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SHORT COMMUNICATION

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Effects of ionization on the phase behavior of poly(*N*-isopropylacrylamide-co-acrylic acid) and poly(*N*,*N*-diethylacrylamide-co-acrylic acid) in water

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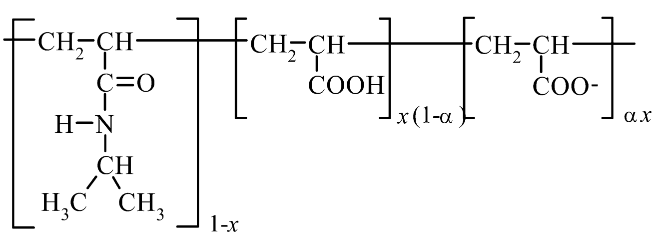
Abstract Phase transitions of poly(*N*-isopropylacrylamide-*co*-acrylic acid) (PiPA-AA) and poly(*N*,*N*-diethylacrylamide-*co*-acrylic acid) (PdEA-AA) in water have been investigated by means of turbidimetry, Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC). The phase transition temperatures (T_p) of these copolymers increase with the degree of ionization (α) of the acrylic acid (AA) units, which in turn is dependent on the pH of the solutions. Apparent values of p K_a for the AA units, determined from the pH dependencies of T_p, are 4.7 and 5.4 for PiPA-AA and PdEA-AA, respectively. Differences between T_p for PiPA-AA and T_p for PiPA homopolymer (ΔT_p) are +1.5 and −0.2 °C/mol% of AA at $\alpha = 1$ and 0, respectively. The values of ΔT_p for PdEA-AA are +2.6 (ionic) and −0.5 (nonionic)°C/mol%, indicating that the incorporated AA units have a larger effect on PdEA than on PiPA. DSC measurements performed with each of these copolymers at different pH values show a linear relationship between T_p and the enthalpy of transition (ΔH). IR measurements of PiPA-AA show that the profiles of IR bands from both iPA and AA units exhibit critical changes at T_p of the copolymer. Heating the solution above T_p leads to shifts of the amide II, C–H stretch, and C–H bend bands from the iPA units toward lower wavenumbers, as well as a shift of the amide I band from the iPA units toward higher wavenumbers. A decrease in the intensity of the symmetric C=O stretch IR band from carboxylate anions (1560 cm^{-1}), and an increase in the intensity of the C=O stretch band from COOH groups (1705 cm^{-1}) suggest that a partial protonation of the carboxylate groups ($COO^- + H^+ \rightarrow COOH$) takes place upon the phase transition.

Keywords IR spectroscopy · Thermo-sensitive polymer · Phase separation · Poly(*N*-isopropylacryamide) · Poly(*N*,*N*-diethylacrylamide)

Introduction

The folding and unfolding of proteins is driven by the cooperative action of many non-covalent interactions between amino acid residues, including hydrogen bonding, electrostatic interactions, and hydrophobic interactions [1, 2]. Poly(*N*-isopropylacrylamide) (PiPA) undergoes structural transition in water at a critical temperature (T_p), and the transition has been regarded as a model for the coil-globule transition of proteins [3]. The phase transition of PiPA has been investigated using many techniques such as turbidimetry, calorimetry [4, 5], fluorescence spectroscopy [6], light scattering [7, 8, 9], and so on [10, 11]. The initial step of the structural transition of PiPA is the dehydration of hydrophobic isopropyl groups of the polymer, which makes an attractive interaction between them dominant and induces collapse of the chain. Poly(*N*,*N*-diethylacrylamide) (PdEA) also exhibits a phase transition at a temperature close to T_p of PiPA, but the phase behavior of PdEA is somewhat different from that of PiPA in some respects [12, 13, 14, 15]. Elucidation of the factors that determine the phase behaviors and of the forces that drive the conformational changes of these polymers is necessary for an overall understanding of phase transition phenomena of aqueous polymer solutions. This would also provide a gateway to the understanding of folding processes of proteins, which contain twenty different comonomers and are structurally more complicated. In particular, ionizable groups have a key importance to the pH dependence of the dynamics of protein folding. The effects of electrostatic interaction between similarly charged groups on the structural transition of polymer chains can be investigated by incorporating ionic comonomers such as AA into PiPA [15, 16, 17, 18, 19, 20, 21], and PdEA, which are analogous to aspartic and glutamic acids in proteins. Moreover, the ionization states of acrylic acid (AA) units are dependent on pH, and the properties of neutral and ionized forms are quite different. That is to say, AA units exist in an ionized form (-COO$^-$) below p K_a and repulsive interactions occur between them, whereas AA units in a protonated form (COOH) can form hydrogen bonds (attractive interactions) with one another. Therefore, the PiPA-AA copolymer is a terpolymer (Scheme 1), and its composition can be controlled by changing the pH of the solutions. The objective of the present study is to reveal effects of the ionization of the AA units that are incorporated into PiPA and PdEA on the properties of their phase transition (transition temperature, heat of transition, and so on) and on the hydration state of these polymers. For this purpose, we have carried out turbidity, DSC and IR measurements of aqueous solutions of PiPA-AA and PdEA-AA at various pH values.



Scheme 1 Structure of PiPA-AA

Experimental

Materials

N-Isopropylacrylamide (iPA, Kohjin, Tokyo, Japan) was purified by recrystallization. *N*,*N*-Diethylacrylamide (dEA, Kohjin) and acrylic acid (Wako, Osaka, Japan) were purified by distillation. Copolymers were synthesized by radical polymerization in methanol (0.1 g-monomer/ml) using 2,2′-azobisisobutylonitrile as an initiator (initiator/monomer = 0.005) at 70 °C. After evaporation, the polymers were fractionated in acetone/*n*-hexane mixtures. The polymers obtained were dialyzed against water for three days with seamless cellulose tubing (Wako), and they were recovered by freeze-drying. The molecular weight distribution of the polymers was determined by gel permeation chromatography using tetrahydrofurane and poly(ethylene oxide) as mobile phase and standard, respectively. AA contents evaluated by elemental analysis were 4.7, 10.2, 5.4 and 9.6 for PiPA-AA5 (weight-average molecular weight $M_w = 19{,}000$, polydispersity $Q = 2.5$), PiPA-AA10 ($M_w = 23{,}000$, $Q = 2.1$), PdEA-AA5 ($M_w = 28{,}000$, $Q = 2.3$), PiPA-AA10, and PdEA-AA10 ($M_w = 21{,}000$, $Q = 2.8$), respectively.

Measurements

Turbidity measurement, differential scanning calorimetry (DSC) and FTIR measurements have been described previously [23, 24]. In brief, a polymer solution was placed between two CaF_2 windows with a spacer (10 μm thick) and the IR spectra were measured using a FTS 3000 spectrometer (Bio-Rad) at a resolution of 2 cm^{-1}. DSC measurements were performed by using a Micro Calorimetry System (MicroCal Inc.) with a scanning rate of 0.75 °C/min. Turbidity and DSC measurements were carried out with 0.5 wt% polymer solutions. Because the copolymers themselves act as buffers, the pH of each solution was adjusted by the addition of small amount of aqueous HCl or NaOH solution.

Results

Phase behavior dependences on pH for PiPA-AA and PdEA-AA

The phase transition temperatures (T_p) of PiPA-AA and PdEA-AA as determined by turbidimetry (unfilled symbols) and DSC (filled symbols) are plotted as a function of pH in Fig. 1. Onset temperatures found from a DSC endothermic peak and a turbidity-vs-temperature curve are defined as the T_p values of a solution. Values of T_p determined by these two different methods agree well. In order to analyze the dependence of T_p on the degree of ionization (α) of AA units, we defined T_p at $\alpha = 0$ and 1 as $T_{p,\alpha=1}$ and $T_{p,\alpha=0}$, respectively. Because the relationship between T_p for copolymers of PiPA and the contents of comonomers have been shown to be linear [25, 26], it is reasonable to assume that T_p is given by an average of $T_{p,\alpha=1}$ and $T_{p,\alpha=0}$ weighted by α. Then, the following equation is surmised:

$$T_p(\alpha) = (1-\alpha)T_{p,\alpha=0} + \alpha T_{p,\alpha=1} \qquad (1)$$

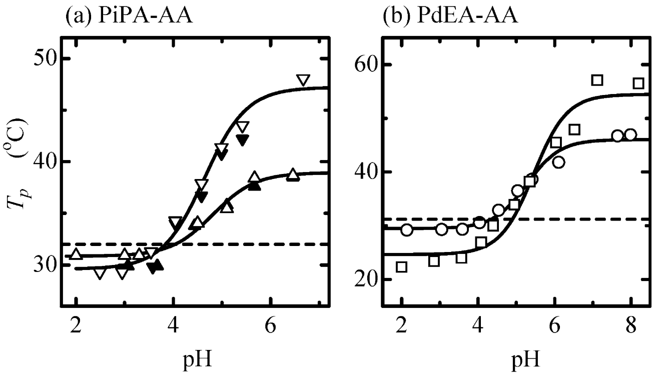


Fig. 1 The pH dependencies of T_p for **a** PiPA-AA5 (filled/unfilled triangles) and PiPA-AA10 (inverted filled/unfilled triangles) and **b** PdEA-AA5 (circles) and PdEA-AA10 (squares), determined by turbidimetry (unfilled symbols) and DSC (filled symbols). Curves were derived using a least-squares regression analysis with Eq. 2. Dashed lines indicate T_p for **a** PiPA, and **b** PdEA homopolymers

Because the dependence of α on pH can be expressed by Henderson-Hasselbalch's equation ($\alpha = 10^{-pH}/(10^{-pKa} + 10^{-pH})$), T_p as a function of pH can be expressed by the following equation:

$$T_p(\mathrm{pH}) = T_{p,\alpha=0} + \frac{\left(T_{p,\alpha=1} - T_{p,\alpha=0}\right) \times 10^{-\mathrm{pH}}}{\left(10^{-\mathrm{p}Ka} + 10^{-\mathrm{pH}}\right)} \qquad (2)$$

Curves in Fig. 1 are obtained by a nonlinear least-squares regression analysis using Eq. 2. Apparent values of p K_a for PiPA-AA and PdEA-AA are 4.7 and 5.4, respectively. Kawasaki et al. has reported that the pK_a value of PiPA-AA determined by potentiometric titration is independent of temperature at < 33 °C (pK_a = 4.9) and increases with temperature at > 34 °C (pK_a = 5.9 at 35 °C) [21]. The p K_a value for AA units in PiPA-AA obtained in the present study is close to the value determined by Kawasaki et al. at $< T_p$.

The effects of incorporated AA units on the T_p for PiPA and PdEA can be evaluated using ΔT_p as defined by the following equation,

$$\Delta T_p = \frac{\left(T_{p,cp} - T_{p,hp}\right)}{x} \qquad (3)$$

where $T_{p,cp}$ and $T_{p,hp}$ are T_p of a copolymer that contains x mol% of the comonomer unit and the corresponding homopolymer, respectively. The values of ΔT_p for PiPA-AA are +1.5 and −0.2 °C/mol% for the ionized and nonionic forms of AA, respectively. The values of ΔT_p for the ionized and protonated forms of AA units in PdEA-AA are +2.6 and −0.5 °C/mol%, respectively. The incorporated AA units have a larger effect in PdEA than PiPA.

Next, DSC thermograms were analyzed in order to reveal effects of α on the enthalpy of phase transition (ΔH). The value of T_p that is defined as the onset temperature of an endothermic peak increased, and ΔH, obtained by integrating the area under the peak, decreased with increasing α (Fig. 2a). Figure 2b shows the relationship between T_p and ΔH for PiPA-AA and PdEA-AA measured at various pH values. The data points for each polymer lie on a single line, suggesting that ΔH is essentially determined by the T_p of the solution, that depends on the compositions (x) and α of the copolymers.

IR spectra of PiPA-AA and PdEA-AA

Figure 3a shows the IR absorption spectrum of deuterated PiPA-AA measured at around $\alpha = 0.5$ and 35 °C. D_2O was used as solvent instead of H_2O to prevent the O–H bending band of water (~1640 cm^{-1}) overlapping with the amide I band of the polymer. The positions of the IR bands arising from the iPA unit in the copolymer are almost the same as those of the homopolymer of iPA, and assignments of these bands have been described in our previous paper [22]. Important IR bands for the deuterated iPA moiety are the amide I′ (mainly the C = O stretch vibration of the deuterated amide group) and amide II′ (a combination of N–D bend and C–N stretch vibrations of the deuterated amide group) bands observed at 1624 cm^{-1} and 1469 cm^{-1}, respectively. IR bands due to the AA moiety are the C = O stretching band of carboxyl group ν(COOH) at 1705 cm^{-1} and the antisymmetric C = O stretching band of carboxylate $\nu(COO^-)$ at 1560 cm^{-1}.

Figure 3c represents the IR difference spectrum obtained by subtracting an IR absorption spectrum of PiPA-AA10 measured at a temperature (T) below T_p from a spectrum measured at $T > T_p$, which is designated as ΔA. In the IR difference spectrum, a negative peak is

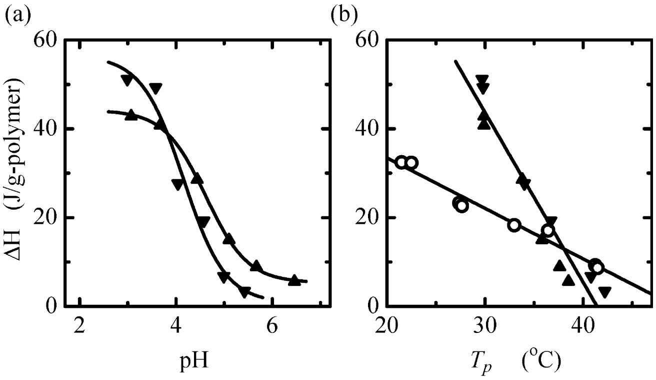


Fig. 2 **a** The pH dependencies of the phase transition enthalpy (ΔH) for PiPA-AA5 (filled triangles) and PiPA-AA10 (inverted filled triangles) plotted against T_p. **b** ΔH values measured at various pH values for solutions of PiPA-AA5 (filled triangles), PiPA-AA10 (inverted filled triangles), and PdEA-AA (circles)

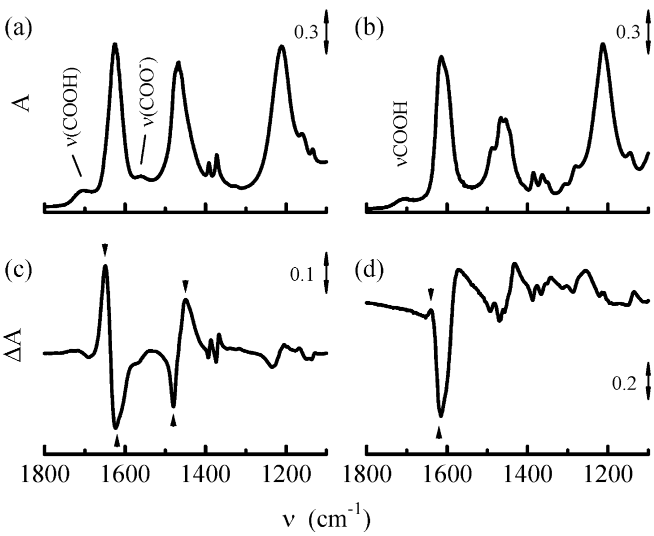


Fig. 3 **a** IR absorption spectrum of PiPA-AA10 ($\alpha = 0.5$) measured at 30 °C in D_2O. **b** IR absorption spectrum of PdEA-AA10 ($\alpha = 0$) measured at 30 °C in D_2O. **c** IR difference spectrum (ΔA_{46-30}) of PiPA-AA10 ($\alpha = 0.5$) measured in D_2O. **d** IR difference spectrum (ΔA_{40-30}) of PdEA-AA5 ($\alpha = 0$) measured in D_2O. Arrows indicate the positive and negative peaks that are use to calculate values of $\Delta\Delta A_{T-\mathrm{To}}(\nu_1,\nu_2)$ as shown in Fig. 4

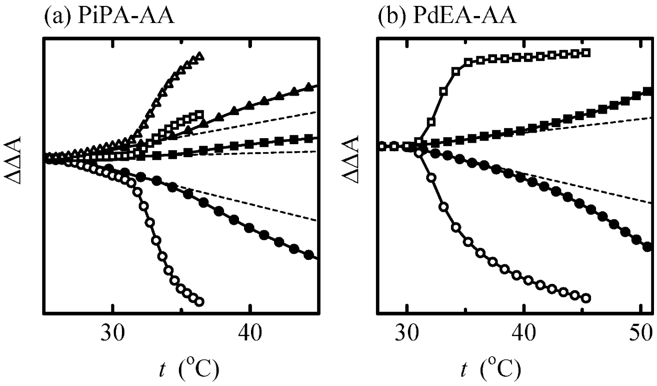


Fig. 4 **a** Values of $\Delta\Delta A(\nu_1,\nu_2)$ for the ν(C–H) ($\Delta\Delta A$(2970,2991): filled, unfilled squares), amide I′ ($\Delta\Delta A$(1625,1650): filled, unfilled circles) and amide II′ ($\Delta\Delta A$(1448,1479): filled, unfilled triangles) modes of PiPA-AA5 (unfilled symbols: $\alpha = 0$, filled symbols: $\alpha = 0.5$) are plotted against temperature (t). **b** Values of $\Delta\Delta A(\nu_1,\nu_2)$ for the ν(C–H) ($\Delta\Delta A$(2959,2984)×5: filled, unfilled squares) and amide I ($\Delta\Delta A$(1615,1640): filled, unfilled circles) modes of PdEA-AA5 (unfilled symbols: $\alpha = 0$, filled symbols: $\alpha = 0.5$) are plotted against temperature (t)

associated with absorption by polymer chains in a coiled structure, and a positive peak represents absorption by dehydrated and collapsed chains. Important features of the difference spectrum from the iPA moiety are red shifts of the C–H stretch ν(C–H) and the amide II′ bands, and a blue shift of the amide I′ band. Changes in the positions of these IR bands indicate the dehydration of the alkyl groups [27] and reduction of the strength of hydrogen bonding for the amide groups [28, 29] as described in our previous paper [22]. Prominent features of the difference IR spectra for AA units are the positive peak around 1725 cm^{-1} and a negative shoulder around 1570 cm^{-1} (Fig. 3c), suggesting that some of the COO$^-$ groups are protonated upon the phase transition. This observation is consistent with the result obtained by potentiometric titration that shows an increase in p K_a of PiPA-AA upon the transition [21]. The protonation of the COO$^-$ groups is partially due to a decrease in the dielectric constant around them (dehydrated iPA units are less polar than water), and also due to a stabilization of resulting COOH groups through hydrogen bonding with one another.

To observe the progress of the phase transition, the difference in the values of ΔA (a function of wavenumber and temperature) at the positive and negative peaks of a selected vibration mode (for example, a negative peak at 1649 cm^{-1} and a positive peak at 1625 cm^{-1} for the amide I mode of PiPA-AA, which are shown in Fig. 3 with triangles) is defined with the following equation:

$$\Delta\Delta A(\nu_1,\nu_2) = \Delta A(\nu_1) - \Delta A(\nu_2) \tag{4}$$

where ν_1 and ν_2 denote wavenumbers at the positive or negative peaks. The values of $\Delta\Delta A$ for the ν(C–H), amide I′ and amide II′ modes of PiPA-AA5 at $\alpha = 0$ (unfilled symbols) and $\alpha = 0.5$ (filled symbols) are plotted against temperature in Fig. 4a. Onsets of $\Delta\Delta A$ vs. temperature curves for PiPA-AA5 at $\alpha = 0$ and 0.5 are located at around 31 and 34 °C, respectively, which are close to the values of T_p determined by turbidimetry. At $\alpha = 0.5$ the change in $\Delta\Delta A$ in the transition temperature region is much smaller than that at $\alpha = 0$. This is partially because the width of the transition region is broadened with an increasing α [19] and partially because dehydration of the iPA moiety is less significant due to the presence of ionized groups nearby. A similar tendency was also observed with PdEA-AA (Fig. 4b). Gradual and linear changes in $\Delta\Delta A$ below T_p are due to a simple temperature effect, which weakens the strength of the hydrogen bond between the amide and water and causes a blue shift of the amide I band and a redshift of the amide II band [30].

To obtain information concerning the hydrogen bonding of the amide group, the amide I′ band of PiPA-AA in D_2O was analyzed using a peak separation method. In our previous study we showed that the amide I′ band of the PiPA homopolymer consists of two components centered at 1625 and 1650 cm^{-1}, which are assigned to the C=O groups that are bound to water (C=O...D–O) and to another amide group of the polymer (C=O...D–N) through a hydrogen bond, respectively [22]. The amide I′ band of PiPA-AA5 and PiPA-AA10 measured below T_p contains only the

1625 cm^{-1} component, and the 1650 cm^{-1} component appears above T_p (Fig. 5a and c). The behavior is similar to that of the amide I′ band of the PiPA homopolymer. The molar fractions (f) of the C = O species of PiPA-AA that is responsible for the 1650 cm^{-1} component (the C = O...D–N species) can be calculated from the relative areas of these components, and are plotted against temperature in Fig. 5b and d. The molar fractions of the C = O...D–N species for PiPA-AA at $\alpha = 0$ are slightly higher than that for the PiPA homopolymer. There is a possibility that hydrogen bonds between the amide C = O groups and the COOH groups of AA units as well as the amide N–H group of iPA units are formed in the globule states.

Discussion

A water-soluble polymer is solvated in a complex hydration shell consisting of various local water structures. A polar and charged region will be surrounded by a coulombic hydration shell, whereas non-polar sites are encapsulated in a hydrophobic hydration shell [31]. The number of hydrogen bonds between water molecules that exist in these shells is affected by the presence of the polymer chain. Indeed, analyses of the O–H stretch Raman band of water in polymer solutions have provided insight into the number of hydrogen bond defects that are introduced into the hydrogen-bonded network structure of water per monomeric unit of the polymer (N) [32]. In general, the values for polyelectrolytes such as poly(acrylic acid) sodium salt ($N = 8.7$) and poly(allylamine) hydrochloride (7.6) are larger than those of neutral polymers such as poly(acrylamide) ($N = 3.2$) and PiPA ($N = 2.1$), because ionized groups and counterions of these polyelectrolytes strongly disturb the structure of water in their hydration shells. In particular, the value of N for poly(AA) depends on α ($N = 8.7$ at $\alpha = 1$and $N = 3.4$ at $\alpha = 0$), indicating that the entropy of water is higher near the ionized AA unit than near the neutral one. Because the phase behavior of an aqueous polymer solution is determined by the balance between an unfavorable decrease in the entropy of water upon dissolution of the polymer and a favorable energy arising from polymer-water interactions such as hydrogen bonding, the incorporation of COO$^-$ groups into a polymer, which reduces net decrease in the entropy of water, raises T_p of the polymer. In contrast, because the COOH groups can form hydrogen bonds with one another and stabilize a collapsed conformation, the values of T_p for PiPA-AA and PdEA-AA at $\alpha = 0$ are slightly lower than those for the corresponding homopolymers.

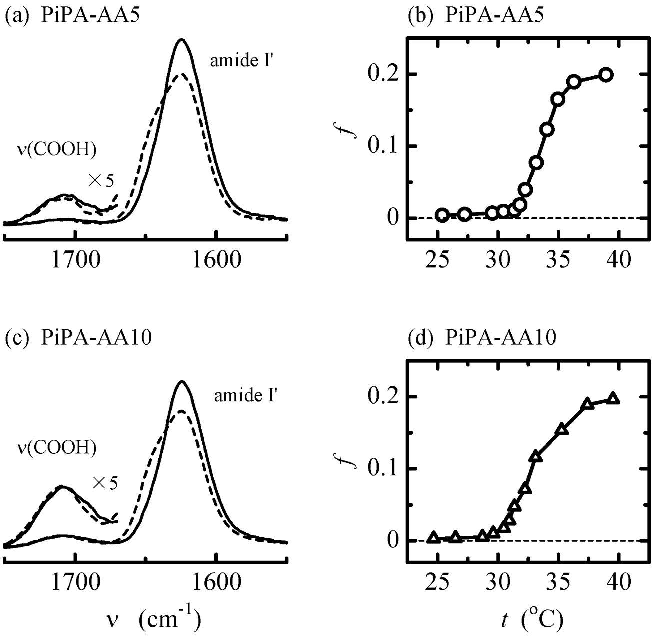


Fig. 5 The amide I′ and ν(COOH) bands of **a** PiPA-AA5 and **c** PiPA-AA10, measured below (solid lines) and above (dashed lines) T_p at $\alpha = 0$. The molar fractions (f) of the C = O groups of **b** PiPA-AA5 and *d* PiPA-AA10, that form hydrogen bonds with the N-D group (the C = O...D–N species) in D_2O at $\alpha = 0$, are plotted against temperature (t)

DSC measurements show a uniform linear relationship between the endothermic heat of the phase transition and T_p for each of the copolymers. A linear relationship between ΔH and T_p indicates that ΔH is essentially determined by T_p, which in turn is dependent on the polymer composition. As shown in Fig. 5, the fraction of polymer-water hydrogen bonds broken upon the phase transition of PiPA is relatively low. Consequently, the contribution from the loss of polymer-water hydrogen bonds to ΔH might be small and, rather, the reduction in hydrogen bonds between water molecules surrounding the hydrophobic moieties of the copolymers are mainly responsible for the value of ΔH [33]. Increasing T_p reduces the value of ΔH because of the relatively low number of hydrogen bonds between water molecules that surround hydrophobic moieties at high temperature. After all, the structure of water around a polymer chain, as well as direct polymer-water interactions such as hydrogen bonding, is important for determining the phase behavior of aqueous polymer solutions.

Conclusions

In the present study, the phase transitions of aqueous solutions of PiPA-AA and PdEA-AA have been investigated by means of turbidimetry, FTIR, and DSC. Apparent values of p K_a for AA units, determined from the pH dependencies of T_p, are 4.7 and 5.4 for PiPA-AA and PdEA-AA, respectively. Ionized and protonated

AA units raise and reduce T_p of the copolymers, respectively, and the units have a larger effect on PdEA than on PiPA. IR measurements showed that red-shifts of the amide II, C–H stretch, and C–H bend bands, and a blue-shift of the amide I band occur during the heating of the solution above T_p. The result indicates the dehydration of the alkyl groups and a decrease in the strength of hydrogen bonding for the amide groups. In addition, a decrease and an increase in the intensities of IR bands due to the COO^- and COOH groups, respectively, suggest that some of the COO^- groups are protonated during the phase transition.

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