NOTE

UCST-Type Cononsolvency Behavior of Poly(2-methacryloxyethyl phosphorylcholine) in the Mixture of Water and Ethanol

By Yasuhiro MATSUDA,[1] Motoyasu KOBAYASHI,[1] Masahiko ANNAKA,[2] Kazuhiko ISHIHARA,[3] and Atsushi TAKAHARA[1,*]

Poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC, Figure 1) is a polyampholyte bearing both positive and negative charge in its phosphorylcholine group. PMPC has so high affinity to water that it never shrinks in aqueous solutions with low ionic strength where polyampholytes usually shrinks or precipitates.[1] In aqueous solutions, PMPC hardly disturbs the network of water molecules formed by their hydrogen bonds,[2] and this special mechanism to dissolve into water is though to be one of the reasons for its high hydrophilic properties. These unique behaviors in aqueous media are the reasons why PMPC attracts much attention from many researchers in addition to its application to medical materials for its high biocompatibility.[3–6]

PMPC also has a very rare property, cononsolvency. Cononsolvency describes the situation that a solute is soluble in two pure solvents, but insoluble in the mixture of the two with specific mixing ratios. Not so many, but a few polymers have been reported to have cononsolvency, for example, polystyrene in N,N-dimethylformamide (DMF) and cyclohexane,[7] poly(methyl methacrylate) in chlorobutane and amyl acetate,[8] poly(ether imide) in N-methyl-2-pyrrolodinone and methylene chloride,[9] poly(vinyl alcohol) in water and dimethyl sulfoxide,[10] poly(N,N-dimethylacrylamide) (PDMAM, Figure 1) in water and 1,4-dioxane, or acetone,[11] and poly(N-isopropylacrylamide) (PNIPAM, Figure 1) in water and methanol, tetrahydrofuran (THF), or 1,4-dioxane.[12,13] The cononsolvency behavior of PNIPAM is used for the controlled release of alcohol soluble drugs from a thermosensitive gel consisting of interpenetrating polymer networks of PNIPAM and a triblock copolymer.[14] Kiritoshi and Ishihara[15] synthesized crosslinked PMPC gel and measured its strange swelling and shrinking behavior in the mixtures of water and ethanol caused by the cononsolvency of PMPC in water and ethanol.

However, there have been few researches on the temperature dependence of cononsolvency. Kiritoshi and Ishihara[15] also carried out the measurements only at room temperature. The temperature dependence of cononsolvency behavior helps to know whether the driving force of the cononsolvency behavior is the entropy or enthalpy change of phase separation and to know the mechanism of phase separation. This knowledge can also give a clue to understand the mechanism of PMPC to dissolve in aqueous media.

In this manuscript, we carried out turbimetry and dynamic light scattering (DLS) measurements for PMPC in the mixtures of water and ethanol varying the temperature, and discussed the unique properties of PMPC in the mixture of water and ethanol.

EXPERIMENTAL

Materials

PMPC was synthesized by atom transfer radical polymerization from the corresponding monomer.[16] The detail of the procedure of synthesis is described elsewhere.[17] Two samples of PMPC were used in this study, because phase separation measurements require so large amount of samples. The molecular weight of the samples was determined by size exclusion chromatography (SEC) recorded on JASCO system which runs through two directly connected polystyrene gel columns (Tosoh super AW3000 and super AW4000, 0.5 mL/min) using water containing 0.01 M of LiBr as eluent. The molecular weight was calibrated using PMPC samples which were synthesized by reversible addition-fragmentation chain transfer reaction to obtain samples with narrow molecular weight distribution.[17] The number average molecular weight M_n of PMPC samples used for the calibration of SEC was determined by the quantification of polymer ends by NMR. M_n and the ratio of weight average molecular weight M_w and M_n were 1.07×10^5 and 1.50 for the sample for DLS, and were 5.95×10^4 and 1.54 for the sample for turbimetry.

Water was purified with Millipore Simpli Lab, and its electrical resistivity was higher than $18 \, \mathrm{M\Omega \, cm}$. Ethanol was purchased from Wako Chemical (> 99.5%) and used without further purification.

[1]Institute for Materials Chemistry and Engineering, Kyushu University, 744 Motooka Nishi-ku, Fukuoka 819-0395, Japan
[2]Department of Chemistry, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan
[3]Department of Materials Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
*To whom correspondence should be addressed (Tel: +81-92-802-2517, Fax: +81-92-802-2518, E-mail: takahara@cstf.kyushu-u.ac.jp).
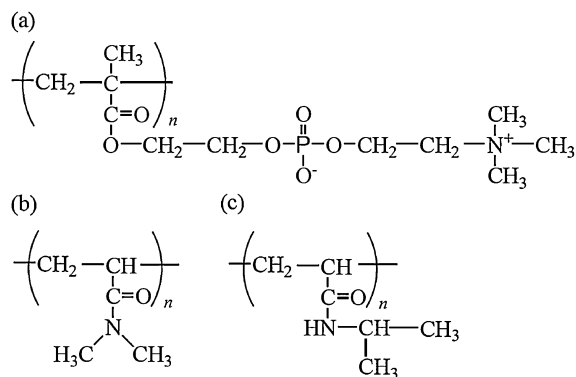
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(a)

(b) (c)

Figure 1. Chemical structure of (a) PMPC, (b) PDMAM, and (c) PNIPAM.

Preparation of Solutions for Turbimetry

The solutions for the estimation of phase diagrams were prepared as follows. First, PMPC was dissolved in pure water and ethanol to adjust the weight fraction of PMPC to solution (the weight of PMPC in solution divided by the weight of whole the solution) w_p to be 0.01. As PMPC is deliquescent, the sample was dried *in vacuo* for more than six hours and handled in a dry box where the relative humidity was less than 20%. The ethanol solution of PMPC and the aqueous solution of PMPC were mixed to prepare solutions with target molar fraction of organic solvent to the whole solvent x_{org}.

The methanol, 1-propanol, and 2-propanol solutions of PMPC were also prepared as descried above. PMPC dissolved easily in methanol, but did not dissolve in 1-propanol and 2-propanol by just adding solvents. PMPC dissolved in 1-propanol and 2-propanol after the sonication for an hour.

Turbimetry

The temperature dependence of transmittance was measured at $x_{org} \sim 0.35$ and 0.80 for PMPC in ethanol-water. The transmittance of the solutions was monitored with a UV-VIS spectrophotometer (Perkin Elmer Lambda 35s UV/VIS spectrometer). The wavelength of irradiation light λ_0 was 500 nm, and the cell length was 0.2 cm. The solution was heated at *ca.* 1.5 K min^{-1} by circulating heated water in a cell holder, and the temperature of the solution was monitored with a digital thermometer.

Dynamic Light Scattering

Dynamic light scattering (DLS) measurements were carried out for the ethanol-water solutions of PMPC at $x_{org} = 0.324$, 0.838, and 1. Mixed solvents of ethanol and water were added to the dried sample, and stirred overnight. The solutions were diluted by the mixed solvents to obtain test solutions for measurements. DLS measurements were carried out at 25 and 50 °C with ALV 5000/E/EPP using He-Ne laser ($\lambda_0 = 633$ nm) as a light source, and the detail of measurements is described in our previous paper.[1]

RESULTS

Rough Estimation of Phase Separation Behavior

In order to estimate the phase separation behavior of PMPC in the mixture of ethanol and water roughly, we carried out the following measurements. The ethanol solution of PMPC was dropwisely added to the aqueous solution of PMPC at room temperature with a syringe to increase the molar fraction of organic solvent to the whole solvent x_{org} form 0 to *ca.* 0.5. The turbidity of the solution was observed by the eye during the addition of the ethanol solution. Then the aqueous solution of PMPC was added to the ethanol solution in the same way to decrease x_{org} from 1 to *ca.* 0.5. The solution of PMPC was turbid at $x_{org} = ca.$ 0.35–0.80 at room temperature (~ 25 °C). The solution at $x_{org} = 0.82$ was clear at room temperature, but got turbid immediately by cooling with ice water.

The turbid solutions at $x_{org} = 0.42$, 0.52, and 0.69 were prepared and allowed to stand at room temperature. The solutions got immediately turbid and remained turbid for some hours, but gradually became clear. A week later, the solution became almost completely clear, but paste-like precipitate stuck on the wall of the glass of sample bottles. These results show that PMPC is insoluble in ethanol-water at $x_{org} = ca.$ 0.35–0.80, has a UCST-type phase diagram, and separates to liquid and (swelled) solid phases, not to liquid-liquid phases.

The same measurements were carried out using methanol, 1-propanol, and 2-propanol instead of ethanol. Cononsolvency was not observed for the methanol-water solution of PMPC, but the 1-propanol/water solution and 2-propanol/water solution were turbid at $x_{org} = 0.14$–0.91 for 1-propanol, and 0.17–0.97 for 2-propanol at room temperature. The turbid solutions of PMPC in the mixture of water and 1-propanol or 2-propanol became clear by heating.

Turbimetry

Figure 2 shows the temperature dependence of the turbidity of the solutions at $x_{org} = 0.359$ and 0.798. While the transmittance of the solutions at $x_{org} = 0.798$ sharply increased by raising the temperature, the temperature dependence of the turbidity of the solution at $x_{org} = 0.359$ was so weak. In the

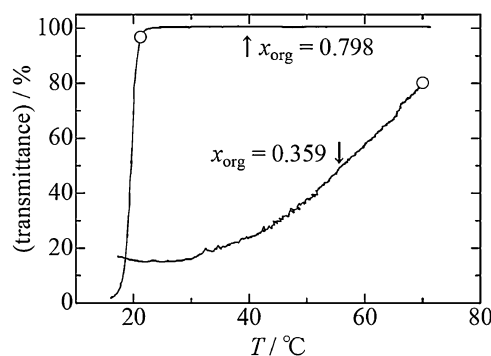Figure 2. The transmittance curves at $x_{org} = 0.351$ and 0.798 ($\lambda_0 = 500$ nm). The temperature was raised at *ca.* 1.5 K min^{-1}. The cloud points are shown as open circles.
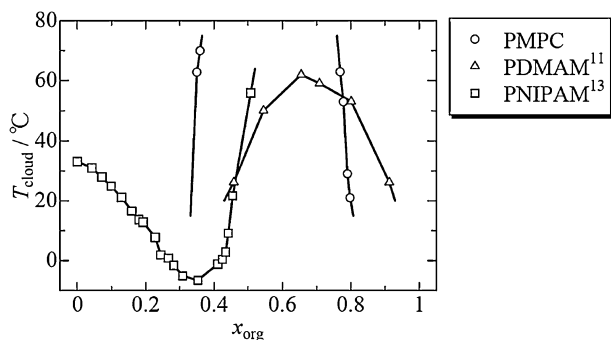
Figure 3. Cloud points of PMPC, PDMAM (redrawn from reference 11) and PNIPAM (redrawn from reference 13) at various solvent composition. The solvents are water and ethanol for PMPC, water and 1,4-dioxane for PDMAM, and water and methanol for PNIPAM, respectively.
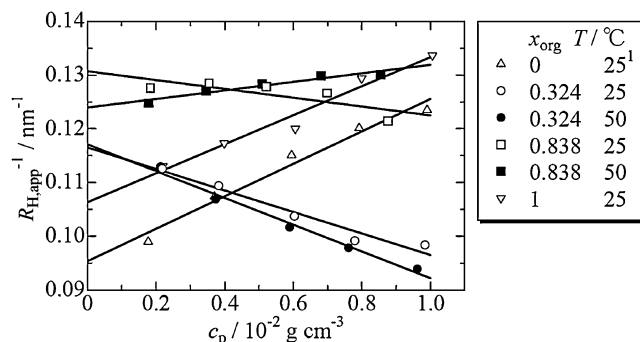

Figure 4. Relationship between the inverse of the average apparent hydrodynamic radius and polymer concentration at various molar fraction of ethanol to solvent, x_{org}. $R_{H,app}$ was calculated by regularizing the mutual diffusion coefficient considering the difference of the temperature and viscosity of solvents (Eq. 1). The data for PMPC in pure water is redrawn from reference 1.
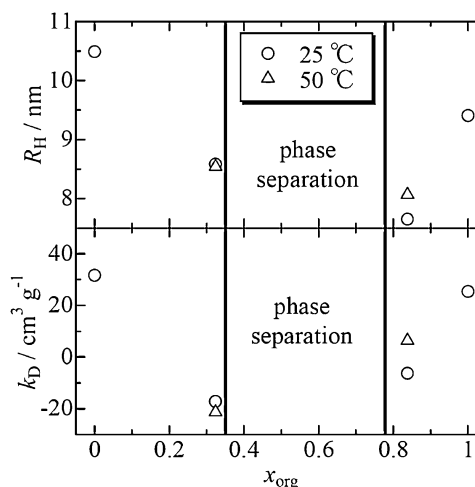
similar way, the transmittance of the solutions at $x_{org} \sim 0.80$ sharply increased, but that of the solutions at $x_{org} \sim 0.35$ gradually increased by raising the temperature. The cloud point T_{cloud} of a solution was determined as the temperature at the onset of turbidity for the solutions at $x_{org} \sim 0.80$, as shown as an open circle in Figure 2. As it was difficult to determine the onset for the solutions at $x_{org} \sim 0.35$ for the gradual decrease of turbidity, cloud points were defined to be the temperatures when the transmittance was 80% at $x_{org} \sim 0.35$.

Figure 3 shows the phase diagram of PMPC in ethanol-water. As the transmittance of the PMPC solution at $x_{org} = 0.331$ was 100% even at 15 °C, T_{cloud} of this solution was below 15 °C. The phase diagrams of PDMAM in 1,4-dioxane-water[11] and PNIPAM in methanol-water[13] are also shown in Figure 3. The mass concentration of the measured solutions of PDMAM and that of PNIPAM were fixed to be 0.4×10^{-2} $g\,cm^{-3}$, and $1 \times 10^{-2}\,g\,cm^{-3}$, respectively, while the mass concentration of the solutions of PMPC measured in this study was *ca.* $0.85 \times 10^{-2}\,g\,cm^{-3}$. T_{cloud} of PMPC, especially at $x_{org} \sim 0.80$ has stronger dependence on x_{org} than that of PDMAM and PNIPAM.

Dynamic Light Scattering

There were two relaxation modes for the auto-correlation functions of PMPC solutions at $x_{org} = 0.324$, 0.838, and 1. However, as the relaxation time of the slower peak was much slower than that of the faster one, and the two peaks can be easily separated by CONTIN analysis, the mutual diffusion coefficients D_m of the faster peak caused by the translational diffusion of non-aggregates which consist of the main component in the solutions were obtained by eliminating the effect of aggregates by the method proposed by Kanao *et al.*[18] Figure 4 shows z-average D_m of PMPC in different solvent conditions. The data for the same PMPC sample in pure water obtained in our previous work[1] are also shown in Figure 4. As D_m depends on not only the dimension of polymer chains, but also the temperature T and the viscosity of solvents η_0, the inverse of average apparent hydrodynamic radius $R_{H,app}^{-1}$ defined as follow was plotted against the mass concentration of polymer c_p to extrapolating D_m to infinite dilution state.

Figure 5. R_H and k_D of PMPC in the solutions at different ethanol content and temperature.

$$R_{H,app}^{-1} \equiv \frac{6\pi\eta_0}{k_B T} D_m = \frac{6\pi\eta_0}{k_B T} D_0(1 + k_D c_p + \cdots)$$
$$= R_H^{-1}(1 + k_D c_p + \cdots) \qquad (1)$$

k_B, D_0, k_D, and R_H are Boltzmann constant, limiting diffusion coefficient, the concentration coefficient of diffusion coefficient, and true average hydrodynamic radius, respectively. While $R_{H,app}^{-1}$ at $x_{org} = 0$ and 1 have positive slope, those at $x_{org} = 0.324$ and 0.838 are almost horizontal or have negative slope. As k_D mainly depends on the second virial coefficient A_2,[19] small k_D in Figure 4 suggest that A_2 in the mixture of water and ethanol was smaller than that in pure water and ethanol, that is, the mixture of water and ethanol was a poorer solvent than pure water and ethanol.

Figure 5 shows R_H and k_D calculated according to Eq. (1). Smaller R_H in ethanol-water than that in pure solvents shows that the free polymer chains of PMPC shrank in poor mixed solvents like the gel of PMPC.[15] While R_H at 50 °C was almost identical to that at 25 °C in ethanol-water at $x_{org} = 0.324$, R_H at 50 °C was larger than that at 25 °C, $x_{org} = 0.838$.

As shown in Figures 2 and 3, PMPC in ethanol-water has a UCST-type phase diagram, which suggests that ethanol-water at lower temperature, is a poorer solvent than that at higher temperature with the same ethanol content. The shrinkage of PMPC chains at 25 °C was caused by the poorer quality of the solvent at lower temperature. The stronger dependence of R_H on temperature at $x_{org} = 0.838$ agreed with the sharper increase of transmittance at $x_{org} \sim 0.8$.

DISCUSSION

The results shown in Figures 2 and 3, and the section of Rough estimation of phase separation show that PMPC has cononsolvency in the aqueous solutions of ethanol, 1-propanol and 2-propanol. The solubility of PMPC increased at higher temperature, that is, PMPC has UCST-type phase diagrams.

The causes for cononsolvency are generally divided into two different thermodynamic mechanisms.[7] One is that there is extremely strong tendency between the two solvents to contact each other comparing with the interaction between the polymer and the solvents. The other is that there is tendency for the solvents to avoid each other.

In the case of PMPC in the mixed solvents of water and alcohols, there is very strong tendency for water and alcohols to contact to form a network by their hydrogen bonds, while the interaction between PMPC and water is not so strong for the unique characteristic of PMPC that it hardly disturbs the network of water molecules.[2] The results obtained by DLS measurements show that both water and ethanol were good solvents to PMPC, but ethanol was a poorer solvent than water, that is, the interaction between PMPC and ethanol was weaker than that between PMPC and water. So, the cononsolvency of PMPC in water-alcohols is caused by the much stronger attractive interaction between water and alcohols than that of PMPC-water and PMPC-alcohols.

By phase separation, water and alcohols can form additional hydrogen bonds which decrease the enthalpy of the system, but the entropy of PMPC decreases by the precipitation. While the dependence of the enthalpy term on the temperature is weak, the entropy multiplied by temperature decreases at lower temperature. So, the advantage of enthalpy overcomes the disadvantage of entropy, and the phase separation becomes easier to occur at lower temperature. This is the reason why our solution system has a UCST-type phase diagram.

As the solvent quality of 1-propanol and 2-propanol was poorer as described above, the advantage of enthalpy by forming hydrogen bonds between water and alcohols overcomes more easily the disadvantage for PMPC to precipitate. This is the reason why the wider cononsolvency regions were observed for PMPC in water and 1-propanol and 2-propanol.

PDMAM[11] and PNIPAM[12,13] are the polymers of which cononsolvency behaviors in aqueous solutions were investigated at different temperature. PDMAM in water and 1,4-dioxane or acetone has UCST-type phase diagrams, while PNIPAM in water and methanol, THF, or 1,4-dioxane has LCST-type phase diagrams. The LCST-type phase diagrams of PNIPAM are caused by the unique property of PNIPAM that the affinity of PNIPAM to water drastically decreases by increasing temperature. On the other hand, PDMAM retains affinity to water over 0–100 °C. So, we will discuss the difference of the cononsolvency behaviors of PMPC in ethanol-water and that of PDMAM in 1,4-dioxane-water, for the affinity of PDMAM to water is more similar to that of PMPC.

The cloud points of PMPC depend more strongly on x_{org} than those of PDMAM. Solution at one phase state separates into two phases, when the free energy of the solution separated into two phases is lower than that of the solution at one phase. So, the stronger dependence of cloud points on x_{org} suggests that the difference of the free energy of solutions at one-phase state and two-phase state changes more drastically by the change of x_{org}.

As water is a better solvent both for PMPC and PDMAM than ethanol or 1,4-dioxane, the preferential adsorption of water molecules near polymer chains occurs in the solutions of PMPC and PDMAM. The precipitation of polymer liberates the water molecules adsorbed near polymer chains, and the liberated water molecules can interact with ethanol or 1,4-dioxane. As this interaction is the driving force of phase separation, stronger dependence of the cloud points of PMPC on x_{org} than that of PDMAM can be caused by two reasons. One is that the interaction between water and ethanol was stronger than that between 1,4-dioxane and water. The other is that there were more water molecules librated by the phase separation of PMPC than those librated by the phase separation of PDMAM. However, as PMPC hardly disturbs the network of water molecules,[2] the number of water molecules librated by the phase separation may be smaller. So, it can be inferred that the strong dependence of the T_{cloud} of PMPC on x_{org} is caused by the strong interaction between water and ethanol.

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