Investigation of Hydrophobic Interactions in Dilute Aqueous Solutions of Hydrogen-Bonding Interpolymer Complexes by Steady-State and Time-Resolved Fluorescence Measurements

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Received May 8, 1997; Revised Manuscript Received October 3, 1997[⊗]

ABSTRACT: Steady-state and time-resolved fluorescence measurements, using pyrene as probe, have been employed to study the contribution of hydrophobic interactions to the stabilization of polymer complexes. Complexes are formed in aqueous solutions through hydrogen bonding. Binary complexes have been studied between poly(acrylic acid) (PAA) and one of the four following polymers: poly(*N*-isopropylacrylamide), (PNIPAAM); poly(acrylamide), (PAAM); poly(vinyl methyl ether), (PVME); and poly-(ethylene glycol), (PEG). From the results obtained, it was concluded that the PNIPAAM/PAA and PVME/PAA interpolymer complexes demonstrate a strong hydrophobic character, that the PEG/PAA complex demonstrates only a limited hydrophobicity, and that the PAAM/PAA complex is not at all hydrophobic.

Introduction

Interpolymer complex formation in aqueous solutions between a proton donor, such as poly(acrylic acid) (PAA), and a proton acceptor, such as poly(ethylene glycol) (PEG) or poly(acrylamide) (PAAM), has been extensively studied during the last decades.[1,2] The formation of these complexes is due to successive hydrogen bonding, but hydrophobic interactions play a major role in some cases.

Fluorescence methods,[3−9] in conjunction with potentiometry,[9−13] viscometry,[10−12] and turbidimetry,[14] have been widely used in the investigation of interpolymer complex formation by incorporating a fluorescent label, such as pyrene, on the chain of one of the two interacting polymers. Excimer fluorescence measurements are used to monitor the local concentration of pyrene labels in the complexes, while the modification of the monomer spectrum provides an indication of the polarity of the environment, showing the existence or not of hydrophobic interactions in the complex.

An inconvenience connected to pyrene labeling is its contribution to the hydrophobic character of the labeled polymer. It is known that increased hydrophobicity of the labeled polymer, due to the interaction of fluorescent hydrophobic markers in the polymer chain, strengthens interpolymer complex stability by hydrophobic interactions.[15,16] However, free pyrene probing has been used in the characterization of surfactants, revealing their micellization through the formation of hydrophobic clusters,[17] as well as in polymer−surfactant interaction studies.[18] A similar exploitation of fluorescence measurements in the study of hydrogen-bonding interpolymer complexes could be proved useful in the investigation of the role of hydrophobic interactions in complex stabilization.

It is known that poly(methacrylic acid) forms an interpolymer complex with PEG showing a considerable hydrophobic character compared to that for the complex formed from PAA and PEG.[7,9,10] Moreover, it has been recently shown[19,20] by viscometric and potentiometric measurements that poly(*N*-isopropylacrylamide) (PNIPAAM) and PAA form a strong interpolymer complex strengthened by increasing temperature while PAAM and PAA form a rather weak interpolymer complex strengthened by decreasing temperature. This temperature dependence of the strength of the complexes has been attributed to the presence or not of hydrophobic interactions. In this work we have used fluorescence measurements through pyrene probing to investigate the existence of hydrophobic interactions in interpolymer complex formation between PAA and PNIPAAM, PAAM, PEG, or poly(vinyl methyl ether) (PVME) without labeling the interacting polymers but incorporating pyrene as a free probe.

Experimental Section

Materials. The PAA sample was a 25 wt % aqueous solution of nominal molecular mass 90.000, obtained from Polysciences. It was diluted to a 10 wt % solution with 0.01 M HCl and purified by dialysis in water and freeze-dried. The PEG sample (Fluka) was of nominal molecular mass 35.000. The PVME sample was a 50 wt % water solution obtained from Polysciences. It was diluted to a 5 wt % water solution, precipitated by heating at 40 °C, and vacuum-dried for 2 days. Its molecular mass was found by light scattering to be equal to 80.000. The synthesis of the PAAM sample used with a molecular mass equal to 148.000 and of the PNIPAAM sample with a molecular mass equal to 230.000 has been described elsewhere.[20]

Sample Preparation. Polymer stock aqueous solutions were prepared at various concentrations. An aliquot of a pyrene 10^{-4} M stock solution in ethanol was first introduced in the flask, and the solvent was evaporated by blowing nitrogen. Then the aqueous polymer solution was added in the flask. The ensuing pyrene concentration was in all cases 5×10^{-7} M. Each solution was stirred at room temperature for 24 h. Millipore water was used in all solutions. In order to achieve a pH value equal to that of the corresponding PAA solution, 0.01 M HCl was added in the case of PNIPAAM, PAAM, PEG, and PVME solutions.

Samples were prepared by mixing polymer stock solutions at various compositions. The mixtures did not exhibit any

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⊗ Abstract published in *Advance ACS Abstracts,* November 15, 1997.

S0024-9297(97)00648-7 CCC: $14.00

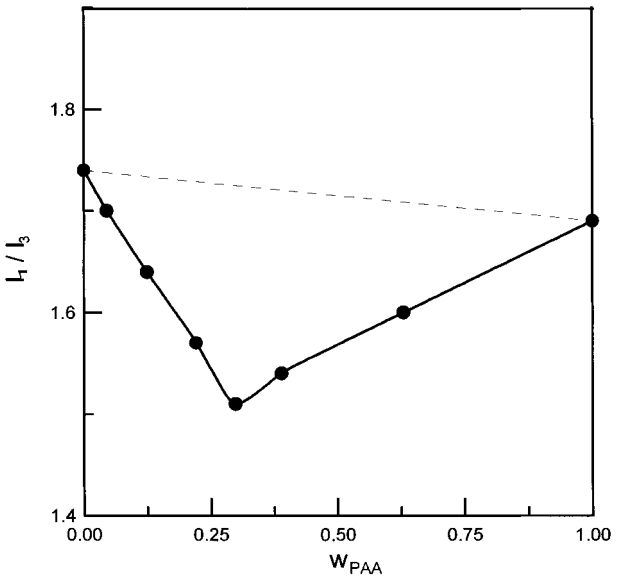

Figure 1. Plot of the I_1/I_3 ratio versus the weight fraction in PAA, w_{PAA}, for the polymer mixture PNIPAAM/PAA in water. The concentration of each pure polymer mixture is $c = 0.01\%$, and the pH is equal to 4.0.

turbidity or phase separation. Samples for time-resolved measurements were degassed for 30 min by gentle bubbling of nitrogen. In the case of the steady-state fluorescence measurements, samples were not degassed, since we found that the vibronic pyrene structure was not affected by the presence of oxygen.

Fluorescence Measurements. Fluorescence measurements were conducted with a home-assembled spectrofluorometer using Oriel parts, and lifetime measurements, with the photon-counting technique using a home-made hydrogen flash and Ortec electronics. Interference filters (Oriel) were used for excitation (335 nm) and emission (380 nm). The I_1/I_3 ratio of pyrene was determined from the emission peak intensities at 373 and 384 nm.

Results

Steady-State Fluorescence Measurements. Fluorescence measurements with 5×10^{-7} M pyrene were conducted in order to examine the existence of hydrophobic interactions in the polymer pairs PNIPAAM/PAA, PAAM/PAA, PVME/PAA, and PEG/PAA in dilute aqueous solutions. At this pyrene concentration, no excimer was detected in the fluorescence spectra. The ratio I_1/I_3 of the pyrene intensity at 373 and 384 nm was used to detect changes in the polarity of the polymer mixtures, since pyrene has the tendency to occupy hydrophobic sites. I_1/I_3 was 1.80 in water, and it decreased with decreasing polarity; for example, $I_1/I_3 = 1.12$ for pyrene solubilized in SDS micelles. In this study the polarity of the polymer mixtures was examined as a function of their PAA weight fraction, w_{PAA}. If interpolymer complexation through hydrogen bonding proceeds without any hydrophobic interaction, then it is expected that the I_1/I_3 ratio of the polymer mixtures should be a linear function of the I_1/I_3 ratio of the two pure components. A decrease of the I_1/I_3 ratio is expected if hydrophobic interaction is present.

The ratio I_1/I_3 as a function of w_{PAA} is shown in Figures 1 and 2 for the PNIPAAM/PAA and PAAM/PAA polymer mixtures, respectively. The I_1/I_3 values considerably deviated from linearity, in the case of the PNIPAAM/PAA complex, in dilute (0.01%) solutions and at a pH of the single component solution equal to 4.0. It attained a minimum value at $w_{PAA} \approx 0.3$. This

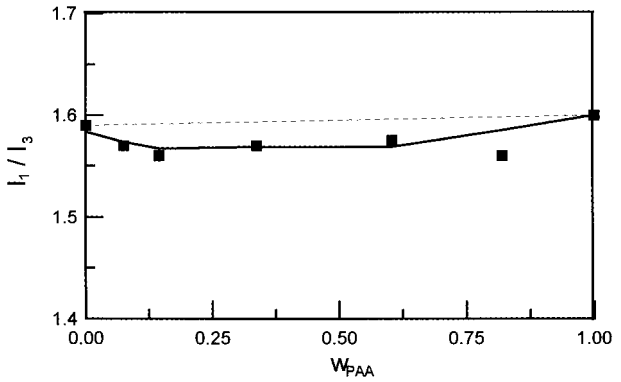

Figure 2. Plot of the I_1/I_3 ratio versus the weight fraction in PAA, w_{PAA}, for the polymer mixture PAAM/PAA in water. $c = 0.3\%$. pH = 3.0.

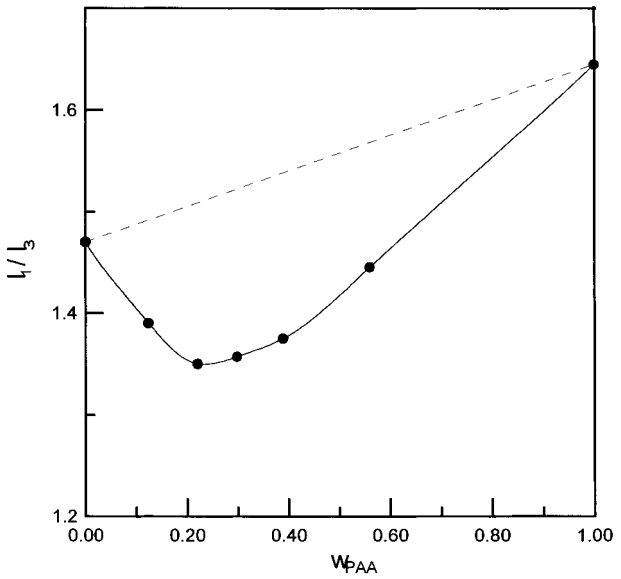

Figure 3. Plot of the I_1/I_3 ratio versus the weight fraction in PAA, w_{PAA}, for the polymer mixture PNIPAAM/PAA in water. $c = 0.1\%$. pH = 3.4.

deviation from the linear function (dotted line) suggests that the interpolymer complex between PNIPAAM and PAA exhibits a hydrophobic character. The minimum I_1/I_3 appears at $w_{PAA} = 0.3$, which, in terms of monomer units molar ratio, corresponds to PNIPAAM/PAA = 1.5. This stoichiometry is in agreement with viscometric and potentiometric evidence,[19,20] and it is related with the particular composition of the hydrophobic aggregates. The hydrophobic isopropyl group of PNIPAAM plays an important role in the hydrophobicity of the formed complex, while simultaneously influencing also the stoichiometry of the complex. On the other hand, in the case of the PAAM/PAA mixture no significant deviations from linearity were observed, even though the polymer concentration was in this case higher, i.e. 0.3%, and the single-component pH was 3.0, so that complexation would proceed to a higher degree.[12,19] As seen in Figure 2, the I_1/I_3 values are only slightly lower than the values of the two pure solutions of PAAM and PAA.

In addition to these observations, concentration effects on the I_1/I_3 ratio were also examined, and the results are shown in Figure 3, where the I_1/I_3 ratio of the PNIPAAM/PAA aqueous system with concentration 0.1% (i.e. ten times higher) is presented. The deviation of the I_1/I_3 ratio from linearity persists. Even though the value of the I_1/I_3 ratio of the pure PNIPAAM solution is now much lower, 1.48 compared to 1.73 in the less

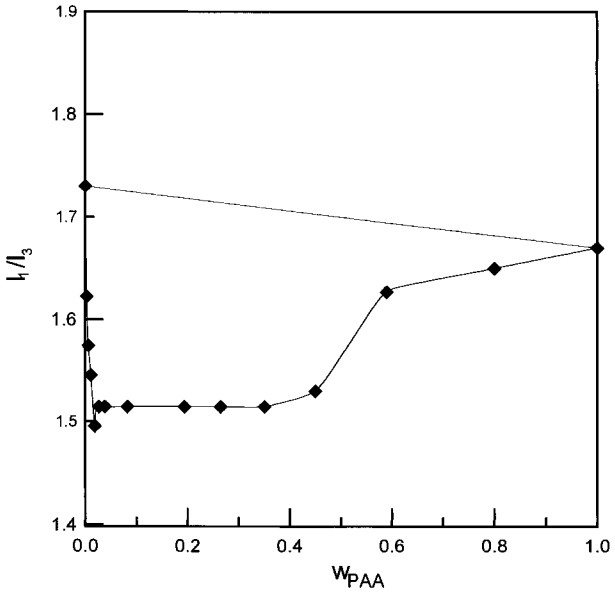

Figure 4. Plot of the I_1/I_3 ratio versus the weight fraction in PAA, w_{PAA}, for the polymer mixture PVME/PAA in water. c = 0.02%. pH = 3.9.

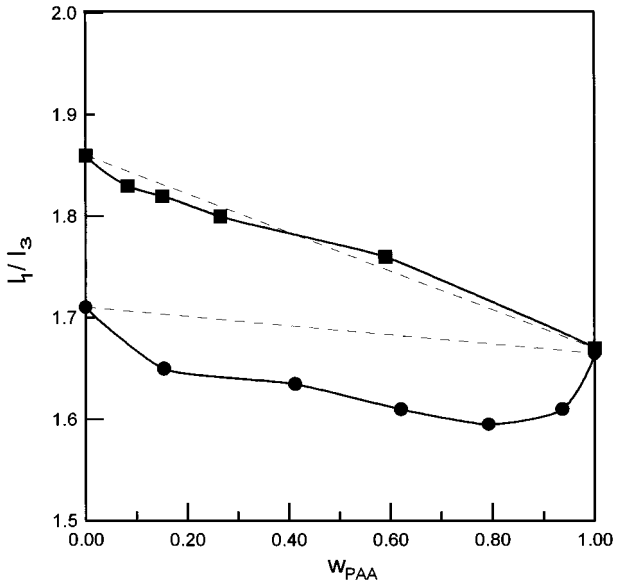

Figure 5. Plot of the I_1/I_3 ratio versus w_{PAA} for the polymer mixture PEG/PAA in water: (■) c = 0.02%, pH = 3.9; (●) c = 0.2%. pH = 3.2.

concentrated solution, the deviation from linearity is still very marked.

The ratio I_1/I_3 of the PVME/PAA mixture as a function of w_{PAA} is shown in Figure 4 for a low polymer concentration, 0.02%, and a pH of the single-component solutions equal to 3.9. The I_1/I_3 ratio decreased rapidly once a small amount of PAA was added in the PVME solution and remained constant until $w_{PAA} \approx 0.5$. When the polymer mixture composition in PAA became higher than 0.5, the ratio I_1/I_3 increased almost to the same value as that of the pure PAA solution. The observed deviation from the linear function (dotted line) implies that the formed interpolymer complex exhibits a profound hydrophobic character and that its overall hydrophobic regions are formed once the polymer mixture composition in PAA exceeds a very low threshold ($w_{PAA} > 0.08$). They exist up to a polymer mixture composition of $w_{PAA} \approx 0.5$. This rapid decrease of the I_1/I_3 ratio seems to be due to an almost complete hydrophobic aggregation of PVME chains after the addition of a small amount of PAA. This aggregation is related to an interpolymer complex formation between PAA and PVME by hydrogen bonding. At higher w_{PAA} compositions the repulsive electrostatic interactions between the negatively charged carboxylate groups of PAA prevail, so that the PVME chains expand and the hydrophobic aggregates are eliminated.

The I_1/I_3 values of the PEG/PAA mixtures at two concentrations and pH values are shown in Figure 5. It is evident that, in the case of the dilute polymer mixture with concentration 0.02% and pH = 3.9 (■), no hydrophobic regions exist, since the ratio I_1/I_3 coincides with the weight average of the ratios I_1/I_3 of the two pure components. Nevertheless some hydrophobic interaction appears as the concentration of the two polymer solutions increases at 0.2% with a simultaneous decrease of their pH to 3.2 (●). It seems that as hydrogen-bonding interactions at higher polymer concentration and lower pH values are favored, favoring interpolymer complex formation,[12] slight hydrophobic interactions also appear.

It is also important to observe from the above results that, contrary to the behavior of the hydrophobic pairs, in the case of the hydrophilic PAAM/PAA complex and

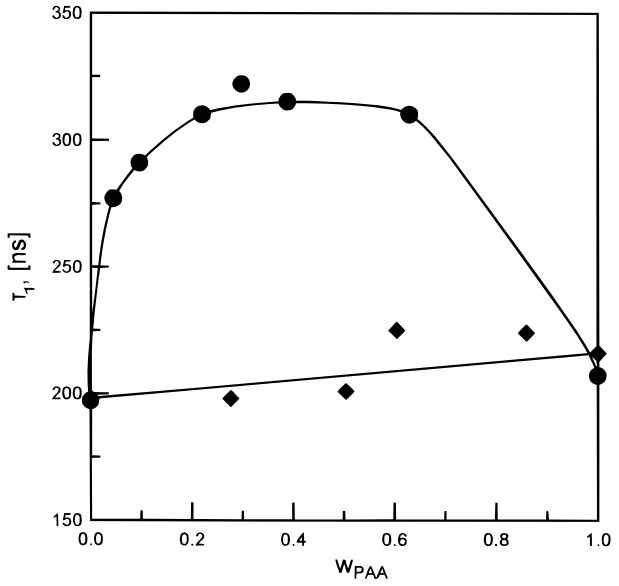

Figure 6. Plot of pyrene fluorescence lifetime, τ_1, versus w_{PAA} for the polymer mixture of PNIPAAM/PAA (●) and PAAM/PAA (◆) in water. c = 0.1%. pH = 3.4.

in the case of the PEG/PAA complex, which demonstrates only a limited hydrophobicity at low pH, no minimum in I_1/I_3 is found at any specific w_{PAA}. The deviation from the I_1/I_3 ratio of the pure components (dashed lines) is similar for both low and high w_{PAA} values.

Fluorescence Lifetime Measurements. The existence or not of a hydrophobic character was also investigated by pyrene fluorescence lifetime measurements. Changes in pyrene fluorescence lifetime were determined in the studied polymer systems, and the results are shown in Figure 6 for PNIPAAM/PAA (●) and PAAM/PAA (◆) and in Figure 7 for PVME/PAA (●) and PEG/PAA (◆ and ■). The pyrene lifetime in two standard systems (water and SDS) was also determined. The decay curve of pyrene fluorescence was always fitted by a biexponential law. In all cases, the value of the first lifetime, τ_1, was between that of pyrene solubilized in water (200 ns) and that of pyrene in SDS micelles (325 ns) and the other, τ_2, was much shorter.

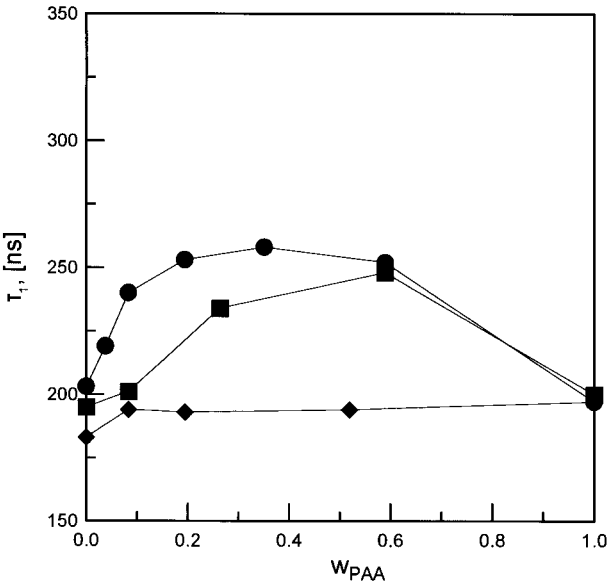

Figure 7. Plot of τ_1 versus w_{PAA} for polymer mixtures in water: PVME/PAA, $c = 0.02\%$, pH = 3.9; (◆) PEG/PAA, $c = 0.02\%$, pH = 3.9; (■) PEG/PAA, $c = 0.02\%$, pH = 3.0.

The shorter component τ_2, which has a small contribution, must be a result of nonspecific interactions between pyrene and its environment. According to Ringsdorf et al.[23] the shorter component of the pyrene lifetime should be a result of quenching by amide groups. The longer lifetime component of the pure polymer solutions, τ_1, was identical to that of pyrene in water (ca. 200 ns). This result was expected, since the polymers are water-soluble and no hydrophobic regions exist at 25 °C. In the case of the polymer mixture solutions the longer component depends on the character of the interpolymer complex formed. For the PAAM/PAA mixture, the pyrene decay time was identical with the one in water, indicating that pyrene is solubilized in a hydrophilic environment, in accordance with the result of the steady-state fluorescence, where no hydrophobic interactions were found to exist in the PAAM/PAA polymer mixture. In all the other polymer mixtures, the pyrene lifetime was longer than the one in water. The longer pyrene lifetime was observed in the case of the PNIPAAM/PAA mixture, and for $w_{PAA} = 0.3$ it was 322 ns, indicative of a strong hydrophobic environment, similar to that provided by surfactant micelles. Hydrophobic interactions are also clearly exhibited in the case of the PVME/PAA mixture, (260 ns). If we compare the results of Figures 1 and 4 and those of Figures 6 and 7, we get the impression that in one case the hydrophobicity of PVME/PAA is favored (Figures 1 and 4), whereas in the case of the τ_1 results (Figures 6 and 7) the PNIPAAM/PAA complex seems to be more hydrophobic. However, no comparison of the results in Figures 6 and 7 can be done in this case, because the corresponding experimental conditions are different. In Figure 6, the polymer concentration is higher and the pH value is lower. It is known that low pH values favor the interpolymer complex formation, and for this reason the hydrophobicity of PNIPAAM/PAA complexes, as seen from the τ_1 results, seems more pronounced than that for the PVME/PAA case.

In the case of the PEG/PAA mixture, the pyrene lifetime was close to that of water (200−225 ns) for the polymer mixture examined at pH = 3.9 (Figure 7 (◆)), which proves that the polymer complex formed has no hydrophobic character. Nevertheless the PEG/PAA mixture of the same concentration examined at pH = 3 (Figure 7 (■)) presents some hydrophobic interactions especially at high w_{PAA} polymer mixture composition.

Discussion

The steady-state fluorescence measurements, in conjunction with time-resolved fluorescence measurements, showed the existence of a strong hydrophobic contribution in the formation of the PNIPAAM/PAA interpolymer complex and the absence of any hydrophobic interaction in the formation of the PAAM/PAA interpolymer complex. These results are in accordance with the results obtained from a potentiometric and viscometric study in a previous work,[19,20] where it has been proposed that the PNIPAAM/PAA hydrogen-bonding interpolymer complex is stabilized by hydrophobic interactions. On the contrary, the PAAM/PAA complex does not exhibit any hydrophobic character and it is exclusively stabilized by hydrogen-bonding interactions. The interpolymer complex formed between PVME and PAA seems to exhibit a strong hydrophobic character while the one formed between PEG and PAA exhibits only a limited hydrophobicity. The above differences in the hydrophobic character of the interpolymer complexes could be explained by the differences in the critical solution temperature behavior of these polymers. It is known that PNIPAAM demonstrates in water a lower critical solution temperature (LCST) behavior, turning cloudy at 34 °C, while PAAM is expected to have an upper critical solution temperature (UCST) behavior[21] with a critical temperature equal to −38 °C. PVME is also a polymer characterized by a LCST behavior,[21] with a critical temperature equal to 32 °C and forming interpolymer complexes in aqueous solution with PAA[22] for which a hydrophobic behavior should be expected. PEG finally is a proton acceptor polymer widely studied in its interactions with PAA,[4−13] demonstrating a LCST behavior[21] but with a critical temperature at 96 °C, far enough from room temperature. Moreover fluorescence measurements have shown that hydrophobic interactions exist only in the case of a polymer with LCST behavior, while the hydrophobicity of the complex formed depends also on the polymer structure. In the case of the PNIPAAM/PAA complex, due to the isopropyl groups of PNIPAAM, its hydrophobic character is very strong, while the PVME/PAA complex is also very hydrophobic especially at low w_{PAA}. The PEG/PAA complex demonstrates only a limited hydrophobicity, and the difference could be explained by the absence of any hydrophobic group except the ethylene group of the polymer chain. It has been found that the hydrophobic character of the PEG/PAA complex is strengthened at low pH, where the hydrogen-bonding interactions are favored. Finally, the PAAM/PAA complex is not at all hydrophobic, and this must be related to the UCST behavior of PAAM as well as to the hydrophilic character of the side group (amide group). Furthermore, the difference in behavior between PEG and PAAM could probably be explained by the higher flexibility of the PEG macromolecular chain, exhibiting a characteristic ratio, $c_\infty = 4.0$,[24] very much lower than that exhibited by PAAM, $c_\infty = 15$.[25]

Acknowledgment. This work has been supported by the HCM CHRX-CT94-0655 program of the European Commission and the ΠΕΝΕΔ program of the Greek General Secretariat for Research and Technology,

Contract No. 1442,3.2. The authors thank Ilias Iliopoulos for useful discussions.

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MA9706485