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Fluorescence Studies of Aqueous Solutions of Poly(*N*-isopropylacrylamide) below and above Their LCST

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ABSTRACT: Pyrene-labeled poly(*N*-isopropylacrylamides) (PNIPAM/Py) have been prepared by reaction of a copolymer of *N*-isopropylacrylamide and *N*-(acryloxy)succinimide with [4-(1-pyrenyl)butyl]-amine. The fluorescence spectra of the labeled polymers in methanol are typical of isolated random-coil polymers. The emission of PNIPAM/Py in aqueous solutions at room temperature is characterized by a strong excimer emission attributed to ground-state pyrene aggregates. At polymer concentrations lower than 1 ppm there is evidence for the formation of single polymer micelles, when the level of labeling is high enough. At higher concentration both inter- and intrapolymeric aggregation takes place. Heating the polymer solutions above their lower critical solution temperature (LCST) results in the disruption of the pyrene aggregates, as evidenced by an increase of the pyrene monomer emission at the expense of the excimer emission. Results are interpreted in terms of interactions between polymer chains and between the polymer and water, which involve hydrogen bonding and hydrophobic interactions. The labeled polymers were also added as polymeric probes to solutions of poly(*N*-isopropylacrylamide). They become incorporated in the polymer-rich phase above the LCST.

Introduction

Polymers prepared from acrylamides exhibit unusual solution properties. The simplest analogue, polyacrylamide, is a water-soluble polymer. Poly(*N*-methylacrylamide) and poly(methacrylamide) are also water soluble, but poly(*N*-*n*-butylacrylamide) and poly(*N*-*tert*-butylacrylamide) are not. Other analogues are soluble in cold water but separate from solution upon heating. Aqueous solutions of poly(*N*-isopropylacrylamide) (PNIPAM) possess this interesting property. They exhibit a lower critical solution temperature (LCST) at 31–32 °C. This thermoreversible phase-separation property of PNIPAM was reported first by Heskins and Guillet.[1] Since then ingenious applications of this behavior of the PNIPAM aqueous solutions have been described. For instance, membranes consisting of PNIPAM or its copolymers with selected *N*-alkylacrylamides possess thermoselective permeability toward a large range of water-soluble permeants.[2] They are such that, as the temperature of the water increases, the flux of permeants through the membrane decreases. Recently Okahata and his co-

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workers have applied the same principle to control thermally the permeability of nylon capsules at or slightly above ambient temperature.[3] PNIPAM was grafted to the external surface of large, ultrathin, porous nylon capsules. The grafted polymer acts as a reversible thermovalve, allowing the permeants to escape from the capsules only at temperatures below the LCST of PNIPAM. PNIPAM-based gels exhibit thermoreversible swelling properties.[4] These have been exploited for example to control enzymatic reactions by a thermal feedback mechanism[5] and to achieve controlled delivery of biomolecules.[6] PNIPAM is ideally suited for this application, since the heat-induced phase transition occurs near physiological temperatures. Other applications of PNIPAM, especially as sun-proofing or shading agents, take advantage of the temperature-induced optical changes incurred by PNIPAM solutions as their temperature crosses the cloud point.

Phenomena occurring at the LCST of aqueous polymer solutions have been observed by a wide variety of experimental techniques. These include calorimetry, viscometry, turbidimetry, and light scattering. Calorimetry provides information on the thermodynamics of phase separation and viscosity about the hydrodynamic consequences of aggregation. Light scattering is an excellent technique for monitoring concentration fluctuations on a spatial scale of ca. 1000 Å. It would be interesting to increase the spatial resolution of the measurements. Such molecular information is often available through experiments based upon neutron and X-ray scattering and fluorescence. This paper describes the application of fluorescence techniques to the study of PNIPAM solution properties through the use of pyrene-labeled polymers.

Two approaches are possible in applying fluorescence techniques to the study of polymer solutions.[7] In probe experiments one simply adds a fluorescent dye to the solution. This type of experiment is useful if the dye binds to the polymer. Alternatively, one labels the polymer by attaching the dye covalently. Labeled-polymer experiments are often more informative because they report on phenomena from the polymer's point of view. For polymer solutions in organic solvents it is usually safe to assume that the dye label does not perturb the properties of the polymer. For water-soluble polymers, the dye may act as a hydrophobic substituent. In this situation is is important to test the consequences of varying the extent of labeling on the behavior of the polymer in solution.

Another problem for water-soluble polymers is that of hydrolysis of the polymer–dye linkage. Ester groups are notorious in that respect. Here the problem is overcome by the use of an amide linkage to attach pyrene groups to the PNIPAM backbone. The customary approach to preparing such a polymer would be to copolymerize *N*-isopropylacrylamide with a pyrene acrylamide, such as [4-(1-pyrenyl)butyl]acrylamide. This method is not very useful here for preparing high molecular weight polymers, since the dye comonomer appears to act as a chain-transfer agent during the polymerization.[8]

As a consequence, an alternative method was developed based upon an idea introduced by Ferruti[9] and Ringsdorf,[10] and applied to PNIPAM by Hoffman,[11] in which one prepares a polyacrylamide containing reactive groups. These groups become sites where pyrene substituents can be attached. Thus a copolymer of *N*-isopropylacrylamide and *N*-(acryloxy)succinimide was prepared and subsequently reacted with [4-(1-pyrenyl)butyl]amine. From the same copolymer, pyrene-labeled PNIPAM samples (PNIPAM/Py) with two different levels of pyrene incorporation were prepared. Fluorescence measurements indicate that, in alcohol solvents, the pyrene labels are predominantly unassociated but form excimers by a dynamic process after photoexcitation. In water a different behavior is observed. The pyrenes stack, so that excimer emission derives predominantly from preformed ground-state dimers or higher aggregates. The existence of such aggregates is extremely sensitive to changes in solution temperature, as shown for example by monitoring the pyrene monomer and excimer emission intensities as a function of solution temperature in the vicinity of the LCST. The temperature-dependent experiments were performed not only with dilute solutions of labeled polymers but also in solutions of PNIPAM to which labeled polymers were added in small amount. In this case PNIPAM/Py played the part of a "polymeric fluorescent probe" of PNIPAM solution properties.

Experimental Techniques

Materials. All commerical chemicals were purchased from Aldrich Chemical Co., unless otherwise mentioned. Pyrene (99%) was purified by repeated recrystallizations from absolute ethanol and subsequent sublimation. *N*-Isopropylacrylamide was obtained from Eastman Kodak Chemicals. *N*-(Acryloxy)succinimide (mp 69–69.5 °C, lit.[10] mp 68–69 °C) was prepared by reaction of acryloyl chloride with *N*-hydroxysuccinimide.[12] [4-(1-Pyrenyl)butyl]amine (3) was prepared from 4-(1-pyrenyl)butanoic acid by the following sequence of reactions: (1) conversion to the acid chloride by treatment with oxalyl chloride;[13] (2) reaction of 4-(1-pyrenyl)butyroyl chloride with aqueous ammonia to yield 4-(1-pyrenyl)butyramide (mp 181–182 °C, lit.[14] 180.5–181 °C)); (3) reduction with $LiAlH_4$ in dry THF. It was isolated as the hydrochloride recrystallized from methanol (mp 262–264 °C, lit.[14] 252–256 °C). For the syntheses, reagent-grade solvents were used without further purification, except for tetrahydrofuran (THF), which was dried by distillation from sodium–benzophenone. Water was deionized with a Millipore Milli-Q water purification system. Spectroscopic-grade solvents were used for all spectroscopic measurements.

Synthesis. Poly(*N*-isopropylacrylamide) (PNIPAM). A solution of *N*-isopropylacrylamide (5.0 g, 0.044 mol) in *tert*-butyl alcohol (25 mL) was heated to 70 °C under nitrogen. AIBN (30 mg) in *tert*-butyl alcohol (1 mL) was added at once. The solution was stirred for 15 h at 70 °C. It was cooled to room temperature. The solvent was evaporated. The residual material was dissolved in THF (5 mL) and reprecipitated into hexane. The material (3.8 g) was purified further by precipitation of a solution in methanol into dry diethyl ether.

***N*-Isopropylacrylamide/*N*-(Acryloxy)succinimide Copolymer (PNIPAM/NASI).** *N*-Isopropylacrylamide (5.0 g, 0.044 mol) and *N*-(acryloxy)succinimide (0.150 g, 0.88 mmol) were dissolved in *tert*-butyl alcohol (30 mL) at 70 °C under nitrogen. AIBN (30 mg) in *tert*-butyl alcohol (1 mL) was added to the solution. The reaction mixture was stirred at 70 °C for 20 h. It was cooled to room temperature. The solvent was evaporated in vacuo. The polymer (2.2 g) was isolated by successive precipitations from a THF solution into hexane and from a methanol solution into dry diethyl ether: IR (KBr) 3435, 2970, 2924, 1814, 1784, 1734, 1652, 698 cm^{-1}.

Pyrene-Labeled PNIPAM (PNIPAM/Py/20). Triethylamine (20 mg, 0.16 mmol) was added to a solution of [4-(1-pyrenyl)butyl]amine hydrochloride (50 mg, 0.16 mmol) and PNIPAM/NASI (0.5 g) in THF (5 mL). The solution was stirred at room temperature in the dark for 21 h. The polymer was isolated by precipitation in hexane (500 mL). It was purified further by two precipitations from a methanol solution (15 mL) into dry diethyl ether (400 mL). It was dried in vacuo for 24 h (380 mg): UV (MeOH) λ_{max} 264, 275, 311, 325, 341 nm; IR (KBr) 3440, 2920, 1734, 1653, 843, 698 cm^{-1}; pyrene concentration 4.4×10^{-4} mol g^{-1} or 1 pyrene per 20 NIPAM units.

Pyrene-Labeled PNIPAM (PNIPAM/Py/200). Triethylamine (10 mg, 0.08 mmol) was added to a solution of [4-(1-

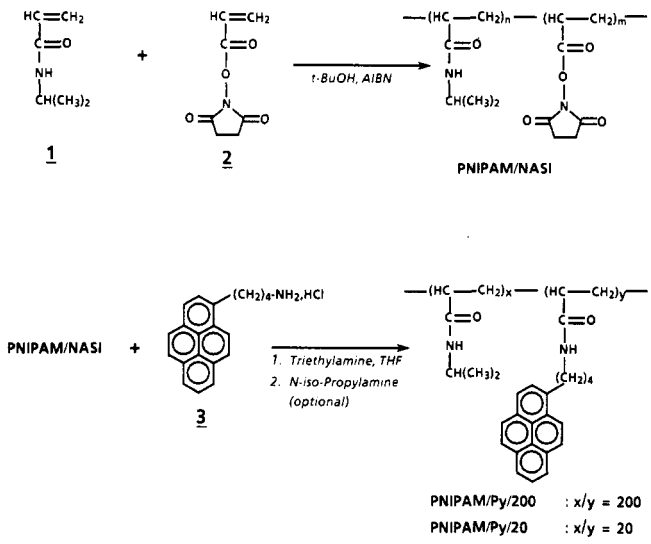

Figure 1. Synthetic scheme for the preparation of pyrene-labeled poly(*N*-isopropylacrylamide).

pyrenyl)butyl]amine hydrochloride (5 mg, 0.016 mmol) and PNIPAM/NASI (0.5 g) in THF (5 mL). The solution was stirred at room temperature for 17 h. Then *N*-isopropylamine (10 mg, 0.17 mmol) in THF (0.5 mL) was added. The reaction mixture was stirred for an additional 2-h period. A workup procedure identical with that described for PNIPAM/Py/20 gave the labeled polymer PNIPAM/Py/200 (410 mg); pyrene content 4.4×10^{-5} mol g^{-1} or 1 pyrene for 200 NIPAM units.

Instrumentation. Melting points are reported uncorrected. IR spectra were recorded with a Nicolet 20DX Fourier transform IR spectrometer. UV spectra were recorded with a Hewlett-Packard 8480A diode array spectrometer. Temperature-controlled experiments were done with a Hewlett-Packard 89100A temperature-control accessory consisting of a digitally controlled thermoelectrically heating and cooling cell holder with sample stirring capability and programmed temperature ramping. The temperature of the sample fluid was measured with a Hewlett-Packard 89102A Teflon-coated temperature-sensing probe immersed in the sample fluid. Heating and cooling rates were chosen between 0.2 and 1 °C min^{-1}. Gel permeation chromatography (GPC) was performed with a Varian instrument equipped with a refractive index detector and a UV detector. Two TSK-G300 columns from Toyo-Soda were used. Deionized water was used as the eluent. Solution viscosities were measured at 30 °C with a Viscotek Model 100 differential viscometer. The polymers were dissolved in THF at concentrations of ca. 50 ppm. Steady-state fluorescence spectra were recorded on a SPEX Fluorolog 212 spectrometer equipped with a DM3000F data system. The temperature of the water-jacketted cell holder was controlled with a Neslab circulating bath. The temperature of the sample fluid was measured with a thermocouple immersed in the sample fluid.

Fluorescence Measurements. The emission spectra were not corrected, except for quantum yield measurements. The excitation spectra were measured in the ratio mode. Solutions in methanol or THF were degassed by vigorous bubbling with solvent-saturated argon for 1 min. Solutions in water were not degassed, since it was determined that for aqueous solutions the fluorescence intensities of pyrene or PNIPAM/Py were not affected significantly by degassing. For measurements of the I_1/I_3 ratio of the pyrene emission the excitation slit widths were set at 3.6 nm (excitation) and 0.9 nm (emission). The excitation wavelength was 330 nm. For measurements of spectra of pyrene-labeled samples the excitation wavelength was set at 330 nm unless otherwise stated. Slit widths ranging from 0.9 to 4.0 nm were chosen, depending on the chromophore concentration. The excimer to monomer ratios were calculated by taking the ratio of the emission intensity at 480 nm to the half-sum of the emission intensities at 376 and 396 nm. It was verified that peak areas and peak heights were proportional. For measurements at different temperatures samples were allowed to equilibrate for 10 min at a given temperature. The heating rate corresponded to approximately 0.2 °C min^{-1}.

Table I
Physical Properties of the Polymers

polymer	$[\eta]$,[a] $cm^3\ g^{-1}$	M_v[c]	pyrene content, mol g^{-1}	cloud point, °C (concn, g L^{-1})
PNIPAM	108 ± 1[b]	1.7×10^6		32.5
	94 ± 1[b]	1.4×10^6		(0.05–10)
PNIPAM/ NASI	104 ± 1	1.6×10^6		32.5 (0.3)
PNIPAM/ Py/20	86 ± 1	850 000	4.4×10^{-4} 1 Py/20 NIPAM	
PNIPAM/ Py/200	103 ± 1	1.1×10^6	4.2×10^{-5} 1 Py/200 NIPAM	33 (0.05)

[a] From THF solutions. [b] Polymers from two different preparations. [c] See text.

Samples for Spectroscopic Analysis. Pyrene-labeled polymer solutions were prepared at room temperature. They were allowed to stand for 24 h before they were diluted to a known volume. Solutions for UV spectroscopy had pyrene concentrations ranging from 0.7×10^{-6} to 5×10^{-6} mol L^{-1}. The concentrations of the samples for temperature-controlled experiments were 0.2–10 ppm for PNIPAM/Py/20 and 2.5–120 ppm for PNIPAM/Py/200. Solutions contains PNIPAM and pyrene-labeled PNIPAM were prepared by mixing solutions of the labeled and unlabeled polymers to cover a concentration range of 0–5 g L^{-1} of unlabeled polymer at a constant concentration of labeled material (5 ppm for PNIPAM/Py/20).

Micropolarity Tests. An aqueous solution of PNIPAM (0.5 g L^{-1}) containing ca. 6×10^{-7} M pyrene was heated from 4 to 40 °C at a rate of ca. 0.2 °C min^{-1}. Emission spectra from pyrene were measured at regular temperature intervals.

Cloud Point Measurements. Cloud points were determined by spectrophotometric detection of the changes in turbidity of solutions heated at a constant rate in a magnetically stirred UV cell, as described previously.[15]

Results

Synthesis and Characterization of Pyrene-Labeled Poly(*N*-isopropylacrylamides). The labeled polymers were prepared by reaction of [4-(1-pyrenyl)butyl]amine (**3**) with a copolymer of *N*-isopropylacrylamide (**1**, NIPAM) and *N*-(acryloxy)succinimide (**2**), PNIPAM/NASI) (Figure 1). This copolymer and PNIPAM itself were prepared by free-radical polymerizations in *tert*-butyl alcohol. This solvent was selected on the basis of its inertness toward *N*-(acryloxy)succinimide and its inefficiency as a chain-transfer agent. When the initial ratio of the imine **3** to the *N*-(acryloxy)succinimide groups of the copolymer was varied, it was possible to prepare PNIPAM/Py samples with different amounts of pyrene incorporation: after all the initial amine had been consumed, the unreacted *N*-(acryloxy)succinimide groups of the copolymer were converted to *N*-isopropylacrylamide groups by treatment with an excess of *N*-isopropylamine. In this manner two PNIPAM/Py samples were obtained. In each sample the amount of pyrene incorporation was calculated from UV absorption data of polymer solutions in methanol, using **3** (λ_{max} 342 nm, ϵ = 32 800)[14] as a model compound. The sample with the highest level of pyrene incorporation (PNIPAM/Py/20) possesses 4.4×10^{-4} mol of pyrene per gram of polymer, or on average 1 pyrene per 20 monomer units. In the second sample, PNIPAM/Py/200, the amount of pyrene linked to the polymer is lower by a factor of 10.

Viscosity average molecular weights of the labeled samples and of PNIPAM were estimated from the intrinsic viscosities of polymer solutions in THF using the viscometric relationship:[16] $[\eta] = 9.59 \times 10^{-3} M_v^{0.65}$. These val-

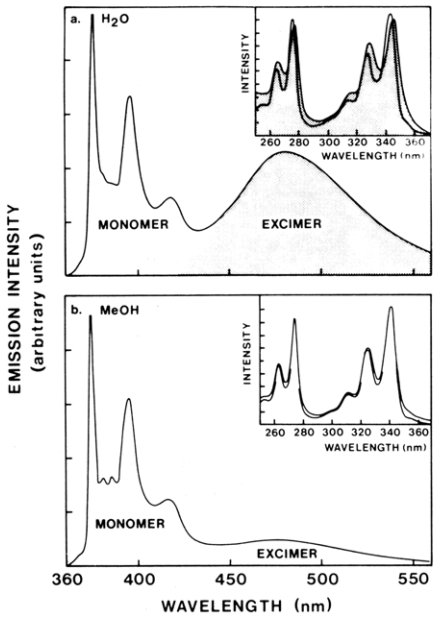

Figure 2. Fluorescence spectra of pyrene-labeled poly(*N*-isopropylacrylamide) (PNIPAM/Py/200, 50 ppm) (a) in water and (b) in methanol. Excitation wavelength 330 nm. Insets are excitation spectra of the solutions monitored for the monomer (emission wavelength 396 nm) and for the excimer (emission wavelength 480 nm, shaded). The excitation spectra were normalized at 345 nm.

ues as well as other physical properties of the polymers are recorded in Table I. The purity of the pyrene-labeled polymers was an important aspect of the experiments. Through the use of tandem UV and RI detectors for GPC analysis, it was established that (1) the pyrene groups were covalently linked to the polymer, (2) the samples contained less than 0.1% low molecular weight UV-absorbing impurities, and (3) the chemical transformation did not alter the molecular weight or the molecular weight distribution of the PNIPAM/NASI copolymer.

Fluorescence Spectra. Emission and excitation spectra of the polymers PNIPAM/Py/20 and PNIPAM/Py/200 were measured in methanol and in water at 25 °C (Figure 2). In methanolic solutions both polymers show an emission due to locally excited pyrene chromophores (intensity I_M, "monomer" emission) with the [0,0] band located at 376 nm and a broad emission centered at 480 nm due to pyrene excimer emission (intensity I_E) (Figure 2b). Identical excitation spectra were obtained for emissions monitored at 396 and 480 nm, and the maxima correspond to those in the UV absorption spectrum. For both polymers, values of the ratio I_E/I_M in methanol remained constant over a large concentration range, implying that in this solvent excimers form intramolecularly.

The spectra of the polymers dissolved in water present a stronger excimer emission relative to monomer emission than the methanolic solutions (Figure 2a), and the excitation spectra for the monomer and the excimer are clearly different. The general features of the spectra are similar, but the former is blue-shifted by about 3 nm. Comparison with the UV spectra of aqueous solutions of the polymers reveals that it is the excitation spectrum for the excimer that corresponds to the UV absorption. The UV spectra of the polymers in water have maxima at 343 nm, compared to 341 nm in methanol. They exhibit a strong hypochromic effect accompanied by peak broadening. The extinction coefficient for the pyrene groups attached to the polymer decreases from its (assumed) value

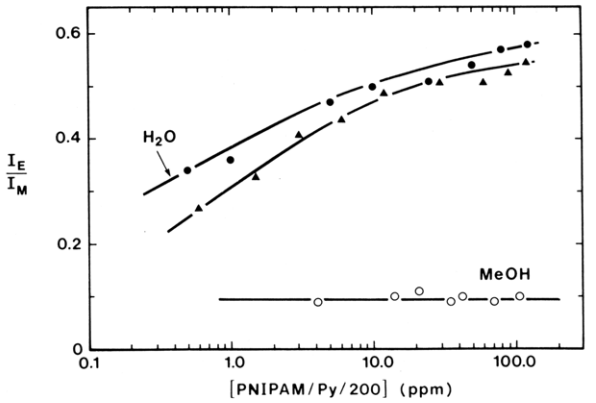

Figure 3. Semilogarithmic plots of the ratio of excimer to monomer emission intensities (I_E/I_M) as a function of polymer concentration for PNIPAM/Py/200 in water (full circle), in methanol (open circle), and in solutions containing a constant amount (120 ppm) of PNIPAM (full triangle). Temperature 25 °C.

of 32 800 in methanol to 22 000 in water. All these observations lead to the conclusion that in aqueous solutions of PNIPAM/Py the excimers originate from pairs of aggregates of pyrenes that exist prior to excitation. This situation has been observed previously in aqueous solutions of other polymers such as pyrene-labeled hydroxypropyl cellulose[17] and pyrene-labeled poly(ethylene oxide).[18]

In an attempt to determine if the pyrene ground-state aggregates occur between chromophores attached to the same polymer or between chromophores on different chains, the effect of polymer concentration on the ratio of I_E/I_M was studied in three different ways. The values of I_E/I_M were determined for solutions of labeled polymers over a range of concentration as wide as possible. This range was limited on the dilute end to 0.2 ppm for PNIPAM/Py/20 and 2.5 ppm for PNIPAM/Py/200 by the inability to measure fluorescence intensity reliably and, at the high concentration limit, by the insolubility of the polymers in water at concentrations greater than 15 ppm for PNIPAM/Py/20 and 200 ppm for PNIPAM/Py/200. For PNIPAM/Py/200 the ratio I_E/I_M increased slightly with increasing polymer concentration, the largest change taking place for solutions of concentration lower than 10 ppm (Figure 3). From this trend it can be inferred that intra- and interpolymeric pyrene aggregates coexist in solution. Unexpectedly, a different trend was observed with solutions of PNIPAM/Py/20: in this case the ratio I_E/I_M decreased abruptly with increasing polymer concentration and, above 5 ppm, it remained unaffected by further increases in polymer concentration. This intringuing result will be discussed further in light of experimental evidence presented in the following sections.

The second set of concentration studies deals with competitions experiments, in which PNIPAM and PNIPAM/Py were mixed, maintaining the total polymer concentration constant but varying the concentration of labeled polymer. In experiments with PNIPAM/Py/20 the total concentration was 10 ppm and the concentration of PNIPAM/Py/20 varied from 0.2 to 10 ppm. With PNIPAM/Py/200 the total polymer concentration was 120 ppm; the concentration of PNIPAM/Py/200 ranged from 2 to 120 ppm. The addition of unlabeled polymer did not affect the trends exhibited by solutions of labeled polymer alone, although in the case of PNIPAM/Py/200 the changes were slightly amplified in the presence of PNIPAM (Figure 3).

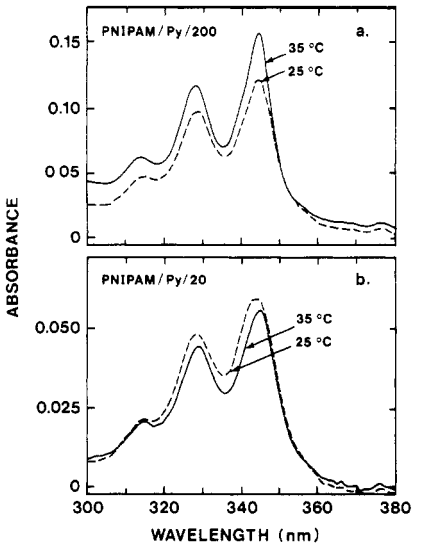

Figure 4. UV absorption spectra of aqueous solutions of pyrene-labeled PNIPAM samples at 25 °C (dashed line) and at 35 °C (full line): (a) PNIPAM/Py/200 (110 ppm); (b) PNIPAM/Py/20 (8 ppm).

In a third set of measurements the labeled polymers were mixed at a fixed concentration (5 ppm for PNIPAM/Py/20 and 60 ppm for PNIPAM/Py/200) with unlabeled PNIPAM from 0.1 to 5 g L^{-1}. No dramatic effects were noticed. The I_E/I_M of PNIPAM/Py/20 solutions fluctuated slightly around a value of 0.39 ± 0.04, while the I_E/I_M of PNIPAM/Py/200 solutions decreased uniformly with increasing PNIPAM concentration from 0.52 to 0.42. Disappointingly, little information was extracted from rather elaborate concentration studies. Clearly, in these systems the ratio I_E/I_M is not a good tool to distinguish between inter- and intrapolymeric pyrene aggregation. However, it proved to be an extremely useful parameter in the experiments described in the next section, which explore molecular aspects of the phenomena taking place at the LCST of PNIPAM solutions.

Temperature Studies. Cloud Point Determinations. Cloud points of PNIPAM and PNIPAM/Py were determined by a spectrophotometric method described in detail elsewhere.[15] The LCST of PNIPAM was 32.5 ± 0.5 °C for polymer concentrations from 0.05 to 10 g L^{-1}, in agreement with reported values.[1] The LCST of PNIPAM/Py/200 was slightly higher (33.0 ± 0.5 °C). Measurement of the LCST of PNIPAM/Py/20 was not possible because of the limited solubility of this polymer in water. Solutions became only faintly turbid above the (presumed) LCST temperature of 33 °C and the onset of turbidity was not sharp.

Pyrene Absorption Spectra. The absorption spectrum of the pyrene label was measured during the heating and cooling of PNIPAM/Py solutions. In the case of PNIPAM/Py/200 a hyperchromic effect took place upon heating (Figure 4a). The intensity of the absorption bands at 345 and 328 nm are enhanced above the LCST by an amount that cannot be accounted for by the small increase in turbidity. There is no measurable shift in the wavelength of maximum absorbance; however, the absorption bands undergo noticeable sharpening. This phenomenon was monitored by changes in the peak to valley ratio, defined here as the ratio of the absorbance at 345 nm (A^{345}) to that at 336 nm (A^{336}). The ratio increased from 1.92 to 25 °C to 2.23 at 33 °C. The curves of A^{345}/A^{336} and of A^{345} as a function of temperature are sigmoidal in shape (Figure 5). The midpoint

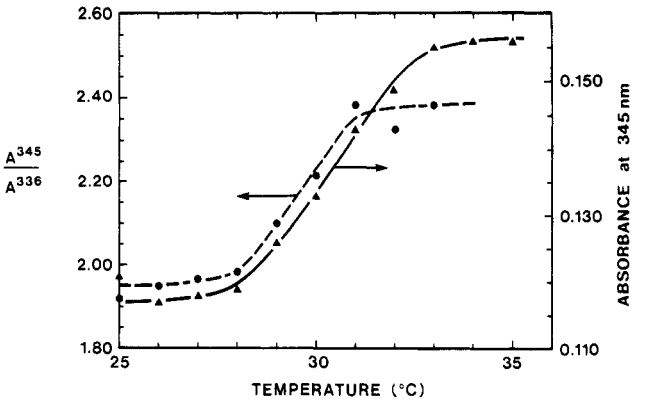

Figure 5. Plots of the absorbance at 345 nm (full line) and of the ratio of the absorbance at 345 nm to the absorbance at 336 nm (A^{345}/A^{336}) of the pyrene chromophore as a function of temperature for an aqueous solution of PNIPAM/Py/200 (110 ppm).

of the transition in each case is ca. 30.5 °C, a temperature slightly lower than the solution LCST. These results indicate that near the LCST the chromophore aggregates are disrupted and above the LCST the chromophores are mostly isolated from each other. In the case of PNIPAM/Py/20 solutions heating affected the pyrene absorption spectrum in a different manner; there was no increase in the absorbance intensity. However, the wavelength of maximum absorbance shifted from 341 nm at 25 °C to 343 nm at 35 °C (Figure 4b). The striking differences between the two polymers are corroborated by the fluorescence experiments described next.

Fluorescence of Pyrene-Labeled Polymers in Water. When a solution of PNIPAM/Py/200 in water was heated from 20 to 40 °C, several events occurred. The ratio I_E/I_M increased slightly to reach a maximum value of 0.46 at 29 °C. Then it decreased sharply to reach a limiting value of 0.01 at 35 °C. The midpoint of the transition was 31 °C. These changes were reversible: upon slow cooling of the solution, the ratio I_E/I_M increased and returned to its initial value. Changes in the excitation spectra were also triggered by the increase in temperature. Below the LCST excitation spectra monitored for the excimer and the monomer were different, the excitation spectrum for the monomer being blue-shifted by about 3 nm compared to that monitored for the excimer. Above the LCST this difference disappeared. Both spectra had maxima at 343.5 nm, and the excitation spectra corresponded to the UV absorption spectrum of the solution.

Deeper insights into the phenomena can be gained by examining the individual quantum efficiencies of monomer and excimer emission. Total quantum yields of emission (Φ_T) were measured for solutions of PNIPAM/Py/200 (100 ppm) as a function of temperature. Intensities were apportioned to monomer and excimer emissions in proportion to their relative areas after conversion of the corrected spectra to frequency units. The total quantum yield was 0.51 at 20 °C. It remained constant from 20 to 28 °C, and then it underwent a pronounced increase between 29 and 33 °C to reach a value of 0.74 (Figure 6). The increase of Φ_T corresponds to a large increase of the monomer emission quantum yield (Φ_M) and a decrease of the excimer emission quantum yield (Φ_E). Between 20 and 29 °C the constant quantum yield derives from a slow increase in excimer quantum yield, offset by a corresponding decrease in the monomer intensity.

Identical experiments were performed with the more highly labeled polymer PNIPAM/Py/20 at concentrations of 0.5, 2, 5, and 10 ppm. In all cases the ratio $I_E/$

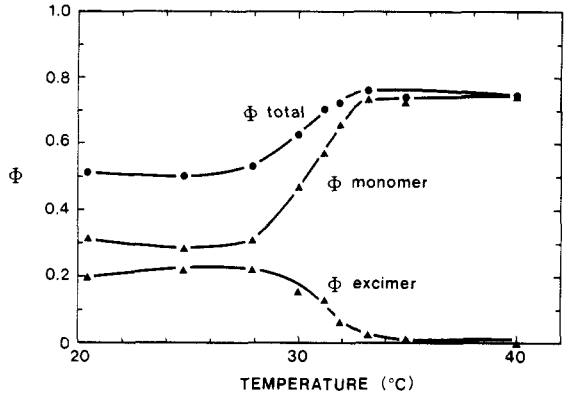

Figure 6. Total fluorescence quantum yields as a function of temperature for solutions of PNIPAM/Py/200 (110 ppm) in water. Also plotted are the fractional contributions of monomer and excimer emissions to that total (see text).

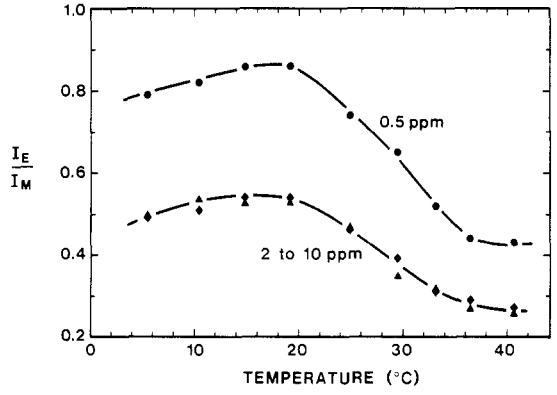

Figure 7. Plot of the excimer to monomer emission intensities ratio (I_E/I_M) as a function of temperature for aqueous solutions of PNIPAM/Py/20 (0.5, 2, 5, and 10 ppm) measured during heating.

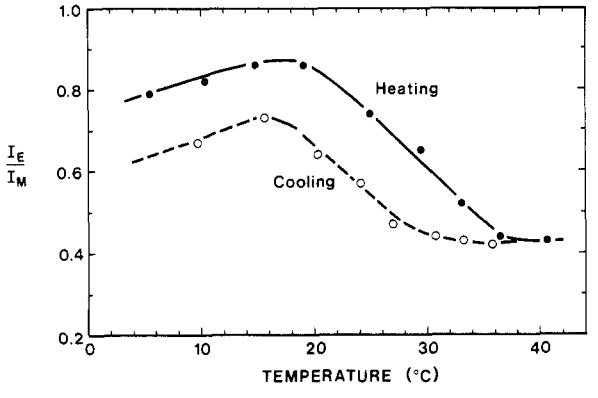

Figure 8. Plot of the excimer to monomer emission intensities ratio (I_E/I_M) as a function of temperature for an aqueous solution of PNIPAM/Py/20 (0.5 ppm) measured during heating (full circle) and cooling (open circle).

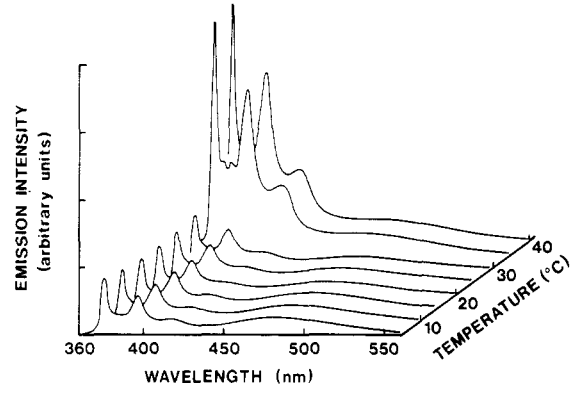

Figure 9. Fluorescence spectra of PNIPAM/Py/20 (5 ppm) in water containing PNIPAM (5 g L^{-1}) measured at several temperatures between 4 and 40 °C. Excitation wavelength 330 nm.

I_M first increased slightly when the solutions were heated from 4 to 20 °C, and then it decreased gradually to reach a constant value at temperatures higher than 35 °C. The magnitude of the changes in I_E/I_M was the largest for the most dilute sample (Figure 7). It is interesting to note that, unlike solutions of PNIPAM/Py/200, solutions of PNIPAM/Py/20 even above their cloud point exhibit important excimer emission. The excitation spectrum monitored for the excimer underwent a small shift, from 345.5 nm for solutions at 20 °C to 344.5 nm for solutions at 35 °C. But even at that temperature, it did not coincide with the excitation spectrum of the monomer, which itself did not undergo any change with temperature. In the case of the most dilute solution (0.5 ppm) the changes in I_E/I_M induced by cooling the solution from 40 to 20 °C differed slightly from those occurring during heating (Figure 8). At all temperatures the ratio measured during the cooling ramp was lower than that measured during the heating ramp. Also, the midpoint of the transition occurred at a lower temperature. Nevertheless, after this sample had been kept at 25 °C for several hours, its emission spectrum became indistinguishable from that of a sample not subjected to the heating/cooling cycle. These observations indicate that the relaxation time characterizing these solutions is on the order of tens of minutes.

Changes in total quantum yield were also measured as a function of temperature for a 10 ppm solution of PNIPAM/Py/20. Φ_T underwent an increase from 0.34 at 20 °C to 0.49 at 40 °C, reflecting a small decrease in excimer emission quantum yield and a small increase in monomer emission intensity, the largest changes occurring between 25 and 35 °C. Interestingly, while Φ_T for PNIPAM/Py/20 solutions is smaller than that of the polymer with the lower level of pyrene substitution, the relative increase in Φ_T caused by heating the solutions above their cloud point is about the same in both instances (ca. 45%).

Fluorescence of Labeled Polymers in PNIPAM Solutions. This section presents experiments where the labeled polymers themselves act as probes of the solution properties of PNIPAM. A small amount of labeled polymer constituting 1% by weight or less of the total polymer, was added to solutions of PNIPAM ranging in concentration from 0.5 to 5 g L^{-1}. When solutions of such concentration are heated above their cloud point, they become highly turbid. As a consequence, it is not possible to measure absorption spectra of pyrene above the LCST or to calculate absolute quantum yields. Relative fluorescence measurements, on the other hand, are not hindered by the turbidity of the sample.[19] For solutions of PNIPAM containing PNIPAM/Py/200 the temperature-induced changes in I_E/I_M followed the trend described previously. Excitation spectra monitored for the excimer and monomer did coincide above the LCST.

In the case of the more highly labeled polymer, an unexpected large increase of the total fluorescence intensity took place when the solution temperature reached the LCST of PNIPAM, as shown in Figure 9, where the emission spectra of PNIPAM/Py/20 (5 ppm) in an aqueous solution of PNIPAM (5 g L^{-1}) are shown at several tem-

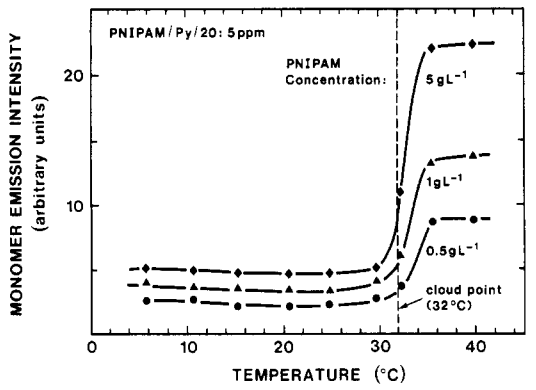

Figure 10. Plot of the pyrene monomer intensity as a function of temperature for aqueous solutions of PNIPAM/Py/20 (5 ppm) and PNIPAM (0.5, 1.0, and 5.0 g L^{-1}).

peratures between 4 and 40 °C. Below the LCST, only minor changes in the relative amounts of monomer to excimer emissions were detected, and they did not affect the total fluorescence intensity. At the LCST, both monomer and excimer emission intensities increased, with monomer emission displaying the larger change. The amplitude of this enhancement of the monomer emission increased as the amount of labeled polymer in the PNIPAM solution decreased from 1.0 to 0.1% by weight of polymer (Figure 10). Excitation spectra monitored for the excimer and monomer were identical at 40 °C. The phenomena were reversible: upon cooling of the solutions their total fluorescence intensity decreased and the ratio I_E/I_M recovered its initial value.

In a last set of experiments pyrene, a small molecule probe, was added to an aqueous solution of PNIPAM. Its fluorescence was monitored as the temperature of the solution was raised above the LCST. This type of measurement yields information on the micropolarity of the probe environment.[20] Specifically, one measures the ratio I_1/I_3 of the intensity of the [0,0] band (374 nm) to that of the [0,2] band (384 nm) of the pyrene emission. This ratio decreases as the micropolarity experienced by the probe decreases. For pyrene in a PNIPAM solution the ratio I_1/I_3 decreased from a value of 1.74 at 20 °C to 1.38 at 40 °C. In the pyrene solvent polarity scale,[21] these values correspond to solvents such as *N,N*-dimethylacetamide (I_1/I_3 = 1.79) at 20 °C and tetrahydrofuran (I_1/I_3 = 1.35) or ethyl acetate (I_1/I_3 = 1.37) at 40 °C. The sharp decrease in micropolarity sensed by pyrene occurs at the LCST.

Discussion

Aqueous Solutions of PNIPAM and PNIPAM/Py at Ambient Temperature. The following description of PNIPAM in solution has emerged from viscosity, osmometry, and light-scattering data. In organic solvents the polymer behaves as a flexible coil, as indicated by the values of the Mark–Houwink exponents for solutions of PNIPAM in tetrahydrofuran or methanol, which are modestly good solvents for the polymer (Table II). The picture of PNIPAM in aqueous solutions is a more controversial issue. From intrinsic viscosities determined for an unfractionated PNIPAM sample, Chiantore and co-workers obtained viscometric relationships for PNIPAM in water (eq 3 and 5, Table II).[22] They interpreted the unusually high Mark–Houwink exponents as an indication that the polymer chains behave as isolated rigid rods. They attributed the stiffening of the chain to hydrogen bonding between water molecules and the amide groups of the polymer. A different Mark–Houwink relationship (eq 5, Table II) was reported recently by Fujishige from intrinsic viscosities of fractionated PNIPAM samples.[16] The Mark–Houwink exponent (0.50) indicates that water is approximately a Θ solvent. Nevertheless, intrinsic viscosity values in water are larger than those in tetrahydrofuran, which is a good solvent for PNIPAM. This implies that the unperturbed dimensions of the polymer are larger in water than in tetrahydrofuran. Fujishige suggested that this difference in size may be ascribed to an elongation of the polymer chain resulting from hydrogen bonding of water molecules to the polymer backbone. Solution properties of PNIPAM near the LCST have been reported by Heskins and Guillet.[1] They put forward a model in which PNIPAM behaves as flexible coils undergoing extensive interpolymeric association. This model is based on an analysis of sedimentation and viscosity measurements at ambient temperature of an unfractionated PNIPAM sample and on the temperature dependence of these properties. Whether interpolymeric association is prevalent also at ambient temperature has not been established unambiguously, but it may be implied from circumstantial evidence, such as solution aging effects on viscosity and osmometry measurements.[23] That water is a special solvent for PNIPAM can also be appreciated qualitatively from the large difference between the Hildebrand solubility parameters of water (δ 23.4)[24] and of PNIPAM (δ 13–14,[25a] 11[25b]). Normally a polymer is soluble only in liquids having solubility parameters within the range $\delta \pm 3$.

Table II
Mark–Houwink Relationships for PNIPAM Solutions

solvent	temp, °C	equation	ref
tetrahydrofuran	27	$[\eta] = 9.59 \times 10^{-3} M_n^{0.65}$ (1)	16
methanol	25	$[\eta] = 2.99 \times 10^{-2} M_w^{0.64}$ (2)	22
water	20	$[\eta] = 4.58 \times 10^{-4} M_w^{0.93}$ (3)	22
	20	$[\eta] = 14.5 \times 10^{-2} M_n^{0.50}$ (4)	16
	25	$[\eta] = 2.26 \times 10^{-4} M_w^{0.97}$ (5)	22

From a thermodynamic point of view, the formation of hydrogen bonds between water molecules and the amide groups of the polymer contributes favorably to the free energy of mixing ($\Delta H_M < 0$) but unfavorably to the entropy of mixing (ΔS_M). Hydrogen bonding between the water and the polymer triggers the formation of a layer of highly organized water molecules around the polymer, which decreases the entropy of the system. Association between polymeric chains via hydrophobic interactions between the alkyl substituents can occur as well. These interactions become significant as the temperature of the solution is increased and the bound water is released. Then the relative values of the thermodynamic functions change: the entropic term becomes dominant, resulting in a positive free energy of mixing (ΔG_M). A two-phase system is favored.

Modified polymers obtained by attaching hydrophobic substituents to water-soluble polymers often differ significantly in their solution properties from the original polymers. The effects are most pronounced in the case of polyelectrolytes, such as *n*-alkyl derivatives of poly(4-vinylpyridine)[26] and hydrolyzed alternating copolymers of maleic anhydride and long-chain alkenes[27] or *n*-alkyl vinyl ethers,[28] but the effect may also be important in the case of neutral polymers, such as hydroxyalkylated cellulose ethers.[29] The hydrophobic substituents promote either interpolymeric association, or single-polymer micelle formation, if the level of substitution is high enough and if the polymer concentration is low enough. Fluorescent labels are hydrophobic substituents and their presence affects the solution properties of

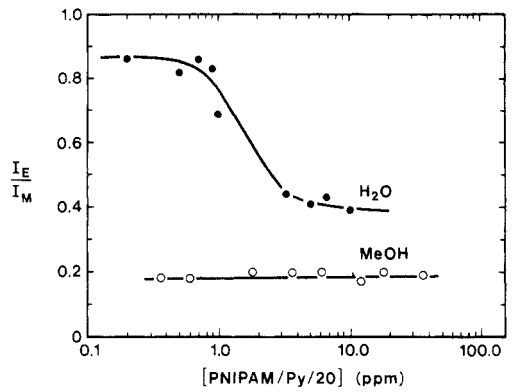

Figure 11. Semilogarithmic plot of the ratio of excimer to monomer intensities (I_E/I_M) as a function of PNIPAM/Py/20 concentration in water (full circle) and in methanol (open circle). Temperature 25 °C.

water-soluble polymers. The results presented here illustrate the extent of the perturbations brought about by linking pyrene groups to PNIPAM side chains, as reflected by the spectroscopy of the labeled polymers in water. It is worth emphasizing that there are no surprising aspects to the behavior of the labeled polymers in methanolic solutions. There is no evidence for association of pyrenes. Upon irradiation, a fraction of the excited pyrenes form intramolecular excimers by diffusion and encounter with other pyrenes. The extent of pyrene excimer formation is higher for the more highly labeled polymer, reflecting the higher local concentration of pyrene groups.

By contrast there is ample evidence that in water at ambient temperature the pyrene groups form ground-state dimers or higher aggregates. Evidence includes (1) the strong hypochromic effect observed in the UV absorption spectrum, (2) the difference between the excitation spectra monitored for the excimer and monomer emission, and (3) the absence of a rising component on the nanosecond time scale in the time profile of the excimer emission.[30] Also a large fraction of the pyrene emission is quenched through the formation of ground-state aggregates, as indicated by the low values of the total quantum yield, especially in the case of PNIPAM/Py/20. From a thermodynamic point of view, the formation of pyrene aggregates can be related to a gain in free energy of mixing through hydrophobic interactions between nonpolar groups.[31] Since these are unable to form hydrogen bonds with water, they are best accommodated in solution as dimers, thus minimizing the number of broken or distorted hydrogen bonds between water molecules. The nonpolar dimers are surrounded by a cage of highly organized water, resulting in a negative entropic contribution. From measurements of I_E/I_M, it is not possible to determine definitively whether pyrene aggregates form between pyrenes attached to the same chain or to different polymers. This issue was discussed in the context of the concentration studies performed with PNIPAM/Py/200. Other fluorescence techniques, especially those based on nonradiative energy transfer between dyes attached to different chains have been used to observe interpolymeric aggregations of neutral aqueous polymers at very low concentrations.[32] Work is in progress to apply such experiments to PNIPAM solutions.

The case of the more highly labeled polymer is puzzling (Figure 11). The sensitivity of the ratio I_E/I_M to polymer concentration has a trend opposite to that reported for most polymers in solution. For extremely dilute solutions of PNIPAM/Py/20 there is a marked increase of the excimer emission relative to monomer emission. One interpretation of this result is that in this concentration range (<5 ppm) interpolymeric pyrene association is no longer possible. The polymer exists only as isolated coils. In order to accommodate this unfavorable situation, more pyrene groups aggregate, resulting in a quenching of the emission originating from isolated pyrenes. Therefore the polymer concentration corresponding to the transition in Figure 11 (1 ppm) may be the highest concentration for which the polymer chains remain unassociated. Below this concentration PNIPAM/Py/20 exists as single molecule micelles in which the pyrene groups form a hydrophobic core, surrounded by the less hydrophobic polymeric backbone, which accommodates the surrounding water molecules through hydrogen bonding. Such polymeric structures have been described in other systems, such as the hydrolyzed copolymers of maleic anhydride and *n*-alkenes,[27] pyrene-labeled polyacrylic acid,[13] and copolymers of sodium styrene sulfonate and 2-vinylnaphthalene.[33] In the latter system the micellelike structures, named "photozymes", were used as hydrophobic molecular reactors.

LCST Behavior of PNIPAM/Py and PNIPAM. The unfavorable entropic term associated with the formation of pyrene ground-state dimers and higher aggregates becomes increasingly dominant, as the temperature of the polymer solution is raised. Thus it can be expected that there will be a temperature at which the pyrene aggregation will be precluded or at least much less prominent than at ambient temperature. This effect is expressed by the spectral changes observed when aqueous solutions of PNIPAM/Py/200 are heated above their LCST. The heat-induced disruption of the pyrene dimers is evidenced by (1) the enhancement of the pyrene absorption at the solution LCST (Figure 6) and (2) the increase in monomer emission intensity at the expense of excimer emission (Figure 7). Two additional facts are noteworthy. First, the ratio I_E/I_M is smaller for aqueous solutions of PNIPAM/Py/200 above their LCST than that for solutions of the same polymer in methanol. Since in both cases the excimer is formed by a dynamic process, the lower value in the former environment may reflect the higher viscosity experienced by the pyrene groups in the polymer-rich phase. Second, the spectroscopic changes (I_E/I_M and excitation spectra) are not affected significantly by the presence of large amounts of PNIPAM. In both cases isolated pyrenes are solubilized in the polymer-rich phase.

The behavior of the more highly labeled polymer is complex but rich in information, especially when it is compared to that of PNIPAM/Py/200. Qualitatively in this case too, the fluorescence spectroscopy (see Figure 7) indicates that increasing the temperature results in a disruption of the pyrene dimers. But this disruption is incomplete. Even above the LCST, excimer emission has an important contribution to the total fluorescence. It still originates mostly from preformed pyrene aggregates, as indicated by the excitation spectra and the UV spectra of PNIPAM/Py/20 solutions (Figure 4b); and as implied by the low values of Φ_T, it may be that the polymer-rich phase that separates above the LCST of PNIPAM/Py/20 has a composition richer in water than that generated from solutions of the other polymers. In such a phase the existence of pyrene dimers or aggregates may be favored, especially if they occur between pyrenes attached to the same chain. The results are also consistent with the presence of single-polymer micelles in the polymer-rich phase.

Spectroscopic measurements truly relevant to the phase separation of PNIPAM itself are those based on probes added in small amounts to a solution of PNIPAM. The important questions here concern the composition, polarity, and structure of the polymer-rich phase that separated above the LCST. The composition of this phase was estimated by Heskins and Guillet to consist of ca. 50% by weight of polymer, a value deduced from the phase diagram of the water/PNIPAM system.[1] In this work experiments with pyrene show that the micropolarity of the polymer-rich phase coincides with that of solvents such as tetrahydrofuran or ethyl acetate.

One of the difficulties in the interpretation of experiments with low molecular weight probes is that the location of the probe is not known with certainty. Even though the probe may be preferentially solubilized in the polymer environment, it is still free to diffuse through the entire sample. Thus the measured spectra are an average emission from probes located throughout the sample. This problem can be overcome with polymeric probes. In such experiments one monitors the spectroscopy of a labeled polymer added in small amount to unlabeled material of identical structure, except for the fluorescent tag. The labeled polymer tracks the behavior of the major, unlabeled, component. A classic case illustrating the power of this technique is that of the thermally induced phase-separation process of polystyrene/poly(vinyl methyl ether) blends.[34] Halary and Monnerie have demonstrated that an anthracene-labeled polystyrene, their polymeric probe, becomes incorporated into a polystyrene-rich phase in the phase-separated blend. In the aqueous solutions investigated here the labeled polymers also become incorporated into the PNIPAM-rich phase above the LCST. This is apparent from the changes in the fluorescence spectra of the labeled polymers above the LCST of PNIPAM. When either PNIPAM/Py/20 or PNIPAM/Py/200 are employed as probes, the following effects are observed: (1) increase in total emission intensity; (2) increase in monomer emission at the expense of excimer emission; (3) dynamic mechanism of excimer emission. The effects are much more pronounced in the case of the more highly labeled polymer. Also the magnitudes of the first two effects depend on the relative amounts of labeled and unlabeled polymers (Figure 10). The midpoints of the transitions in the curves of monomer intensity as a function of temperature correspond in all cases to the LCST of PNIPAM. It is striking to note that the spectra of the labeled polymers in the PNIPAM-rich phase above the LCST show all the features of the spectra of these polymer in good solvents; even the fine structure of the pyrene monomer emission is recovered (compare Figure 9, spectrum run at 35 °C, and Figure 2b). These results are consistent with a phase-separation mechanism that occurs via association of flexible polymer chains into larger aggregates stabilized by interpolymeric hydrogen bonds and hydrophobic interactions. They illustrate how much information can be derived from the use of trace amounts of labeled polymers, an idea that is gaining prominence in the study of polymeric systems by fluorescence.[35]

Summary

Investigations of the spectroscopy of pyrene-labeled PNIPAM in water and in PNIPAM solutions, below and above the LCST, have led to the following conclusions: (1) In water, at ambient temperature, solutions of PNIPAM/Py are characterized by the occurrence of ground-state pyrene dimers or higher aggregates. These take place between pyrene attached to the same chain and to different chains. In the low concentration limit (<1 ppm) and for highly labeled polymers evidence exists for the formation of single-polymer micelles. (2) Heating solutions of labeled PNIPAM above their LCST results in a disruption of the pyrene aggregates. This dissociation is complete in the case of PNIPAM/Py/200 but only partial in solutions of the more highly labeled polymer. (3) When the labeled polymers are added in trace amount to PNIPAM solutions, at ambient temperature, there is no indication of interactions between labeled and unlabeled polymers, but, above the LCST, the labeled polymers are incorporated into the PNIPAM-rich phase. The picture of the PNIPAM-rich phase that emerges is that of large aggregates consisting of associated flexible polymer chains.

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Longest Relaxation Times of Linear Polymers in Concentration Regions between the Critical Concentrations for Zero-Shear Viscosity and for Steady-State Compliance

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ABSTRACT: The longest relaxation times τ_m of linear polymers in concentration regions between the critical concentrations for the zero-shear viscosity η° and for the steady-state compliance J_e, that is, in a semidilute region for η° and in a dilute (not entangled) region for J_e, were studied by measuring the stress relaxation after cessation of steady shear flow. It is concluded that the weight-average relaxation time τ_w ($= \eta^\circ J_e$) is well represented by τ_m and the magnitudes of the contribution of the terminal relaxation processes to η° are the same in semidilute solutions for η°, whether the entanglements are effective to J_e or not.

Introduction

The longest relaxation time τ_m, which is a measure of the time required to free a whole molecule from the restraints imposed, is one of the central problems in the study of viscoelastic properties of polymer chains in entangled regions.[1,2] Hence, τ_m or, more in detail, the terminal relaxation spectra[3-5] as well as other viscoelastic parameters such as the zero-shear viscosity η° and the steady-state compliance J_e[6-8] have been extensively studied. In concentrated solutions and melts where polymer chains are highly entangled with each other, the terminal relaxation processes can be well separated from the faster processes owing to the high entanglement densities. In the highly entangled regions, the viscoelastic properties at the terminal relaxation process, so far published, can be summarized as follows[1,2]

$$\eta^\circ \propto M^{3.4}C^b \tag{1a}$$

$$J_e \propto M^0C^{-2} \tag{1b}$$

$$\tau_m \propto M^{3.4}C^{b-2} \tag{1c}$$

where M and C are the molecular weight and the concentration of the polymer, respectively, and b is the exponent specifying the concentration dependence of zero-shear viscosity. As shown in these equations the weight-average relaxation time τ_w defined as the product of η° and J_e can be well represented by τ_m.[1,3] Moreover, these relaxation times are found to be related to the characteristic time specifying the onset of non-Newtonian viscosity.[1,6]

The entanglements decrease with decreasing concentration, and the effects of entanglements on viscoelastic properties disappear at certain critical concentrations, which are different in different properties.[1,2,8] As shown in the molecular weight-concentration diagrams for η° and J_e of linear polymers in good solvents in previous papers,[7,8] the critical concentrations from dilute to semidilute regions for η° and from dilute (not entangled) to entangled regions for J_e, denoted by C_c^η and C_c^J, respectively, are given by

$$(C_c^\eta)^{1.27}M = 1.5_5 \times 10^4 \tag{2a}$$

$$(C_c^J)M = 1.4 \times 10^5 \tag{2b}$$

These relationships can be also read as the corresponding critical molecular weight-concentration relationships. If we compare the critical concentrations or the critical molecular weights for η° with those of J_e, the latter is 4-6 times higher than the former.[7,8]

In the concentration region between the two critical concentrations, i.e., in semidilute solutions for η° and in dilute solutions for J_e, τ_w is proportional to $M^{4.4}$ instead of to $M^{3.4}$, since η° is proportional to $M^{3.4}$ while J_e is still proportional to M in this region. Moreover, the concentration dependence of τ_w is also different above and below C_c^J, according to the change in the concentration dependence of J_e (C^{-2} to C^{-1}). We already reported that the characteristic time for the onset of non-Newtonian viscosity is proportional to τ_w also in this weakly entangled region, where the entanglements are effective to η° but not to J_e.[9] However, it has not been studied whether τ_w is represented by τ_m or not in this region. The purpose of this work is, therefore, to study the relationships between τ_w and τ_m in the region.

Experimental Section

Samples used here were linear polystyrenes with narrow molecular weight distributions purchased from Tosoh Corp. Molec-

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