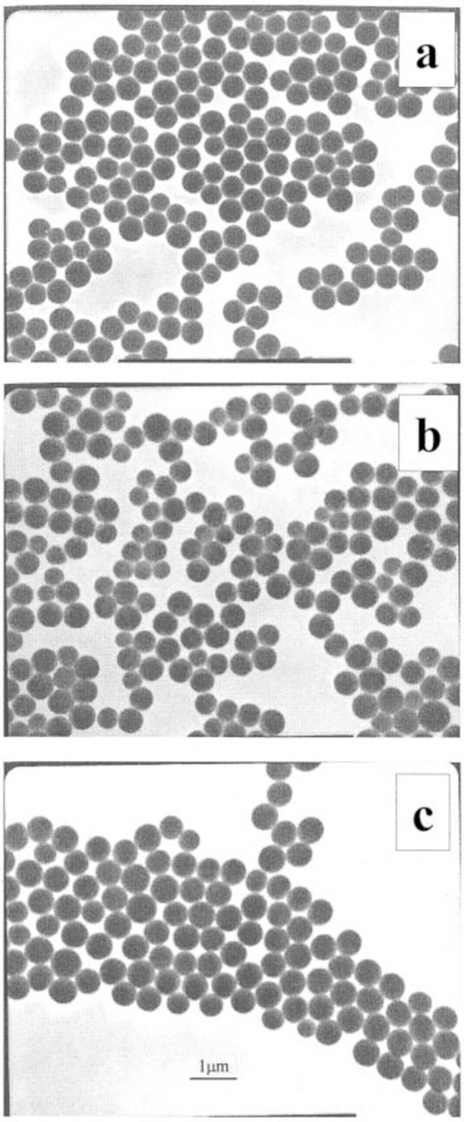


Figure 1. TEM images of S1 series nanoparticles prepared by the dispersion copolymerization of PNIPAAm macromonomer with St in mixed ethanol/water (7/3, v/v) solvents. (a) Sample S1–1; (b) S1–2; (c) S1–3 in Table II.

the dynamic LLS measurement at different temperatures of the dispersed solution. The hydrodynamic diameters (d_h) of the PSt nanoparticles were dependent on the dispersion temperature. When the temperature was raised to 35 °C, the hydrodynamic diameters of the samples S1–1, S1–2, and S1–3 were decreased. The d_h of the nanoparticles at 20 and 35 °C were summarized in Table II. A significantly larger change in d_h was observed for S1–1 with the highest PNIPAAm content on the nanoparticle surface. The difference in the d_h of S1–1 was 210 nm, varied from 680 nm at 20 °C to 470 nm at 35 °C. The phase transition temperature of linear PNIPAAm chains was ~32 °C lower than 35 °C. In fact, a main change in d_h was observed around the LCST of PNIPAAm.[22] The change in d_h decreases as PNIPAAm content decreases. We found the PSt nanoparticle with lower PNIPAAm segments on their surface was less sensitive to the varying temperature of the dispersed solution. Similar results have been obtained in the case of physically adsorbed PNIPAAm on surfactant-free PSt nanoparticles above LCST.[19] When more PNIPAAm segments were adsorbed on the particle surfaces, larger decrease in d_h was observed in our previous study.[19] Comparing the temperature dependence of d_h for sample S1–1 and S1–3, it is clear that the PNIPAAm content can affect dynamic diameter of the PSt nanoparticles. The nanoparticles with higher PNIPAAm content on their surfaces showed the larger decrease in d_h at 35 °C.

The PNIPAAm macromonomer used as a steric stabilizer in the above dispersion copolymerization has the number-average molecular weight of 3600. On the basis of the bonding distance of C–C, the chain length of the macromonomer was calculated to less than 5 nm in a coiled state when the molecular weight was < 5000.[25] In our recent study, we also found the facts that poly(ethylene glycol) (PEG) macromonomer grafted nanoparticles have an outer layer (corona) by TEM. The thickness of the corona layer obtained from TEM images of the thin sliced nanoparticles seems to be reasonable if we consider the surface-grafted polymer chain to be the almost full extension.[32] The size in PNIPAAm grafted nanoparticle became small above LCST due to shrinkage of a corona layer. However, the size change in hydrodynamic diameters was much larger than the total length of two PNIPAAm chains. Only considering the surface grafting, it is impossible that the size of nanoparticles changes more than 100 nm at above LCST. One of the causes may be that some thermosensitive graft copolymers constitute a mixture layer in outside of the PSt rich core. If the nanoparticles have the core-corona structures similar to PEG grafted particles,[32] the larger decrease in diameter suggests an induced shrinkage of the mixture layer on the PSt core due to the shrinkage of a corona layer at 35 °C. The surface densities of grafted PNIPAAm were increased by increasing PNIPAAm macromonomer amount to St in feed, in which change of PSt core can become larger in diameter at 35 °C.

Furthermore, comparisons of hydrodynamic diameter (d_h) of S1–1, S1–2, and S1–3 in dispersed solution at 35 °C with the number-average diameter (D_n) of the dry nanoparticles from the TEM micrographs were found that d_h was approximately the same as D_n. The result also suggested the shrinkage of the PSt core in the nanoparticles.

Effect of Molecular Weight on Thermosensitive Behavior

We analyzed the thermosensitive properties of the

PSt nanoparticles having different PNIPAAm chain lengths on their surfaces. From the d_h data in Table III, we found that the hydrodynamic diameters were significantly decreased at 35 °C. Although sample S2–1 had the shortest PNIPAAm chain on the surface, the largest decrease was observed, which was from 616 to 530 nm, indicating a 14% decrease of the hydrodynamic diameter compared to that at 20 °C. The d_h of sample S2–2, which had a medium molecular weight of grafted PNIPAAm on the surface, clearly decreased from 598 to 536 nm, which corresponded to a 10% decrease in diameter. The d_h of sample S2–3, which had a longer PNIPAAm chain on the surface, slightly decreased from 506 to 462 nm, which corresponded to a 9% decrease in diameter. Sample S2–4, with the longest PNIPAAm chain on the surface also showed a small decrease in diameter, which change from 460 to 414 nm. The percentage of d_h decrease was 10% at 35 °C in hydrodynamic diameter. From the experimental results, the larger decrease in hydrodynamic diameter can be observed at 35 °C, when prepared nanoparticle diameters were increased. Similar behaviors were observed as discussed in the next section (see Table IV). In the case, total surface area (**S**) was calculated for the nanoparticles of series 2. We found that **S** of sample S2–1 was smaller than that of S2–4. This means that the density of grafted PNIPAAm in corona layer was higher for sample S2–1 than one of S2–4 because their molar number of grafted PNIPAAm on the nanoparticles was similar. In Table II, the nanoparticles showed larger decrease in d_h when the densities of grafted PNIPAAm were increased. On the other hand, Napper *et al.* reported the thermosensitive behavior of PSt particles. The decrease of hydrodynamic diameter depended only on the molecular weight ($> 10^5$) of the PNIPAAm on the PSt particle surfaces which occurs coil-to-globule transition above LCST.[18] A larger decrease in d_h was expected when longer PNIPAAm chain lengths are grafted to the nanoparticle surfaces. Although the decrease in diameter for S2–4 was slightly larger than that for S2–3, no significant difference in percentage decrease of d_h was observed among these samples of S2–2, S2–3, and S2–4. This may be attributed to different intensity of shrinkage of PSt cores. Comparing these shrinkage of the PSt core and PNIPAAm corona layer, it can be considered that the molecular weight of grafted PNIPAAm might slightly affect the thermosensitive property of the nanoparticles. The effect of the chain length of PNIPAAm onto nanoparticle surface can be analyzed by LLS easily if a molecular weight of a macromonomer larger than 50000 can be synthesized. In this case, the nanoparticles prepared by the dispersion copolymerization of PNIPAAm macromonomer with St seem to be not significantly effective for decreasing d_h. The contribution of coil-to-globule transition of grafted PNIPAAm chains to the decrease in d_h is not easily detected for these with small molecular weights of PNIPAAm.

Effect of Nanoparticle Size on Thermosensitive Behavior

In order to demonstrate the effects of nanoparticle sizes on the thermosensitive properties, the nanoparticles with both different diameters and equal chain lengths on their surfaces have been prepared by the dispersion copolymerization method. The temperature dependence of the hydrodynamic diameter was examined as shown in Table IV. The hydrodynamic diameters were significantly decreased when the temperatures of dispersed solutions were raised from 20 to 35 °C. The d_h of sample S3–1 with the smallest size was decreased from 436 to 418 nm, which corresponded to only a 4% decrease in diameter. On the other hand, the d_h of sample S3–3, which had the larger particle size, decreased from 816 to 712 nm. The decrease in the hydrodynamic diameter corresponded to a 13% decrease in diameter. However, the d_h of sample S3–4 with the largest size was decreased from 1278 to 846 nm, which corresponded to a 34% decrease in diameter. Although sample S3–4 had the lowest molar number of grafted PNIPAAm units in the nanoparticles, the decrease in hydrodynamic diameter was larger than in samples S3–1 to S3–3 which corresponded to the 45 times larger decrease to the total chain length. The result also suggested the shrinkage of PSt core in the nanoparticles. Comparing sample S3–4 with S3–1, the diameter of S3–4 increase by three times when the content of PNIPAAm units in the nanoparticles was decreased by half. Also, the percentage change in d_h increase by about nine times. In this case, the surface area and the volume of S3–4 were 8.5 times and 25.6 times larger than that of S3–1, respectively. According to the surface area and the volume of S3–4, we found that the density of grafted PNIPAAm was higher than that of S3–1. The higher density can affect the thermosensitive property of the nanoparticles, which showed a larger decrease in d_h. In addition, a possible reason can be inferred that grafted PNIPAAm chains on the nanoparticle surfaces went through a coil-to-globule transition, while hydrophobic PSt cores might concentrate to exhibit a decrease in diameter when the temperatures of dispersed solutions were raised above LCST of PNIPAAm. As a consequence, the thermosensitive properties of the nanoparticles significantly depend on their diameters when the diameters overtook certain range. The PSt nanoparticles with larger sizes showed larger

Table V. DSC data of PSt nanoparticles

SampleNo.	T_g(°C)	ΔH (mJ mg^{-1})
S1–1	115.0	3.47
S1–2	113.8	3.18
S1–3	113.4	1.43

decrease in hydrodynamic diameter due to shrinkage of PNIPAAm corona layer.

Glass Transition Temperature

The thermal behavior of PNIPAAm-g-PSt nanoparticles was examined in DSC scans. All samples were scanned from 0 to 180 °C for S1–1, S1–2, and S1–3. The resulting glass transition temperature (T_g) of the nanoparticles is shown in Table V. The T_g was 115.0, 113.8, and 113.4 °C based on the temperature of the peak bottom, respectively. From the DSC thermogram of nanoparticles, clear endotherms are resolved for the S1 series having different densities of grafted PNIPAAm on their surfaces. In the case of polymeric particles, the enthalpy of dissociation, ΔH, represents the endothermic amount of fusion. The values of ΔH were 3.47, 3.18 and 1.43 mJ mg^{-1} for sample S1–1, S1–2, and S1–3, respectively. In our previous study, the PSt nanoparticles with a molecular weight of about 10^5 were polymerized by the dispersion copolymerization of PNIPAAm macromonomer with St using 1 mol% AIBN (to total monomer) as an initiator at 60 °C.[22] Although lower molecular weights of grafted copolymers can be obtained when using higher initiator concentration and higher polymerization temperature for samples S1–2 and S1–3. The differences of T_g cannot only be due to the different molecular weights, because there should be little effect of molecular weight on T_g. Ford and Pilcher also reported the effects of tacticities on the T_g for the poly(methyl methacrylate) (PMMA) particles.[33] The difference in ΔH seems to be dependent on the compositions of the nanoparticles such as distribution and amount of PSt segment in PNIPAAm-g-PSt mixed layer. The thicker mixture layer of PNIPAAm-g-PSt is, the higher the ΔH is. The larger endotherm was observed for sample S1–1, with the largest decrease in hydrodynamic diameter. The DSC data are consistent with the results of dynamic LLS measurements.

Structural Model of Nanoparticle

The solubility of the polymers (PSt oligomers and graft copolymers) generated in reaction solution depended on their molecular weights and the composition of the graft copolymers. The grafted polymers with a large molecular weight in the present study will precipitate and coagulated to form particles. At the same time, the corona layer of grafted PNIPAAm can be formed, which is based on the self-assembly of the graft polymers. The density of grafted PNIPAAm chains at the corona layer will be increased during the self-assembly of graft polymers by using high PNIPAAm macromonomer concentration. One of the possible reasons is that the reactivity of the PNIPAAm macromonomer was lower than that of St in the dispersion copolymerization system. Therefore, PNIPAAm segments can be polymerized onto the outer surface based on the radical growth or termination reaction in the dispersion copolymerization process. The apparent thermosensitivity can be observed from the percent decrease in d_h of the nanoparticles with higher densities of grafted PNIPAAm on their surfaces, as shown in Table II (S1–1 and S1–2). When high molecular weight of macromonomer was used, the grafted PNIPAAm segments might be accumulated in the corona layer with more which depended on the solubility of the graft copolymers and their self-assembly. From the results shown in Table III (S2–4), it seems that the chain lengths of grafted PNIPAAm can slightly affect the thermosensitive property of the nanoparticles, in which the larger percentage decrease in d_h was observed. Although PNIPAAm content in larger nanoparticles was lower based on the total PSt segments, the stable nanoparticles can be formed in the dispersion copolymerization process (S3–4) owing to the self-assembly of graft copolymers. The density of grafted PNIPAAm in the corona layer was higher than smaller nanoparticles (S3–1), while thickness of corona was independent on a particle size.[32] According to the previous three thermosensitive behaviors, the PSt nanoparticles may be alternatively viewed (as is shown in Figure 2) as consisting of a hydrophobic PSt core (diameter dc) and a “soft” corona layer of grafted PNIPAAm segments (hydrodynamic diameter d_h). The d_h depended on the temperature of dispersed solutions. The dc was

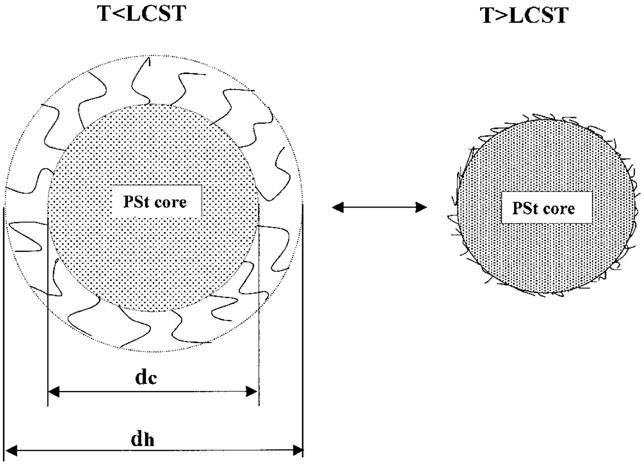


Figure 2. Schematic illustration of PNIPAAm grafted nanoparticles.

slightly larger than Dn at a dried state observed by TEM micrograph.

The nanoparticles with PNIPAAm segments on their surfaces are useful in the adsorption of protein.[34] Furthermore, the PSt nanoparticles were applied to the oral peptide delivery system[35] and improvement of immobilization of metal catalyst.[36, 37] The catalyst of platinum colloids have shown high activity on recycling in the aqueous hydrogenation of allyl alcohol. In chemical analysis based on electron spectroscopy, the hydrophilic grafted polymer chains were favorably located on the surfaces of the PSt nanoparticles.[20, 22, 24] It means that the nanoparticle has a PSt core and PNIPAAm corona layer structure, while the PNIPAAm corona layer was accumulated on the surface to stabilize the nanoparticle formed.

CONCLUSION

We successfully prepared PSt nanoparticles with different grafted densities and chain length of PNIPAAm on their surfaces by the dispersion copolymerization of various PNIPAAm macromonomers with St in ethanol/water mixed solvents. The obtained PSt nanoparticles have a narrow size distribution under the various polymerization conditions. The decrease in the hydrodynamic diameters was dependent on the surface densities, and sizes of nanoparticles when temperature of dispersed solution was raised to above LCST of PNIPAAm. Higher molecular weights of PNIPAAm macromonomer seem not to affect thermosensitive property of resulting nanoparticles because the change was small for coil-to-globule transition of grafted PNIPAAm chains than shrinkage of PSt core. Using low molecular weights or high concentrations of the PNIPAAm macromonomer as a steric stabilizer in the dispersion copolymerization, the nanoparticle showed more clear thermosensitive property in the d_h decrease. The obtained nanoparticles can be widely used in the catalysis, biomedical and biochemical systems.

Acknowledgments. This work was financially supported in part by a Grant-in-Aid for Scientific Research (No. 10555326 and 11480259) from The Ministry of Education, Culture, Sports, Science and Technology, Japan. The authors would like to thank Mr. Terumi Kakoi for his experiment support in regards of TEM. The authors also appreciatively thank Dr. Akio Kishida for his vital helpful discussion.

REFERENCES

1. a) M. Heskins and J. E. Guillet, *J. Macromol. Sci. Chem.*, **A 2**, 1441 (1968).
   b) A. S. Hoffman, *Res. Soc. Bull.*, **16**, 42 (1991).
   c) H. G. Schild, *Prog. Polym. Sci.*, **17**, 163 (1992).
2. a) S. Fujishige, K. Kubota, and I. Ando, *J. Phys. Chem.*, **93**, 3311 (1989).
   b) H. G. Schild and D. A. Tirrell, *J. Phys. Chem.*, **94**, 4352 (1990).
3. C. Wu and S. Zhou, *Macromolecules*, **28**, 8381 (1995).
4. X. Wang, X. Qiu, and C. Wu, *Macromolecules*, **31**, 2972 (1998).
5. F. M. Winnik, *Macromolecules*, **23**, 2223 (1990).
6. C. W. Chen and M. Akashi, *Langmuir*, **13**, 6465 (1997).
7. P. S. Stayton, T. Shimoboji, C. Long, A. Chilkoti, G. Chen, J. M. Hsrris, and A. S. Hoffman, *Nature*, **378**, 472 (1995).
8. a) H. Feil, Y. H. Bae, J. Feijen, and W. Kim, *Macromolecules*, **26**, 2496 (1993).
   b) Y. G. Takei, T. Aoki, K. Sanui, N. Ogata, T. Okano, and Y. Sakurai, *Bioconjugate Chem.*, **4**, 341 (1993).
   c) K. Suwa, K. Morishita, A. Kishida, and M. Akashi, *J. Polym. Sci., Part A: Polym. Chem.*, **35**, 3087 (1997).
9. G. Chen and A. S. Hoffman, *Nature*, **373**, 49 (1995).
10. R. Yoshida, K. Uchida, Y. Kaneko, K. Sakai, A. Kikuchi, Y. Sakurai, and T. Okano, *Nature*, **374**, 240 (1995).
11. L. C. Dong and A. S. Hoffman, *ACS Symp. Ser.*, **350**, 236 (1987).
12. a) A. Afrassiabi, A. S. Hoffman, and L. A. Cadwell, *J. Memmr. Sci.*, **33**, 191 (1987).
    b) H. Kawaguchi, K. Fujimoto, and Y. Mizuhara, *Colloid Polym. Sci.*, **270**, 53 (1992).
13. H. Kanazawa, K. Yamamoto, Y. Matsushima, N. Takai, A. Kikuchi, Y. Sakurai, and T. Okano, *Anal. Chem.*, **68**, 100 (1996).
14. F. Seto, K. Fukuyama, Y. Muraoka, A. Kishida, and M. Akashi, *J. Appl. Polym. Sci.*, **68**, 1773 (1998).
15. S. Cammas, K. Suzuki, C. Sone, Y. Sakurai, K. Kataoka, and T. Okano, *J. Controlled Release*, **48**, 157 (1997).
16. K. Makino, S. Yamamoto, K. Fujimoto, H. Kawaguchi, and H. Ohshima, *J. Colloid Interface Sci.*, **166**, 251 (1994).
17. C. D. Jones and A. Lyon, *Macromolecules*, **33**, 8301 (2000).
18. a) P. Zhu and D. H. Napper, *J. Colloid Interface Sci.*, **164**, 489 (1994).
    b) P. Zhu and D. H. Napper, *J. Colloid Interface Sci.*, **168**, 380 (1994).
19. J. Gao and C. Wu, *Macromolecules*, **30**, 6873 (1997).
20. a) M. Akashi, I. Kirikihira, and N. Miyauchi, *Angew. Makromol. Chem.*, **132**, 81 (1985).
    b) M. Akashi, T. Yanagi, E. Yashima, N. Miyauchi, *J. Polym. Sci. Part A: Polym. Chem.*, **27**, 3521 (1989).
    c) M. Akashi, D. Chao, E. Yashima, and N. Miyauchi, *J. Appl. Polym. Sci.*, **39**, 2027 (1990).
    d) M. Riza, S. Tokura, M. Iwazaki, E. Yashima, A. Kishida, and M. Akashi, *J. Polym. Sci., Part A: Polym. Chem.*, **33**, 1219 (1995).
    e) I. Capek, M. Riza, and M. Akashi, *J. Polym. Sci., Part A: Polym. Chem.*, **35**, 3131 (1997).

f) M. Akashi, T. Niikawa, T. Serizawa, T. Hayakawa, and M. Baba, *Bioconjugate Chem.*, **9**, 50 (1998).

g) M. Q. Chen, A. Kishida, T. Serizawa, and M. Akashi, *J. Polym. Sci., Part A: Polym. Chem.*, **38**, 1811 (2000).

h) T. Uchida, T. Furuzono, K. Ishihara, N. Nakabayashi, and M. Akashi, *J. Polym. Sci., Part A: Polym. Chem.*, **38**, 3052 (2000).

i) T. Serizawa, S. Yasunaga, and M. Akashi, *Biomacromolecules*, **2**, 469 (2001).

J) M. Q. Chen, T. Kaneko, C. H. Chen, and M. Akashi, *Chem. Lett.*, **2001**, 1306.

21. T. Serizawa, M. Q. Chen, and M. Akashi, *J. Polym. Sci., Part A: Polym. Chem.*, **36**, 2581 (1998).
22. M. Q. Chen, A. Kishida, and M. Akashi, *J. Polym. Sci., Part A: Polym. Chem.*, **34**, 2213 (1996).
23. M. Q. Chen, T. Serizawa, and M. Akashi, *Polym. Adv. Tech.*, **10**, 120 (1999).
24. M. Q. Chen, T. Serizawa, A. Kishida, and M. Akashi, *J. Polym. Sci., Part A: Polym. Chem.*, **37**, 2155 (1999).
25. C. Wu, M. Akashi, and M. Q. Chen, *Macromolecules*, **30**, 2187 (1997).
26. M. B. Nugroho, S. Kawaguchi, K. Ito, and M. F. Winnik, *Macromolecular Reports*, **A32**, 593 (1995).
27. S. Kawaguchi, K. Ito, and M. A. Winnik, *Macromolecules*, **28**, 1159 (1995).
28. a) K. Ishizu and N. Tahara, *Polymer*, **37**, 1729 (1996).
    b) K. Ishizu and N. Tahara, *Polymer*, **37**, 2853 (1997).
29. T. Serizawa, M. Q. Chen, and M. Akashi, *Langmuir*, **14**, 1278 (1998).
30. M. Li, Y. Zhang, M. Jiang, L. Zhu, and C. Wu, *Macromolecules*, **31**, 6841 (1998).
31. A. J. Paine, W. Luymes, and J. McNulty, *Macromolecules*, **23**, 3104 (1990).
32. T. Serizawa, A. Takehara, and M. Akashi, *Macromolecules*, **33**, 1759 (2000).
33. S. C. Pilcher and W. T. Ford, *Macromolecules*, **31**, 3454 (1998).
34. H. Kitano, C. H. Yan, and K. Nokanura, *Makromol. Chem.*, **192**, 2915 (1991).
35. a) S. Sakuma, N. Suzuki, H. Kikuchi, K. Hiwatari, K. Arikawa, A. Kishida, and M. Akashi, *Int. J. Pharm.*, **149**, 93 (1997).
    b) S. Sakuma, N. Suzuki, H. Kikuchi, K. Hiwatari, K. Arikawa, A. Kishida, and M. Akashi, *Int. J. Pharm.*, **158**, 69 (1997).
    c) S. Sakuma, Y. Ishida, R. Suda, N. Suzuki, H. Kikuchi, K. Hiwatari, A. Kishida, and M. Akashi, *Int. J. Pharm.*, **159**, 181 (1997).
    d) S. Sakuma, R. Suda, N. Suzuki, H. Kikuchi, M. Akashi, and M. Hayashi, *Int. J. Pharm.*, **177**, 161 (1999).
    e) S. Sakuma, M. Hayashi, and M. Akashi, *Adv. Drug Deliv. Rev.*, **47**, 21 (2001).
36. C. W. Chen, M. Q. Chen, T. Serizawa, and M. Akashi, *Chem. Commun.*, 831 (1998).
37. C. W. Chen, M. Q. Chen, T. Serizawa, M. Akashi, *Adv. Mater.*, **10**, 1122 (1998).