pH-Sensitivity and Air/Water Interfacial Activity of Poly(*N*-isopropylacrylamide-*co*-methacrylic acid-*co*-octadecyl acrylate)

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ABSTRACT: The copolymers of *N*-isopropylacrylamide and methacrylic acid (P(NIPAM-*co*-MAA)), and the copolymers of *N*-isopropylacrylamide, methacrylic acid, and octadecyl acrylate (P(NIPAM-*co*-MAA-*co*-ODA)) were synthesized by a free radical reaction. MAA was copolymerized as a pH-sensitive residue and ODA as a hydrophobic moiety. The transmittances of P(NIPAM-*co*-MAA) solutions were almost constant in the pH 2.0–8.5. On the other hand, the transmittances of P(NIPAM-*co*-MAA-*co*-ODA) solutions were reduced in the pH 2–5, whereas the values were almost invariable in the pH 5.0–8.5. In the low range of pH, most of carboxyl groups are in unionized forms. Therefore, the hydrophobic interactions among ODAs might be greater than the electrostatic repulsions. Self-assemblies such as polymeric micelles could be formed, leading to the reduced transmittances. In the measurement of air/water interfacial tensions, the surface activities of both P(NIPAM-*co*-MAA) and P(NIPAM-*co*-MAA-*co*-ODA) were lower in alkali pHs than in acidic conditions. This is possibly because that the copolymers are more hydrophilic at higher pHs due to the ionization of the carboxyl groups. P(NIPAM-*co*-MAA-*co*-ODA) was more surface-active than P(NIPAM-*co*-MAA) in the full range pH, pH 2.0–8.5, due to the hydrophobic effect of ODA. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 108: 3707–3712, 2008

Key words: *N*-isopropylacrylamide; methacrylic acid; octadecyl acrylate; copolymer; pH-sensitivity; surface-activity

INTRODUCTION

Stimuli-sensitive polymers have played an important role in designing drug delivery systems. Either pH- or temperature-sensitive polymers are the most commonly studied ones.[1,2] Poly (*N*-isopropylacrylamide) (PNIPAM) has been utilized as a thermosensitive polymer in developing temperatures-sensitive carriers. The polymer maintains an expanded form below its lower critical solution temperature (LCST) of around 32°C, and attains a contracted form above the temperature. Hydrogel of crosslinked PNIPAM is in a swollen state at room temperature and it contracts upon raising temperature beyond LSCT. Active ingredients entrapped in the gels could release by a squeezing-out mechanism.[3,4] Besides hydrogels, temperature-sensitive liposomes were prepared by coating liposomes with hydrophobically modified PNIPAM.[5–10] Release of the materials entrapped in the inner aqueous phase of liposomes is caused by the interaction of the polymer and lipid membranes. On the other hand, copolymers contain-ing methacrylic acid (MAA) were synthesized, and they were used as a modulator to control the release from liposomes.[11–13] Since MAA has a titrable group, carboxylic acid, the copolymers of MAA take different conformations depending on pH. Under acid conditions, carboxyl groups are in unionized forms and accordingly the copolymer takes random coils. In alkali conditions, carboxyl group is deprotonated and the polymer would stretch out due to intramolecular electrostatic forces. Based on these characteristics, pH-sensitive drug delivery systems such as self assembly,[14] microparticle,[15,16] liposome,[12,13,17] and nanosphere[18] have been developed.

To develop a delicate drug delivery system using a stimuli-sensitive polymer, the sensitivity of the polymer with respect to environmental change should be well established. In this study, poly(*N*-isopropylacrylamide-*co*-methacrylic acid-*co*-octadecylacrylate) (P(NIPAM-*co*-MAA-*co*-ODA) with variable content of MAA was synthesized. MAA was copolymerized as a pH-sensitive residue and ODA as a hydrophobic moiety. To employ the copolymers in developing pH-sensitive drug delivery systems, one of the most important properties the copolymer should have is the pH-sensitivity. The sensitivity was investigated by observing the pH-dependent transmittances of the copolymer solutions. The pH-induced conformational changes of the copolymers

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lead to the change in the transmittance. Another important property is the surface activities of the copolymers. To be tightly adsorbed onto liposomes or other drug carriers, and to be self-assembled into micelles, the copolymer should be surface-active. The surface activities of the copolymers were also observed with varying pH.

EXPERIMENTAL

Materials

Octadecylacrylate (ODA), a hydrophobic anchor for water-soluble PNIPAM, was purchased from Adrich Chemical (Milwaukee, WI). Monomers of NIPAM and methacrylic acid (MAA) was purchased from Tokyo Chemical Industry (TCI, Tokyo, Japan). Water was doubly distilled in a Milli-Q water purification system (Millipore, Bedford, MA) until the resistivity was 18 MΩ/cm. All other reagents were in analytical grade.

Methods

Synthesis of NIPAM copolymers

Poly(NIPAM-co-MAA-co-ODA) were prepared by a free radical reaction.[10] NIPAM (9.9 mmol), MAA (0, 0.34, 0.70, or 1.49 mmol), ODA (0.1 mmol), and azobisisobutyronitrile (0.05 mmol) were dissolved in 20 mmol of freshly distilled dioxane. The contents of MAA were varied from batch to batch and the values were 0, 2.5, 5, and 10% based on the total mass of the monomers. The content of ODA was 1% in all preparations. The solution was degassed by bubbling N_2 for 1 h and then heated to 65°C for 12 h. The copolymers were precipitated upon the addition of diethylether. For purification, the precipitated polymers were dissolved in dioxane and reprecipitated with diethylether.

Determination of molecular weight

Gel permeation chromatography was performed in a high-performance gel permeation chromatograph system (WATERS M 910) equipped with columns of HR3 (MW = 500–30,000) and HR4E (MW = 50–100,000). The eluent was tetrahydrofuran, and polystyrenes of various molecular weights were used as a standard polymer.

Observation of FTIR spectra

The FTIR spectra of ODA, MAA, PNIPAM, P(NIPAM-co-ODA), P(NIPAM-co-MAA), and P(NIPAM-co-MAA-co-ODA) were taken in KBr pellet using Perkin Elmer Fourier Transformed Infrared (FTIR) spectrophotometer instrument.

Measurement of pH-dependent transmittance of PNIPAM copolymer solutions

PNIPAM, P(NIPAM-co-ODA), P(NIPAM-co-MAA), or P(NIPAM-co-MAA-co-ODA) were dissolved in distilled water so that the concentration is 5%. The pH of copolymer solutions were adjusted to 2, 3, 4, 5, 6, 7, and 8. The transmittance of the solutions was measured at 600 nm on a UV spectrophotometer (JENWAY 6505).

Surface tension measurement

PNIPAM, P(NIPAM-co-ODA), P(NIPAM-co-MAA), or P(NIPAM-co-MAA-co-ODA) was dissolved in distilled water at various concentrations. Measurements were made using the ring method with a tension meter (SEO D60A, Korea). The pH of the polymer solutions was adjusted to 2.1, 3.2, 5.1, and 8.3 using 1N HCl and 1N NaOH. The measurement was repeated three times under a specific condition, and the values were averaged.

RESULTS AND DISCUSSION

Determination of molecular weight

According to the gel permeation chromatography profile, the molecular weight of PNIPAM was ~ 50,000. The elution profiles and the retention times of P(NIPAM-co-ODA), P(NIPAM-co-MAA), and P(NIPAM-co-MAA-co-ODA) were almost the same as those of PNIPAM. The reported value of PNIPAM obtained under reaction conditions similar to ours was 87,000.[19]

Observation of FTIR spectra

The FTIR spectra of ODA, MAA, PNIPAM, P(NIPAM-co-ODA), P(NIPAM-co-MAA), and P(NIPAM-co-MAA-co-ODA) are shown in Figure 1. In spectrum (a), the strong peak at 1720 cm^{-1} are due to stretching of ester carbonyl groups in ODA. In spectrum (c), the broad strong band at 1651 cm^{-1} is ascribed to stretching of amide carbonyl groups in PNIPAM. The marked difference between PNIPAM spectrum (c) and P(NIPAM-co-ODA) spectrum (d) is that the peak at 1709 cm^{-1} was observed with P(NIPAM-co-ODA). This peak comes from stretching of ester carbonyl groups in ODA. Therefore, it is believed that ODA was successfully copolymerized with NIPAM. On the other hand, the strong peak at 1690 cm^{-1} was observed in spectrum (b) and it corresponds to the carboxylic carbonyl group of MAA. In spectrum (e), the broad peak of the carboxylic carbonyl group of MAA was observed with the characteristic peaks of PNIAPM. Thus, MAA is thought to be successfully copolymerized with NIPAM. In spectrum (f), a broad
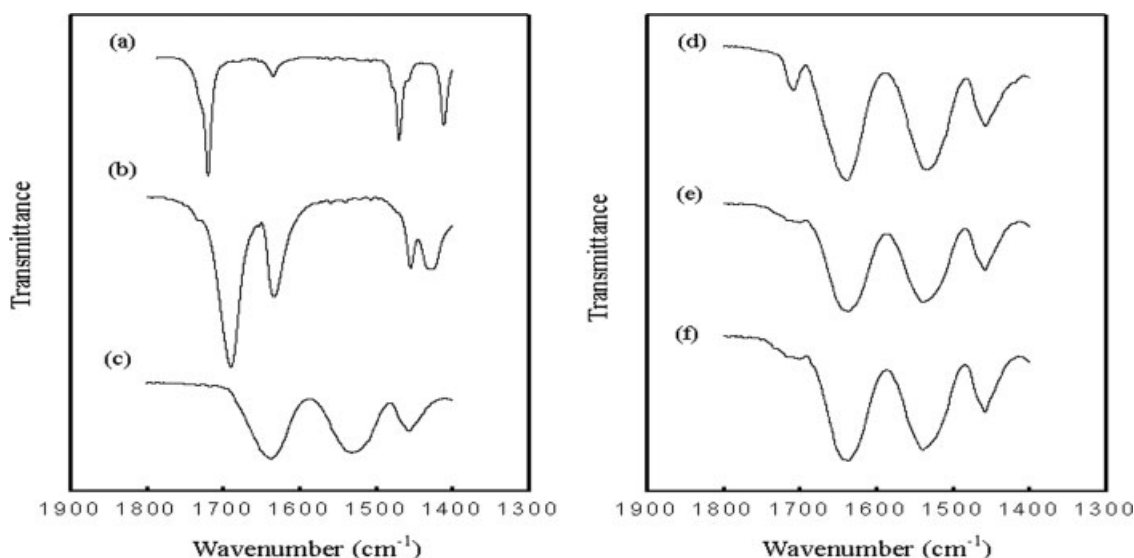
Figure 1 FTIR spectra of ODA (a), MAA (b), PNIPAM (c), P(NIPAM-*co*-ODA) (d), P(NIPAM-*co*-MAA) (e), and P(NIPAM-*co*-MAA-*co*-ODA) (f).

peak around 1701 cm^{-1} was observed along with the peaks of PNIPAM. The ester carbonyl groups of ODA and the carboxylic carbonyl groups of MAA are responsible for the peak. Accordingly, it is concluded that MAA and ODA have been copolymerized with NIPAM. Diez-Pena et al. synthesized P(NIPAM-*co*-ODA) with the molar feed ratios of NIPAM to MAA being 85/15, 70/30, 50/50, 30/70, and 15/85, and the compositions of the copolymers were determined by an elemental analysis.[20] According to the results, the molar ratios of the NIPAM residues to MAA ones in the copolymers were almost the same as the feed ratios. Hence, it could be assumed that the feed compositions are the same as those of the produced copolymers.

pH-Dependent transmittance of PNIPAM copolymer solutions

Figure 2 shows the transmittance variations of P(NIPAM-*co*-MAA) solutions (5%) with pH. Whatever the contents of MAA were, the transmittances were almost 100% in the full range of pH. In fact, the polymer solutions were apparently transparent at all the pHs. On the other hand, Figure 3 shows the transmittance variations of P(NIPAM-*co*-MAA-*co*-ODA) solutions (5%). In the pH range of 5.0–8.5, the values were almost constant with respect to pH. In the acidic condition, the transmittance was reduced as pH decreased. The pK of carboxyl group of MAA is around 5.5. Above the pK value, the carboxyl group tends to be ionized and the copolymers would take an expanded form due to an electrostatic intramolecular repulsion. In P(NIPAM-*co*-MAA-*co*-

ODA), there are two dominant forces determining the conformation of the copolymers. One is electrostatic repulsion forces because of ionizable carboxyl groups and the other is hydrophobic interactions among ODAs. In the higher range of pH, the electrostatic repulsion force would be greater than the hydrophobic interaction, leading to an expanded solubilized form of the polymer chains. Below the pK value, most of carboxyl groups are unionized forms. Therefore, the hydrophobic interactions might
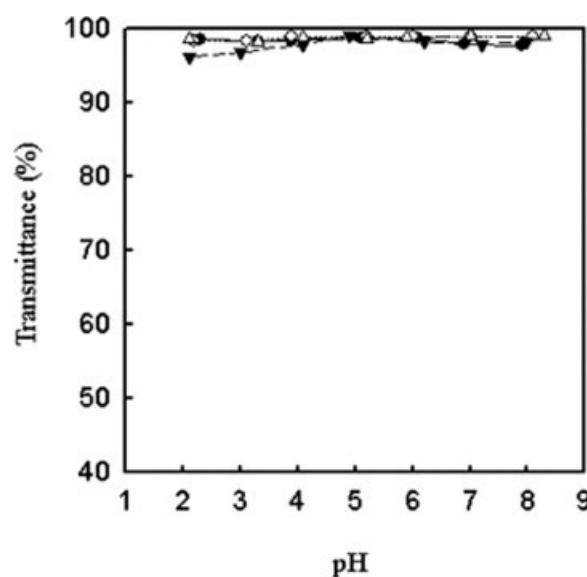
Figure 2 Transmittance variations of P(NIPAM-*co*-MAA) solutions with pH. The concentrations of polymers were 5%. The contents of MAA were 0% ($\triangle$), 2.5% ($\bullet$), 5% ($\bigcirc$), and 10% ($\blacktriangledown$).
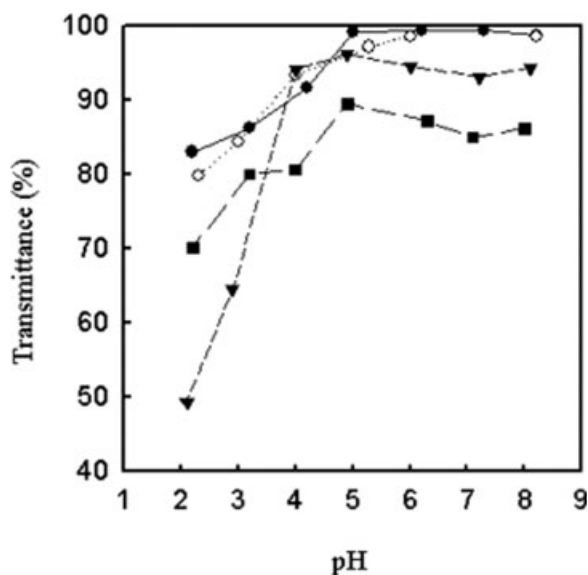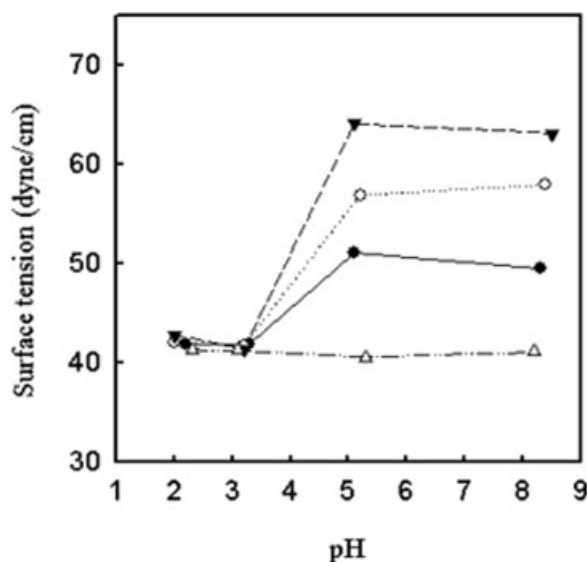
Figure 3 Transmittance variations of P(NIPAM-*co*-MAA-*co*-ODA) solutions with pH. The concentrations of polymers were 5%. The contents of MAA were 0% (■), 2.5% (●), 5% (○), and 10% (▼).

be greater than the electrostatic repulsions. Because of the hydrophobic interaction, self assemblies such as polymeric micelles and nano/micro particles could be formed and they would be responsible for the reduced transmittance. According to the data shown in Figure 3, the pH-sensitivity of the copolymers increased with the content of MAA. In case of P(NIPAM-*co*-MAA), hydrophobic interactions is negligible since no hydrophobic anchor exists in the

copolymers. Thus, the P(NIPAM-*co*-MAA) would take expanded solubilized forms irrespective of the pHs. Because of this, the copolymer solutions are transparent in the full range of pH.

pH-Dependent surface tension of PNIPAM copolymer solutions

Figure 4 shows the surface tension variations of P(NIPAM-*co*-MAA) solutions (0.00125%) with pH. In case of homo-PNIPAM solution, the surface tension was almost constant with respect to pH and a decreased value, ~ 40 dyne/cm, was obtained in the full range of pHs. PNIPAM has no titrable group. This could account for the pH-independent value of the surface tension. It has a hydrophilic segment and a hydrophobic segment. The back bone of ethylene chains is hydrophobic and the pendant groups of amides are hydrophilic. Thus, PNIPAM is surface-active and it reduced the surface tension down to 40 dyne/cm at the concentration of 0.00125%. On the other hand, P(NIPAM-*co*-MAA) were surface-active as much as PNIPAM homopolymers at lower pHs such as pH 2.2 and 3.3. At higher pHs such as 5.2 and 8.3, the surface activity of the copolymer decreased. A titrable group, carboxyl group, is in the side group of the copolymer and it tends to be ionized at the higher pHs. In other words, the copolymers become more soluble at higher pHs and, in turn, they lose their surface activity. In terms of change in the surface activity, the pH-sensitivity of the copolymer was higher as the content of MAA was more. This is because the titrable group is
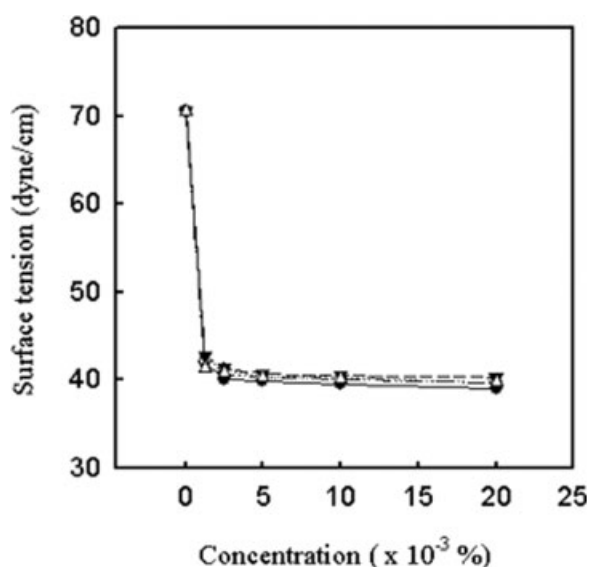

Figure 4 Surface tension variations of P(NIPAM-*co*-MAA) solutions with pH. The concentrations of polymers were 0.00125%. The contents of MAA were 0% (△), 2.5% (●), 5% (○), and 10% (▼).
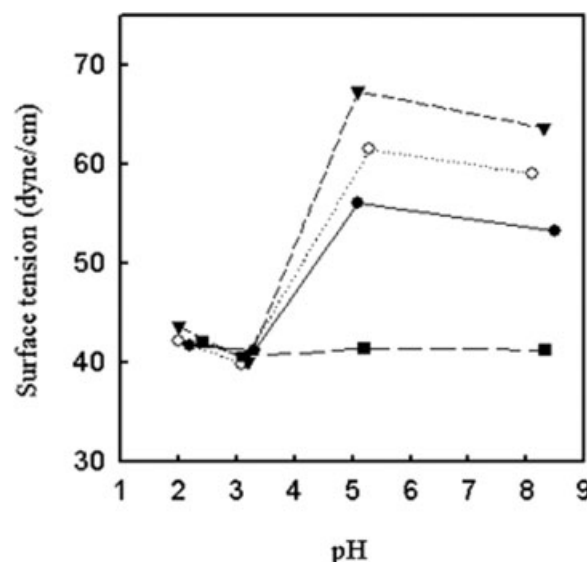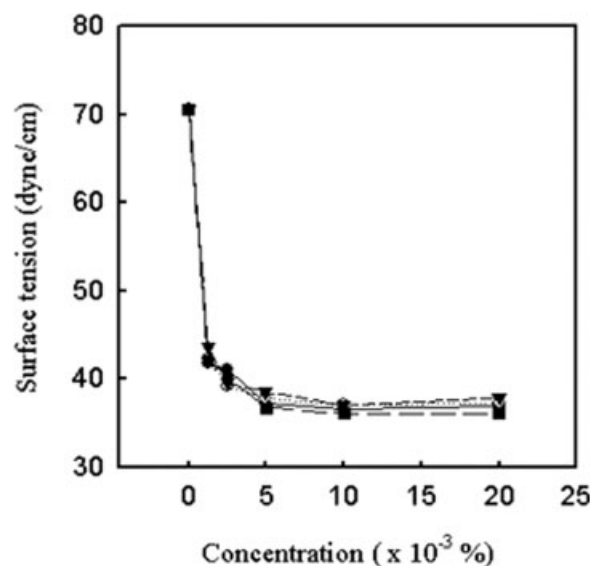

Figure 5 Surface tension variations of P(NIPAM-*co*-MAA-*co*-ODA) solutions with pH. The concentrations of polymers were 0.00125%. The contents of MAA were 0% (■), 2.5% (●), 5% (○), and 10% (▼).

proportional to the content of MAA. Figure 5 shows the surface tension variations of P(NIPAM-*co*-MAA-*co*-ODA) solutions (0.00125%) with pH. The behaviors of pH-dependent surface activity were similar to those of P(NIPAM-*co*-MAA). The ODA is a hydrophobic side chain, so the copolymers bearing ODA was thought to be more surface-active than the corresponding copolymers free of ODA. According to the results, however, no significant difference in the surface tension was observed among them. For example, the surface tension of homo-PNIAPM was almost the same as those of P(NIAPM-*co*-ODA). The concentration of the polymers, 0.00125%, would not be enough to exhibit the effect of ODA on the surface activity. Therefore, the surface activities of the copolymers with or without ODA were investigated varying the concentration. Figures 6 and 7 show the surface tension of P(NIPAM-*co*-MAA) solutions and P(NIPAM-*co*-MAA-*co*-ODA) solutions, respectively, at variable concentrations, where the pHs were adjusted to 2.1. Whether ODA, a hydrophobic moiety, is incorporated in the polymer or not, the surface tension markedly decreased by adding a small amount of the polymers (see the second data points where the concentration of polymers is 0.00125%). Furthermore, at the low concentration, the effect of ODA on the surface tension was negligible. That is, the values of P(NIPAM-*co*-MAA) solutions were almost the same as those of P(NIPAM-*co*-MAA-*co*-ODA) solutions. At concentrations higher than 0.0025%, the effect of ODA became marked. ODA is a hydrophobic side chain and thus it would bring solubilized copolymers to air/water interface, lead-

Figure 7 Surface tension of P(NIAPM-*co*-MAA-*co*-ODA) solutions at variable concentrations. pHs were adjusted to 2.1. The contents of MAA were 0% (■), 2.5% (●), 5% (○), and 10% (▼).

ing to a reduced surface tension. In parallel, while keeping the pH of the solution at pH 8.3, the surface tensions of P(NIPAM-*co*-MAA) solutions and P(NIPAM-*co*-MAA-*co*-ODA) solutions were measured at variable concentrations (data are not shown here). Although the effect of ODA on the surface tension was less than in case of pH 2.1, ODA obviously increased the surface activities of the copolymers. The less effect of ODA at 8.3 is possibly because MAA in the copolymer is ionized and accordingly P(NIPAM-*co*-MAA-*co*-ODA) is more soluble at the higher pH. That is, the increased hydrophilicity caused by the ionization would dominate the surface tension rather than the hydrophobicity coming from ODA.

CONCLUSIONS

The transmittances of P(NIPAM-*co*-MAA) solutions were almost constant in the full range of pH, pH 2.0–8.5, but the transmittances of P(NIPAM-*co*-MAA-*co*-ODA) solutions were reduced as pH decreased in the range of pH 2–5. On the other hand, P(NIPAM-*co*-MAA-*co*-ODA) was more surface-active than P(NIPAM-*co*-MAA) in the range of pH 2.0–8.5, and the difference in the surface activity between the two kinds of the copolymers become marked at lower pHs. According to the results of the transmittances and the surface activities, it is concluded that P(NIPAM-*co*-MAA-*co*-ODA) was more pH-sensitive than P(NIPAM-*co*-MAA). The hydrophobic interactions between ODAs would play a major role in the contractions of the polymer chains at acidic pHs.

Figure 6 Surface tension of P(NIPAM-*co*-MAA) solutions at variable concentrations. pHs were adjusted to 2.1. The contents of MAA were 0% (△), 2.5% (●), 5% (○), and 10% (▼).

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