Characterization of Photo- and Thermoresponsible Amphiphilic Copolymers Having Azobenzene Moieties as Side Groups

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ABSTRACT: Two series of amphiphilic copolymers, poly(HPMA-*co*-MPAP) I–V with n = 0.05–0.29 of the molar ratio of MPAP and poly(HPMA-*co*-MPAH)-I–V with n = 0.05–0.23 of the molar ratio of MPAH, were prepared by radical copolymerization of *N*-(2-hydroxypropyl) methacrylamide (HPMA) with azo-monomers such as 4-(4-methoxyphenylazo) phenyl methacrylate (MPAP) and 6-[4-(4-methoxyphenylazo) phenoxy] hexyl methacrylate (MPAH) using 2,2′-azobisisobutyronitrile as an initiator. Self-organization of these copolymers in water was confirmed by disappearance of the proton signal of the methoxyazobenzene in ^{1}H-NMR spectra measured in the solvent system of D_2O and CD_3OD. It was also found from the λ_{max}, located near 344 nm, that azobenzene groups self-organized to form the dimeric chromophore type of aggregate. The aqueous solutions of poly(HPMA-*co*-MPAP) and poly(HPMA-*co*-MPAH) exhibited the lower critical solution temperature (LCST) from at 68 to 40°C and from at 70 to 52°C in the dark state, respectively, with increasing the molar ratios of azo-monomers. On the other hand, the LCST measured in the photostationary state showed the higher temperature by 2–4°C compared with that in the dark state. It was found that the adsorption of poly(HPMA-*co*-MPAP)-V (n = 0.29) on polystyrene microspheres was photoregulated. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 81: 3056–3063, 2001

Key words: photoresponsible; thermoresponsible; amphiphilic polymer; self-organization; *N*-(2-hydroxypropyl) methacrylamide; azobenzene moiety

INTRODUCTION

There has been increasing attention focused on stimuli-sensitive polymers showing a reversible phase separation in an aqueous solution in response to change in temperature. Aqueous polymer solution such as poly(*N*-isopropylacrylamide),[1] poly[*N*-(3-ethoxypropyl) acrylamide],[1,2] and poly(*N*-vinylisobutyl amide)[3] exhibit a phase separation at elevated temperature, which is called the lower critical solution temperature (LCST). These thermosensitive polymers and gels have been widely applied to drug delivery system, liquid chromatography, and others.[4–7] On the other hand, the light-induced change in the configuration of azobenzene groups in side chains and/or crosslinks of macromolecules has been suggested as a tool for reversible optical storage,[8] photomechanical transformation of energy,[9] photoregulation of enzyme activity,[10] photocontrol of membrane permeability,[11] and antibody-antigen recognition.[12–14] Recently, we found the phase separation of aqueous solution of copolymers of *N*-(2-hydroxypropyl) methacrylamide (HPMA) and alkyl metha-

crylates (RMA, alkyl: methyl or butyl) with the molar ratio of RMA more than 0.08.[15] It was also found that the adsorption of protein on poly(methyl methacrylate) microspheres modified with the HPMA moiety changed in response to change in temperature.[16] The HPMA polymer is possibly applied in the biomedical field being used for fusion solution,[17] protein carrier,[18-20] drug delivery systems,[21] and biorecognizable polymers.[22] In the course of our study on the functionalized HPMA polymer,[15,16] the present article deals with an introduction of the hydrophobic methoxyazobenzene moiety to the HPMA polymer to prepare the materials having photochemical and thermal response in the adsorption. These materials are expected to apply for a scaffold in tissue engineering constructs.[23] The effects of stimuli on the LCST of aqueous solution of the HPMA copolymers and on the adsorption of them on polystyrene microspheres were discussed.

EXPERIMENTAL

Materials

6-[4-(4-Methoxyphenylazo)phenoxy] hexyl methacrylate (MPAH) was prepared according to the method described in previous article.[24] 4-(4-Methoxyphenylazo) phenyl methacrylate (MPAP) was synthesized by the reaction of methacryloyl chloride with 4-(4-methoxyphenylazo) phenol in the presence of triethylamine in chloroform: m.p. 116.2–117.5°C. Anal. $C_{17}H_{16}N_2O_3$ (296.326). Calc. C : H : N = 68.91% : 5.44% : 9.45%, Found C : H : N = 68.63% : 5.59% : 9.20%. N-(2-Hydroxypropyl) methacrylamide (HPMA) was prepared according to the method of Strohalm and Kopeček.[25] Styrene was purified by a standard method and distilled under reduced pressure of nitrogen.[26] The polypropylene film (d÷1 mm) was obtained from the Sanplatec Corp. 2,2′-Azobisisobutyronitrile (AIBN) was recrystallized from methanol. Distilled and deionized water was used throughout the experiments.

Polymerization Procedures

Ten milliliters of ethanol solution containing 2 g (14 mmol) of HPMA, a definite quantity of azomonomer (MPAP or MPAH : 0.07–0.75 mmol) and 0.02 g (0.12 mmol) of AIBN in a glass tube was degassed by the freeze–technique. The sealed tube was shaken at 60 ± 0.2°C. After polymeriza-

tion for 10 h, the content of the tube was poured into a large amount of diethylether to precipitate the solid. The crude polymer was reprecipitated from acetonitrile solution with diethylether twice. In the copolymer, no trace of vinyl groups could be detected by IR and NMR spectroscopic techniques. The molar ratios of azo-monomer in the copolymers (n) were determined by the N-content obtained from the elemental analysis. The number-average molecular weights (M_n) of copolymers were calculated by the gel permeation chromatography (GPC) measurements.

Preparation of Polystyrene Microspheres[26]

Polystyrene microspheres were prepared by emulsifier-free emulsion polymerization of styrene initiated with potassium peroxodisulfate (KPS) in water at 70°C under a nitrogen stream for 24 h, stirring mechanically with a 350 rpm speed of agitation. Polystyrene microspheres were purified according to the method described in a previous article.[26] Particle size of polystyrene microspheres was found to be 840 ± 100 nm and 990 ± 10 nm obtained from the SEM measurements of dried sample and the particle size distribution measured in water, respectively.

Measurements of Turbidity

The optical transmittance of aqueous copolymer solution (C = 2 wt %) was measured for each 2°C rise or cooling down in temperature from 10 to 90°C in the dark state by spectrophotometry, measuring the absorption at 600 nm. In the case of a photostationary state, aqueous copolymer solution in a 100-mL quartz tube was placed on a merry-go-round Rayonet photochemical reactor Model-RMA-400 equipped with four RPR-253.7 nm lamps. The distance between the tube and the lamps was 11 cm. After UV irradiation for 4 h, the solution was submitted to the optical transmittance measurements in a similar manner as mentioned above. The LCST was defined as the temperature at the beginning of turbid.

Adsorption of Copolymer on Polystyrene Microspheres

A mixture of 10 mL of a known concentration (0.005–0.25 g/dL) of aqueous solution of poly(HPMA-*co*-MPAP)-V (see Table I) and 10 mL of polystyrene microspheres (0.54 g/dL) in a stoppered polyethylene tube was equilibrated at 25°C for 6 h, followed by centrifugation at 13,500 rpm for

Table I   Results of Copolymerization and Characterization of Copolymers[a]

Copolymer	Azo-Monomer in Feed mmol	Yield %	Molar Ratio[b] in Copolymer $m : n$	GPC Analysis[c] M_n	M_w/M_n	LCST °C In the Dark State	LCST °C In the Photostationary State
Poly(HPMA-*co*-MPAP)							
I	MPAP : 0.09	88.1	0.95 : 0.05	1.89×10^4	2.80	—	—
II	0.19	85.0	0.90 : 0.10	2.11×10^4	2.87	68	70
III	0.28	86.6	0.85 : 0.15	1.93×10^4	2.40	60	62
IV	0.37	86.7	0.78 : 0.22	2.22×10^4	2.87	50	54
V	0.75	85.3	0.71 : 0.29	2.02×10^4	2.53	40	44
Poly(HPMA-*co*-MPAH)							
I	MPAH : 0.07	84.2	0.95 : 0.05	1.43×10^5	1.68	—	—
II	0.14	84.5	0.94 : 0.06	1.11×10^5	2.27	—	—
III	0.21	83.7	0.81 : 0.19	1.07×10^5	2.39	70	72
IV	0.28	83.9	0.79 : 0.21	1.15×10^5	2.25	60	62
V	0.56	83.2	0.77 : 0.23	1.28×10^5	2.11	52	56

[a] Condition: HPMA; 14.0 mmol, AIBN; 0.14 mmol, Ethanol; 10 mL, 60°C, 10 h.
[b] Calculated from elemental analysis.
[c] Calculated from GPC by use of polystyrene standards.

15 min. A parallel experiment without any poly-styrene microspheres was also run to eliminate the effect of poly(HPMA-*co*-MPAP)-V adsorption onto the walls of the tube and filter discs. The amount of the copolymer adsorbed on polystyrene microspheres in the dark state was calculated from absorbance differences in 348 nm of a characteristic wavelength of the azo-monomer between the initial and equilibrium concentrations of supernant solution obtained after centrifugation at 13,500 rpm using a Sigma Laborzentrifugen.[2–15] Adsorption experiments were also carried out for poly(HPMA-*co*-MPAP)-V after UV irradiation for 2 h. Each adsorption experiment was repeated two times, and the difference in the adsorbed amounts from two runs was less than 5%. The adsorbed amounts described in Figure 7 were the average of two runs of respective copolymer.

Adsorption of Polystyrene Microspheres Covered with Poly(HPMA-*co*-MPAP) on Polypropylene Film

The polystyrene microspheres covered with poly-(HPMA-*co*-MPAP)-V were obtained as follows: a mixture of 10 mL of 0.25 g/dL of aqueous copolymer solution and 10 mL of 0.54 g/dL of polystyrene microspheres was equilibrated at 25°C for 6 h, followed by centrifuging to isolate the particles. The polypropylene film was well rinsed with methanol, and immersed in the emulsion of polystyrene microspheres covered with and without poly(HPMA-*co*-MPAP)-V (0.54 g/dL) at 25°C for 4 h. After that, the polypropylene film was rinsed carefully to remove unadsorbed particles, followed by freeze drying at −78°C. The adsorption of polystyrene microspheres on the polypropylene film was evaluated by means of scanning electron microscopy (SEM) measurements.

MEASUREMENTS

GPC measurements were performed in dimethyl-formamide solution with HHR 6000, 5000, 4000, 3000, and 2000 columns at 40°C, using TOSOH LC-8020 GPC apparatus. Absorption was recorded on a Shimadzu UV-160A spectrophotometer. Temperature-controlled experiments were done with a circular jacket equipped with a temperature controller, RKC Instrument Inc., REX - P100. The particle size of polystyrene microspheres was determined by SEM at 25 kV with an Hitachi S-2150. Size distribution of the particles was measured with a Pacific Scientific NICOMP 370.
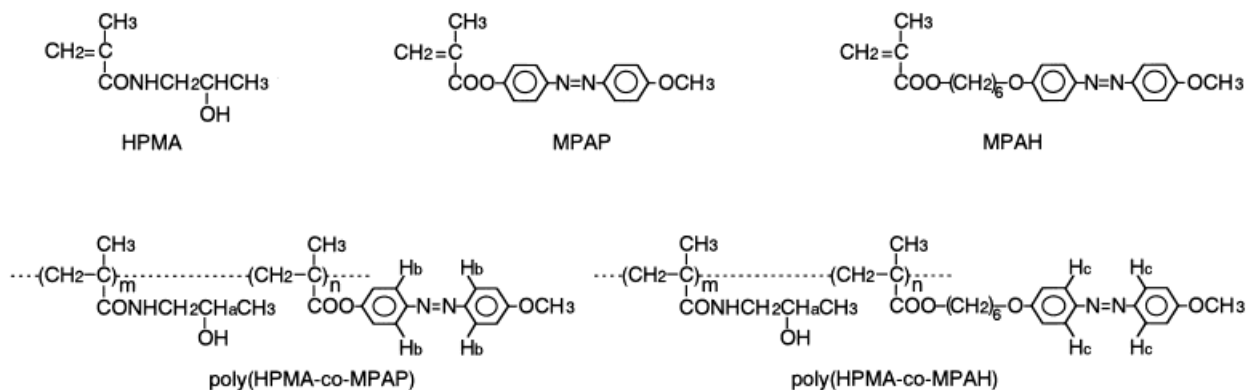
Figure 1 Structures of monomers and amphiphilic copolymers used.

RESULTS AND DISCUSSION

Preparation of Amphiphilic Copolymers

The structures of monomers and amphiphilic copolymers, HPMA, MPAP, MPAH, poly(HPMA-*co*-MPAP), and poly(HPMA-*co*-MPAH), are shown in Figure 1. The HPMA is known as a biocompatible monomer,[18,19,27] and gives not only a thermoresponsible homopolymer (PHPMA) but copolymers[15,16] with alkyl methacrylates as comonomers. As an appropriate hydrophobic–hydrophilic balance of the copolymer is considered an important factor in controlling LCST, it can be said that an introduction of the optimum amount of the hydrophobic alkyl methacrylate in the copolymers results in providing the thermoresponsibility to the water-soluble PHPMA. By considering the balance, we prepared two series of PHPMA copolymers having both a photosensitive and thermoresponsive character, by changing the molar ratio of hydrophobic azo-monomers. In addition to poly(HPMA-*co*-MPAP), we prepared poly(HPMA-*co*-MPAH), in which the MPAH moiety has a hexamethylene group, to know the effect of the decoupling spacer on both the phase transition behavior and the molecular assembly of the copolymers in water. The results are summarized in Table I. The copolymers were obtained in a moderate yield, with the number-average molecular weight of $M_n = 1.89–2.22 \times 10^4$ and $M_n = 1.07–1.43 \times 10^5$ for poly(HPMA-*co*-MPAP) and poly(HPMA-*co*-MPAH), respectively. These amphiphilic copolymers have a completely random sequence distribution of monomer units: the monomer reactivity ratios were found to be $r_1 = 0.56$ and $r_2 = 1.60$, which were obtained from the Fineman-Ross plot applied to copolymers prepared from copolymerization of HPMA (M_1) and MPAH (M_2) carried out separately in less than 10% yield.

Self-Organization

Self-organization of copolymers below the LCST was confirmed by means of ^{1}H-NMR measurements. The spectra of poly(HPMA-*co*-MPAP)-V and poly(HPMA-*co*-MPAH)-V in CD₃OD, D₂O and the mixed solvent of CD₃OD and D₂O are shown in Figure 2. The proton signals of the methoxyazobenzene moiety (H_b and H_c) appearing in CD₃OD disappeared completely in D₂O, whereas that of H_a in HPMA showed no change (see H_a, H_b, and H_c marked in Fig. 1). The half-widths of the two independent peaks as a function of the D₂O content in CD₃OD are shown in Figure 3. The half-widths of H_b ($\delta = 7.84–7.95$ ppm) and H_c ($\delta = 7.83–7.98$ ppm) broadened gradually with an increase in the D₂O content, and the peak disappeared completely in 50 and 100% (vol/vol) of D₂O, respectively. On the other hand, the half-widths of the peak of H_a ($\delta = 3.94$ ppm) did not change at all with changing the D₂O content. Line broadening of the proton signals of the methoxyazobenzene group in the copolymers in an aqueous medium is ascribed to the restricted molecular motion of methoxyazobenzene groups upon self-aggregation.[8,29] The mobility of the hydrophilic segments of HPMA was held even if the solvent polarity increased. This means that these copolymers self-aggregate and form polymer micelles consisting of a rigid core of methoxyazobenzene groups and a mobile shell of PHPMA segments. It is well known that azobenzene-containing amphiphiles form stable bilayer membrane in water and the absorption spectrum reflects the aggregate structure such as H-aggregate(λ_{max} ca. 300 nm), dimeric chromophore type (330–340 nm), the iso-
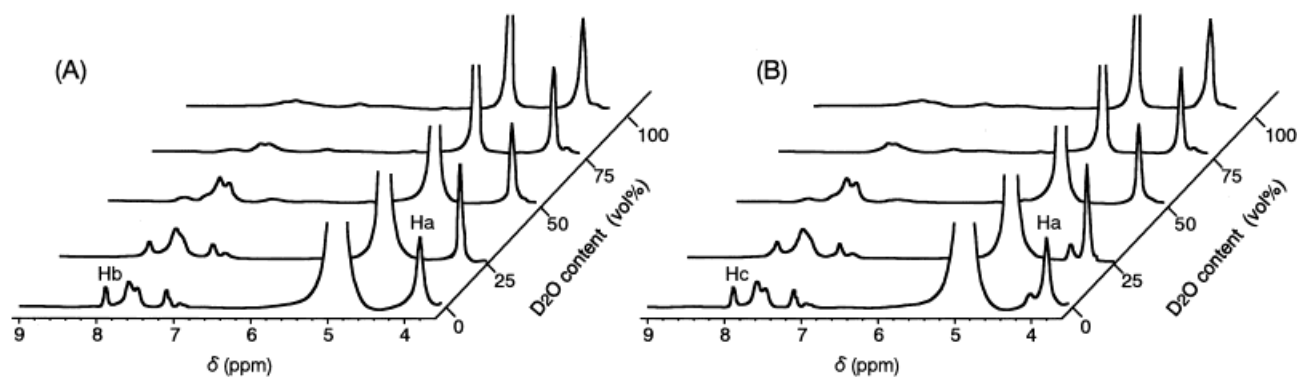
Figure 2 ¹H-NMR spectra of the copolymer measured in D₂O and CD₃OD. Concentration of copolymer is 1 wt %: (A) poly(HPMA-*co*-MPAP)-V; (B) poly(HPMA-*co*-MPAH)-V.

lated chromophore type (ca. 355 nm), and J-like aggregate (350–390 nm).[30,31] The absorption spectra of aqueous copolymer solution show the λ_{max} at 343.5 nm (346 nm in ethanol) and 344.5 nm (347 nm in ethanol) for poly(HPMA-*co*-MPAP) and poly(HPMA-*co*-MPAH), respectively. The blue shifts of λ_{max} observed in aqueous solution relative to those in ethanol are attributed to the stacking of azobenzene groups. Therefore, the azobenzene groups in copolymer seem to exist in dimeric chromophore type of aggregate in water. The difference in the effect of D₂O content on the half-widths of H_b and H_c describes the difference in facility of the self-organization of copolymers in water. From the comparison of effect of water content on the half-width of poly(HPMA-*co*-MPAH) with that of poly(HPMA-*co*-MPAP), it can be said that the former can self-aggregate in water

more easily than the latter. The hexamethylene group in the MPAH moiety is, therefore, useful to decouple the intermolecular interaction of methoxyazobenzene groups to form a rigid core from a disruptive motion of the PHPMA segment, while the MPAP moiety contributes to an increase over all hydrophobicity of the poly(HPMA-*co*-MPAP).

Photoisomerization

The change of configuration of the methoxyazobenzene moiety was photochemically studied. *Trans* to *cis* isomerization of the methoxyazobenzene group in the copolymers (3.0×10^{-5} mol/L) occurred upon UV irradiation reached the photostationary state within 2 h by monitoring λ_{max} = 350 nm. Irradiation for 2 h was sufficient to achieve the photostationary state with predominantly *cis*-configuration. The rate of *cis*–*trans* relaxation of the methoxyazobenzene group was found to be very slow: the half-life period $t_{1/2}$ = 12 h. The $t_{1/2}$ is enough to enable the experiments carrying out the condition either *trans*- or *cis*-configuration of the methoxyazobenzene moiety.

Phase Transition

The transmittance of the aqueous 2 wt % copolymer solution was measured for each 2°C rise and cooling down in temperature from 10 to 90°C by spectrophotometry, measuring the absorption at 600 nm. The poly(HPMA-*co*-MPAP)-II–V with n = 0.10–0.29 and poly(HPMA-*co*-MPAH)-III–V with n = 0.19–0.23 exhibited a remarkable phase change in response to the change of temperature within 30 and 80°C. The solution of PHPMA as
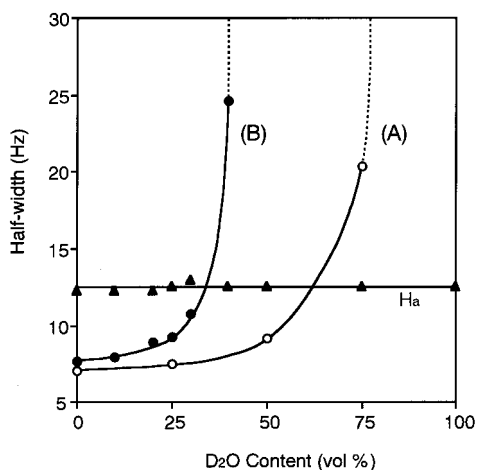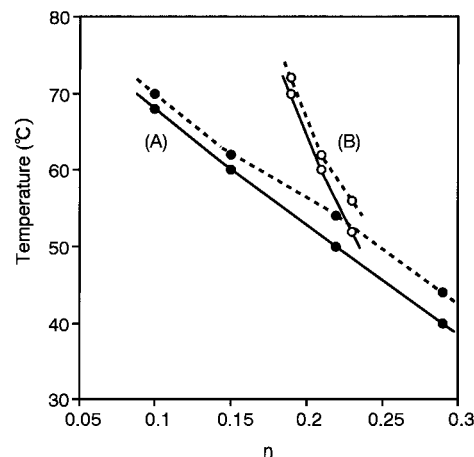

Figure 3 Change of the half-widths ¹H-NMR proton signal as a function of D₂O content in CD₃OD: (A) poly(HPMA-*co*-MPAP)-V; (B) poly(HPMA-*co*-MPAH)-V.
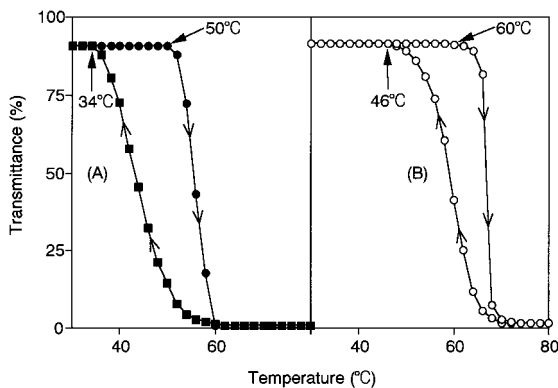
Figure 4 Effect of temperature on the transmittance of 2 wt % aqueous polymer solution measured in the dark state: (A) poly(HPMA-*co*-MPAP)-IV; (B) poly(H-PMA-*co*-MPAH)-IV.

Figure 5 Comparison of the effect of the molar ratio of the azo-monomer (*n*) on the LCST measured in the dark state (solid line) with that in the photostationary state (dotted line) on the heating cycle: (A) poly(HPMA-*co*-MPAP)-IV: (●); (B): poly(HPMA-*co*-MPAH)-IV: (○).

well as copolymers with the molar ratio of azo-monomer less than $n = 0.06$ exhibited no phase transition, where n represents the molar ratio of azo-monomers. The poly(HPMA-*co*-MPAP)-IV with $n = 0.22$ shows, for instance, transparency at below 50°C, and it sharply turns opaque when the temperature is raised to 60°C, while the phase transition of the poly(HPMA-*co*-MPAH)-IV with $n = 0.21$ occurs at 60°C as shown in Figure 4, together with the transmittance change when the temperature is cooling down. It was found that an hysteresis effect occurred: on cooling cycles, poly(HPMA-*co*-MPAP)-IV and poly(HPMA-*co*-MPAH)-IV showed the LCST at 34 and 48°C, respectively. The poly(HPMA-*co*-MPAP)-II–V showed the LCST at 68–40°C with increasing the molar ratio of MPAP from $n = 0.10$ to $n = 0.29$. The LCST of aqueous solution of poly(H-PMA-*co*-MPAH)-III–V decreased from at 70 to 52°C with increasing the molar ratio of MPAH.

The introduction of a hydrophobic comonomer and an increase in its molar ratio thus result in a lowering of the phase transition temperature, as shown in Figure 5. Comparing the LCST of poly-(HPMA-*co*-MPAP) with that of poly(HPMA-*co*-MPAH), it is noteworthy that the latter shows a high LCST than the former, although the latter is expected to be less hydrophobic than the former. This means that the hexamethylene group attached to the methoxyazobenzene group was decoupled from forming a dehydrated and compact chain conformation of PHPMA at elevated temperature. The LCST was also measured in the photostationary state. Figure 6 shows the transmittances of aqueous solution of the 2 wt % poly-(HPMA-*co*-MPAP)-IV with $n = 0.22$ and poly(H-

PMA-*co*-MPAH)-IV with $n = 0.21$ as typical examples, of which LCSTs show at 54 and 62°C on the heating cycle, whereas LCSTs show at 36 and 52°C on the cooling cycle, respectively. The LCST measured in the photostationary state also decreases with increasing the molar ratio of azo-monomers, as shown in Figure 5. Comparing the LCST measured in the dark state (Fig. 4) with that in the photostationary state (Fig. 5), the latter is higher than the former. A high polarity of the *cis*-isomer of the methoxyazobenzene group due to a high dipole moment seems to be the reason for the increase in the LCST: the higher the polarity stabilizes, the more hydrogen bonding.
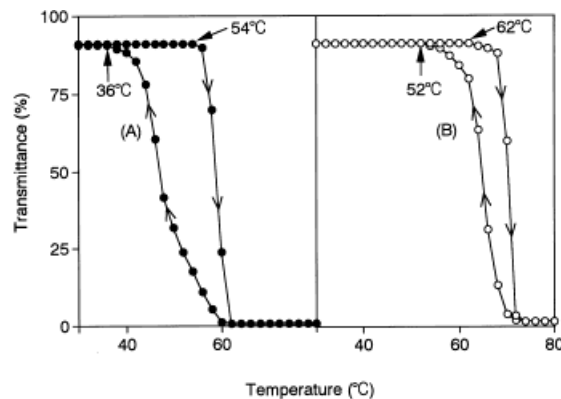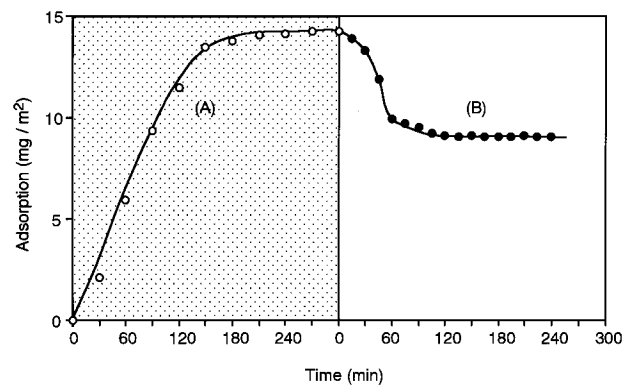

Figure 6 Effect of temperature on the transmittance of 2 wt % aqueous polymer solution mesured in the photostationary state: (A) poly(HPMA-*co*-MPAP)-IV; (B) poly(HPMA-*co*-MPAH)-IV.

Photoregulated Adsorption

Because polymer microspheres have advantages such as an extremely large surface area, and controlled surface change density, the binding ability of polymer was studied using various lattices as adsorbents.[16,25] The adsorption of poly(HPMA-co-MPAP)-V on the polystyrene microsphere was examined in water, varying the concentration of the copolymer from 0.005 to 0.25 g/dL. Figure 7 shows the adsorption of poly(HPMA-co-MPAP)-V on polystyrene microspheres in the dark state and in the photostationary state at 25°C. The amount of the copolymer adsorbed drastically decreased in the photostationary state. The difference in the amount of the adsorbed copolymer is explained by the difference in polarity change based on the trans–cis isomerization of the methoxyazobenzene group. The adsorbed copolymers on polystyrene microspheres in the dark state is, therefore, anticipated to desorb upon UV irradiation. Figure 8 exhibits the adsorption of poly(HPMA-co-MPAP)-V on polystyrene microspheres in the dark state and desorption behavior in the photostationary state as the time passes. The amount of adsorption gradually increases in the dark state and finally reaches saturation, while it begins to decrease upon UV irradiation and reaches equilibrium after about 2 h. This time period is similar to that required for completion of the trans to cis isomerization of the methoxyazobenzene group. It can be said that the polymers adsorbed on the polystyrene microspheres through hydrophobic interaction are desorbed as a result
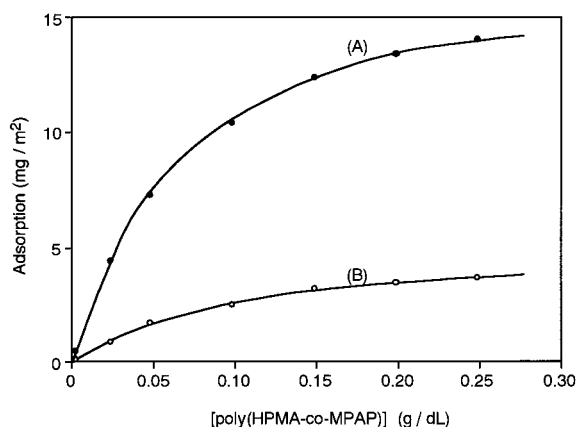
Figure 8 Adsorption and desorption of poly(HPMA-co-MPAP)-V on polystyrene microspheres. [poly(HPMA-co-MPAP)-V] 0.25 g/dL, at 25°C: (A) in the dark; (B) upon UV irradiation.

of decreased hydrophobicity of the polymers. Figure 9 shows the SEM micrographs of polypropylene film after being immersed in polystyrene microspheres covered with and without poly(HPMA-co-MPAP)-V. Untreated polystyrene microspheres with a hydrophobic surface adsorbes on the polypropylene film, whereas polystyrene microspheres covered with hydrophilic copolymers was found to reduce adsorption.

CONCLUSION

From the results obtained here, the following conclusion can be drawn: amphiphilic copolymers, poly(HPMA-co-MPAP) and poly(HPMA-co-MPAH) showed the LCST within the molar ratio of azo-monomer of $n = 0.10–0.29$ and $n = 0.19–0.23$, respectively, in water, and the LCST decreased with increasing the molar ratio of azo-monomers in copolymers. Amphiphilic copolymers can self-aggregate in water below the LCST to form the polymer micelle consisting of the rigid core of methoxyazobenzene groups and a mobile shell of PHPMA. The decoupling effect of the hexamethylene group attached to the methoxyazobenzene group on both the LCST and the polymer micelle formation was obserbed. The LCST measured in the photostationary state is higher temperature by 2–4°C compared with that measured in the dark state. The adsorption of poly(HPMA-co-MPAP) on the polystyrene microspheres are also photoregulated. Polystyrene microspheres covered with poly(HPMA-co-MPAP) was found to reduce the adsorption on the polypropylene film. Because the adsorption and desorption can be

Figure 7 Adsorption of poly(HPMA-co-MPAP)-V on the surface of polystyrene microspheres. [polystyrene microspheres] 0.54 g/dL, the concentration of poly(HPMA-co-MPAP)-V was varied for 6 h at 25°C: (A) in the dark state: (●); (B) in the photostationary state: (○).
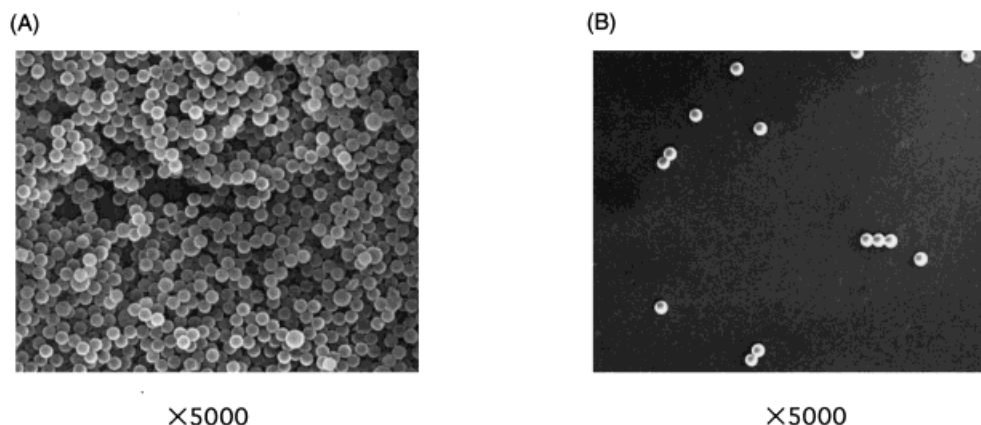
(A)

(B)

×5000   ×5000

Figure 9 SEMs of polypropylene film after being immersed in the emulsion at 25°C for 4 h. [polystyrene microspheres] = 0.54 g/dL: (A) polystyrene microspheres; (B) polystyrene microspheres covered with poly(HPMA-*co*-MPAP)-V (14.2 mg/m^2).

controlled photochemically and thermally, these copolymers are expected to apply for a scaffold materials in tissue engineering constructs.

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