Hydrophobically-Modified Poly(*N*-isopropylacrylamides) in Water: A Look by Fluorescence Techniques at the Heat-Induced Phase Transition

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ABSTRACT: The effect of sample preparation, aging, and thermal history on the intermixing of hydrophobically-modified poly(*N*-isopropylacrylamides) in water has been examined for mixtures of random copolymers of *N*-isopropylacrylamide (NIPAM) and *N*-[4-(1-pyrenyl)butyl]-*N*-*n*-octadecylacrylamide (PNIPAM–C_{18}Py/200), and *N*-[2-(1-naphthyl)ethyl]-*N*-*n*-octadecylacrylamide (PNIPAM–C_{18}Na/200). Solutions were prepared by codissolution of the two copolymers, by mixing of aqueous stock solutions of each copolymer, and from a stock solution of the copolymers in tetrahydrofuran. They were subjected to one, or several, heating/cooling cycle in a temperature range (20–35 °C) which includes the lower critical solution temperature (LCST) of the copolymers (31 °C). From fluorescence experiments using nonradiative energy transfer between excited naphthalene and pyrene and from changes in the ratio of pyrene excimer to monomer intensity, it has been established that (1) the exchange of copolymers between micellar structures is slow in cold water, (2) efficient intermixing of the copolymers takes place above the LCST, and (3) statistical intermixing of the copolymers is preserved upon cooling the solutions to 25 °C, regardless of the copolymer distribution prior to heating.

Introduction

Though common among neutral water-soluble polymers, the heat-induced phase separation of aqueous polymer solutions remains intriguing. The macroscopic phenomenon is the same for all polymers: a clear solution suddenly becomes "milky" when it is heated to a specific temperature. On a molecular level however quite distinct mechanisms can be operative for each class of polymers. The process is directly controlled by molecular level interactions between the polymer chains and surrounding water molecules, as dictated by the chemical structure of the monomer units. Poly(*N*-isopropylacrylamide) (PNIPAM) exhibits thermoreversible phase separation at a temperature of 31 °C, its cloud point or lower critical solution temperature (LCST).[1] Through the use of techniques which probe molecular scale distances, such as static and dynamic light scattering[2-4] and fluorescence spectroscopy,[5] it has been ascertained that the phase separation of PNIPAM occurs in two steps: (1) the collapse of individual polymer chains from an extended coil into a globule and (2) the aggregation of the globules which triggers macroscopic phase separation. The polymers do not form interchain aggregates in cold water solutions. Aqueous solutions of poly(*N*-isopropylacrylamides) that carry along their backbone a few long alkyl chains also exhibit this phase separation phenomenon at temperatures in the vicinity of 31 °C. However, experimental evidence from several sources points to the fact that, on a molecular scale, the phenomena involved are quite distinct from those in action in the case of the homopolymer. The objectives of the present study are to gain a deeper understanding of the phase separation mechanisms at play in solutions of hydrophobically-modified NIPAM copolymers.

The work was prompted by a previous investigation on the application of fluorescence techniques to probe the morphology of aqueous solutions of amphiphilic copolymers of NIPAM.[6] In these studies we established the existence of polymeric micelles,[7] with the following salient features: (1) they consist of a highly viscous hydrophobic core formed by the alkyl substituents and a loose corona made up by the polymer main chain; (2) they exist in extremely dilute solutions, and they always involve several polymer chains; (3) they seem to be stable indefinitely in cold water, but they are disrupted severely when the solutions are heated above their LCST. The original core is destroyed and the hydrophobic groups are accommodated as isolated entities within the separated polymer-rich phase. These conclusions were drawn from a combination of results based on experiments with extrinsic probes, pyrene,[8] perylene,[8] and dipyme,[9] with pyrene-labeled copolymers, and with mixtures of pyrene- and naphthalene-labeled copolymers.[6] They corroborate conclusions reached by Schild and D. Tirrell from an independent study of similar amphiphilic NIPAM copolymers.[10]

Particularly powerful are experiments with mixtures of differently labeled NIPAM copolymers based on the photophysical process of direct nonradiative energy transfer (NRET) between two chromophores.[11,12] The process originates in dipole–dipole interactions between an energy donor in its excited state and an energy acceptor in its ground state. The probability of energy transfer between the two chromophores depends sensitively on their separation distance and to a lesser extent on their relative orientation.[12] Therefore, in mixed solutions of polymers carrying either donor chromophores or acceptor chromophores the extent of energy transfer between the two labels can be related to the extent of interpolymeric association. The application of NRET to probe interpolymeric interactions was pioneered by Nagata and Morawetz.[13] It has proven to be powerful in probing detailed features of polymeric mixtures in solutions[14,15] or in the bulk.[16,17] By monitoring the extent of NRET in mixed labeled polymer solutions, we established for the

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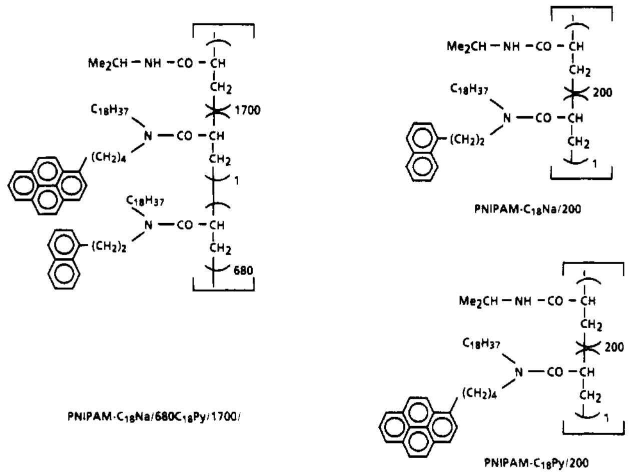

Figure 1. Chemical structures of the polymers used in this study.

first time the interpolymeric nature of the aggregates in cold aqueous solutions. Important issues on the architecture of the aggregates still remained unanswered. They concern, for example, the changes in aggregate morphology during the heat-induced phase separation process and the stability of the aggregate morphology during aging of solutions below and above their cloud point. Other questions arise too on the influence on the aggregate morphology and stability of parameters such as the length of the alkyl substituent, the degree of substitution of the copolymers, and the distribution of the alkyl groups along the polymer backbone.

Here we focus our attention on the heat-induced phase separation process. The objectives of the study are (1) to monitor the effect of heating solutions through their cloud point on the photophysical properties of the labeled polymers in mixed solutions, (2) to test the reversibility of the heat-induced morphological changes, and (3) to probe the stability with time of the aggregates in cold solutions and in the phase-separated samples.

Experimental Section

Materials. Spectroscopic grade solvents were purchased from Caledon. Water was deionized with a Millipore Milli-Q water purification system (specific conductance 0.056 μmhos/cm at 25 °C). The polymers were prepared by AIBN-initiated free-radical polymerization in dioxane (PNIPAM–C_{18}Py/200,[8] PNIPAM–C_{18}Na/200,[6] and PNIPAM–C_{18}Na/680–C_{18}Py/1700[6]). Their structures are shown in Figure 1. Important physical properties are summarized in Table I.

Instrumentation. UV spectra were recorded with a Varian UV–vis–near-IR Cary-5 spectrophotometer. Solution viscosities of THF solutions (3 g L^{-1}) were determined with a 53810/I Ubbelohde microviscometer linked to an AVS 300/P22 Schott automatic viscometer. GPC measurements were performed with a Waters WISP 700 system equipped with a Waters RI 410 refractive index detector. Four ultrastyragel columns (10 000, 5000, 500, and 100 Å) were used. The eluent (THF) was used at a flow rate of 0.8 mL min^{-1}. Quasi-elastic light scattering (QELS) measurements were performed at a fixed 90° scattering angle, using a Brookhaven Instrument Corp. particle sizer, model BI-90, equipped with a He/Ne laser. Cloud points were determined by spectrophotometric detection of changes in turbidity of solutions heated at a constant rate (0.2 °C min^{-1}) in a magnetically stirred UV cell, as described previously.[19] Steady-state fluorescence spectra were recorded on a SPEX Fluorolog 212 spectrometer equipped with a DM3000F data system. The temperature of the water-jacketed cell holder was controlled with a Neslab circulating bath connected to a Neslab MTP-6 programmer. Excitation spectra were measured in the ratio mode. Emission spectra were recorded with an excitation wavelength of 330 nm (pyrene) and 290 nm (naphthalene). They were not corrected.

Determination of the Spectroscopic Parameters. The pyrene excimer to monomer ratios $[I_E/I_M]^{Py}$ were calculated by taking the ratio of the intensity (peak height) at 480 nm to the half-sum of the intensities at 379 and 399 nm. The extent of pyrene emission due to NRET from naphthalene was determined by integration of spectra from 378 to 500 nm, after correcting for emission due to direct pyrene excitation. Corrections were performed by subtracting from each spectrum the emission spectrum of PNIPAM–C_{18}Py/200 (λ_{exc} = 290 nm) of identical pyrene concentration.

Samples for Spectroscopic Analysis. Several sample preparation protocols were employed. In all cases polymer powders were allowed to dissolve in the solvent (water or THF) for 1 h at room temperature, and then the solutions were kept at 5 °C for 24 h to ensure complete dissolution of the polymers. Before spectroscopic measurements the solutions were kept at room temperature for 2 h. They were not degassed. In all samples the polymer concentration was such that the absorbance at the excitation wavelength (290 or 330 nm) was below 0.05. The compositions of the solutions are listed in Table II.

(a) Stock Solutions. Aqueous solutions of the individual polymers (5 or 1 g L^{-1}) were prepared following the general procedure. Aliquots of the stock solutions were diluted to the desired concentration.

(b) Mixed Solutions. From Aqueous Stock Solutions. A stock solution of PNIPAM–C_{18}Py/200 (0.15 mL, 1.0 g L^{-1}) and a stock solution of PNIPAM–C_{18}Na/200 (0.15 mL, 5.0 g L^{-1}) were added to deionized water (2.0 mL).

By Codissolution in Water. Copolymer powders obtained by freeze-drying of aqueous solutions [PNIPAM–C_{18}Py/200 (2.8 mg) and PNIPAM–C_{18}Na/200 (14.8 mg)] were dissolved together in deionized water (100 mL). The solution was kept at room temperature for 2 h, and then at 5 °C for 24 h. It was allowed to stand at room temperature for 2 h prior to fluorescence measurements.

From a Stock Solution in THF. PNIPAM–C_{18}Py/200 (1.7 mg) and PNIPAM–C_{18}Na/200 (10.4 mg) were dissolved in THF (2.0 mL). This stock solution (1 mL) was added to deionized water (50 mL). The resulting solution was kept at 5 °C for 24 h. It was allowed to stand at room temperature for 2 h prior to fluorescence measurements.

Heating/Cooling Cycles. The temperature of a sample in the spectrometer sample compartment was measured with an immersed thermocouple. During a heating or cooling scan, the temperature of the samples was increased (or decreased) by increments of 1 or 2 °C from 20 to 35 °C. The samples were allowed to equilibrate for 10 min at a given temperature before recording their spectra. Thus, overall the heating/cooling rate corresponded to approximately 0.2 °C min^{-1}.

Results

Structure and Composition of the Copolymers. Three copolymers were used in this study (Figure 1). They are PNIPAM–C_{18}Py/200, a random copolymer of *N*-isopropylacrylamide (NIPAM) and *N*-[4-(1-pyrenyl)butyl]-*N*-*n*-octadecylacrylamide; PNIPAM–C_{18}Na/200, a random copolymer of *N*-isopropylacrylamide and *N*-[2-(1-naphthyl)ethyl]-*N*-*n*-octadecylacrylamide; and a doubly labeled copolymer (PNIPAM–C_{18}Na/680–C_{18}Py/1700), a random copolymer of *N*-isopropylacrylamide, *N*-[4-(1-pyrenyl)butyl]-*N*-*n*-octadecylacrylamide, and *N*-[2-(1-naphthyl)ethyl]-*N*-*n*-octadecylacrylamide. The molar ratio of chromophores to NIPAM units is small in all cases, for example, 1:200 for PNIPAM–C_{18}Py/200 (Table I). Their synthesis and characterization have been described previously, together with a detailed analysis of the photophysical properties of their solutions in cold water and in methanol.[6]

Spectroscopy of the Copolymers: A Brief Review. Upon excitation at 330 nm PNIPAM–C_{18}Py/200 in water exhibits an emission due to locally isolated excited pyrene

Table I
Molecular and Physical Properties of the Polymers

polymer	composition (unit mol) (NIPAM:C_{18}Na:C_{18}Py)	[Na] (mol g^{-1})	[Py] (mol g^{-1})	M_v[a]	LCST (°C)
PNIPAM–C_{18}Na/200	230[b] (205)[c]:1:–	4.0×10^{-5}	–	279 000	30.4
PNIPAM–C_{18}Py/200	206 (181):–:1		4.4×10^{-5}	390 000	30.6
PNIPAM–C_{18}Na/680–C_{18}Py/1700	1704:2.5:1 530:1 (C_{18})[b]	1.3×10^{-5} [Na]/[Py] = 2.5	0.51×10^{-5}	263 000	30.7

[a] From viscosity measurements in THF, using $[\eta] = 9.59 \times 10^{-3} M_v^{0.65}$; see ref 21. [b] From NMR measurements.[6] [c] From UV measurements.[6]

Table II
Composition of the Solutions Used in the Energy Transfer Experiments

	polymer concentration (g L^{-1})			chromophore concentration (mol L^{-1})	
preparation method	PNIPAM–C_{18}Na/200	PNIPAM–C_{18}Py/200	total	[Na]	[Py]
from aqueous stock solutions	0.326	0.065	0.391	7.11×10^{-6}	1.42×10^{-6}
	0.081	0.016	0.097	1.78×10^{-6}	0.35×10^{-6}
by codissolution in water	0.148	0.028	0.176	3.23×10^{-6}	0.61×10^{-6}
from a THF stock solution	0.104	0.017	0.121	2.27×10^{-6}	0.37×10^{-6}

chromophores (intensity I_M, pyrene "monomer" emission) with the (0, 0) band located at 378 nm, together with a broad featureless emission centered at 480 nm. This emission (intensity I_E) originates from pyrene excimers. Identical excitation spectra are obtained for emissions monitored at 378 and 480 nm, and their maxima correspond to the UV absorption spectrum. Therefore, both monomer and excimer emissions orginate from excited isolated pyrenes. The dynamic nature of the excimer is confirmed by the time-dependent fluorescence profile of the excimer. It shows both a growing-in component (τ = 20 ns) and a decaying component ($<\tau>$ = 110 ns). To allow two chromophores to interact during the lifetime of the excited pyrenes, either individual polymer chains must adopt a conformation that keeps the pyrene groups in close proximity (unimolecular polymeric micelles) or several polymer chains must aggregate to form multimolecular micellar structures. That the latter situation takes place predominantly was established in a previous study of the photophysical properties of naphthyl- and pyrenyl-labeled copolymers in mixed solution.[6] These are reviewed next.

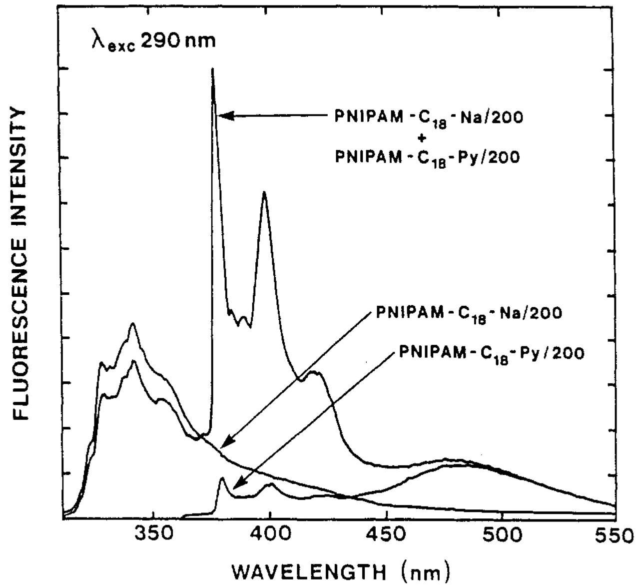

Figure 2. Fluorescence spectra of solutions in water of PNIPAM–C_{18}Na/200, PNIPAM–C_{18}Py/200, and a mixture of the two polymers (total polymer concentration 395 ppm; see Table II for the relative polymer concentration in the mixture, λ_{exc} = 290 nm), reproduced with authorization from ref 6.

The pyrene–naphthalene pair of chromophores is known to interact as energy donor (naphthalene) and energy acceptor (pyrene) by nonradiative energy transfer with a characteristic distance, R_0, of 29 Å. With λ_{exc} = 290 nm, a wavelength at which most of the light is absorbed by naphthalene, one can detect both the direct emission from excited naphthalene and the emission from pyrene excited through NRET from Na*. Thus, under circumstances where the pyrene and naphthalene groups are in close enough proximity to satisfy the NRET requirements, excitation at 290 nm will result in a complex emission consisting of the emission from Na* (310–400 nm) and the emission from Py* excited by transfer of energy from Na*. This is the case in solutions containing PNIPAM–C_{18}-Na/200 and PNIPAM-C_{18}Py/200 (total polymer concentration >10 mg L^{-1}). The effect is exemplified in Figure 2 where the spectra obtained by excitation at 290 nm of solutions of the pure polymers and of a mixture of the two polymers are presented. By comparing the emission of the mixed solution to the spectra of the solutions of each polymer, one observes a significant quenching of the naphthalene emission and a dramatic enhancement of the pyrene monomer emission, even though the concentration of each chromophore is the same for corresponding solutions. Emission of pyrene upon excitation at 330 nm, a wavelength to which naphthalene is transparent, should not be perturbed by the presence of naphthalene from a photophysical point of view. However, in the mixed solution the pyrene emission is affected: the excimer emission is much weaker compared to a solution of PNIPAM–C_{18}Py/200 in the absence of PNIPAM–C_{18}Na/200. This decrease was ascribed to a local dilution of the pyrene groups in the hydrophobic core of the polymeric micelles as a consequence of the addition of naphthyl-substituted polymers, thus further confirming the interpolymeric nature of the micelles.

The emission spectrum of the doubly labeled copolymer PNIPAM–C_{18}Na/680–C_{18}Py/1700 dissolved in cold water (100 mg L^{-1}) exhibits the features characteristic of efficient energy transfer between Na* and Py: a lower intensity of the naphthalene emission, compared to that of a solution of PNIPAM–C_{18}Na/200 of identical naphthalene concentration, and a much stronger pyrene emission, compared to the corresponding PNIPAM–C_{18}Py/200 solution. The energy transfer takes place between chromophores from different polymer chains as well as between chromophores attached to the same polymer.

Temperature Effects. Mixed Polymer Solutions. A dilute solution (97 mg L^{-1}; see Table II) of a mixture of the two differently labeled polymers, PNIPAM–C_{18}Na/200 and PNIPAM–C_{18}Py/200, was prepared by diluting in water aliquots of stock solutions of each polymer. It was heated from 20 to 40 °C in the spectrometer sample

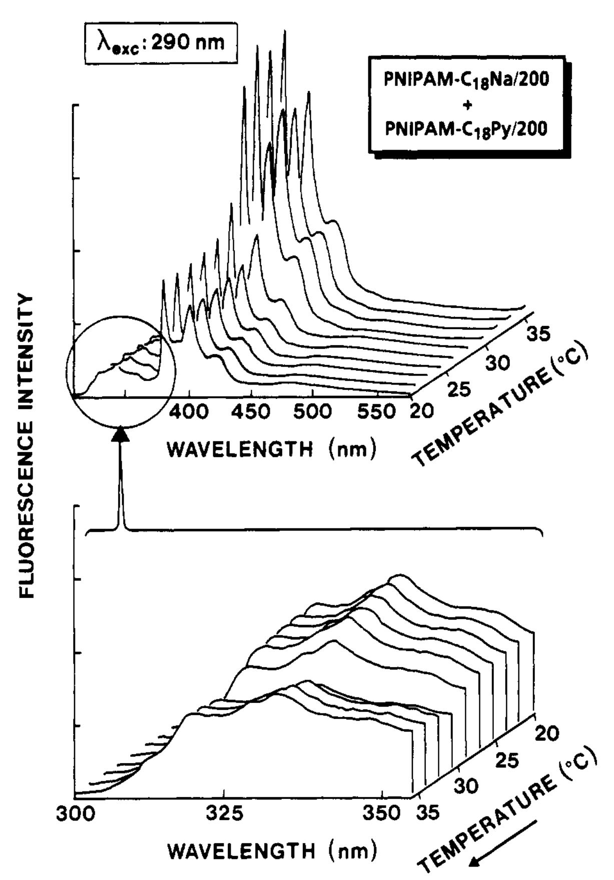

Figure 3. Fluorescence spectra of a solution in water of PNIPAM–C_{18}Na/200 and PNIPAM–C_{18}Py/200 (prepared from aqueous stock solutions of each copolymer) at several temperatures between 20 and 35 °C; λ_{exc} = 290 nm.

compartment. Fluorescence spectra were monitored as a function of temperature. A sudden increase in the Na* to Py energy transfer efficiency was observed as the solution temperature reached the macroscopic cloud point (31 °C). The effect is illustrated in Figure 3 where the emissions of naphthalene and of pyrene (λ_{exc} = 290 nm) at several temperatures during the temperature scan are presented. A decrease in the naphthalene emission intensity took place, concomitant with an abrupt increase in the intensity of the pyrene monomer emission. Comparison of spectra from solutions at 20 and 32 °C indicates that the total pyrene emission is enhanced by a factor of ca. 2.5 in solutions above their LCST. Fluorescence spectra resulting from excitation at 330 nm were recorded also, to monitor the heat-induced changes in the emission of directly excited pyrene chromophores during the temperature scan: (1) the pyrene monomer emission intensity increased at the expense of the excimer, and (2) the total emission intensity increased (Figure 4). Qualitatively similar changes in pyrene fluorescence were observed also while heating solutions of PNIPAM–C_{18}Py/200 in the absence of PNIPAM–C_{18} Na/200.[8]

Next, the solution was cooled to 20 °C at a rate approximately identical to the heating rate. As the temperature of the solution passed the LCST, the spectra underwent the opposite changes from those observed in the heating scan: the efficiency of NRET (λ_{exc} = 290 nm) decreased abruptly, and the direct pyrene emission exhibited a decrease in total intensity as well as an increase in pyrene excimer emission at the expense of the monomer emission. To our surprise, however, spectra of the solution subjected to the heating/cooling treatment differed from those of the initial solution with respect to two key features: (1) the efficiency of the Na* to Py energy transfer (λ_{exc} = 290 nm) was *larger* in the cooled sample, as indicated by a smaller I_{Na}/I_{Py} value (0.15 vs 0.28, Figure 5), and (2) the amount of pyrene excimer emission relative to pyrene monomer emission (λ_{exc} = 330 nm) of the sample was much *smaller* after the temperature scan, as indicated by a smaller I_E/I_M value (0.18 vs 0.54). These results were unexpected since in solutions containing only PNIPAM–C_{18}Py/200 the ratio I_E/I_M at a given temperature is not affected by the sample thermal history.[8] In this context it is worth recalling that the spectral properties of the mixed solutions are stable over periods of several days at a given temperature, such as 25 °C before and after heat treatment, or 35 °C. QELS measurements as well confirm that no change in the mean aggregate sizes occurred over long period of time at 25 °C.

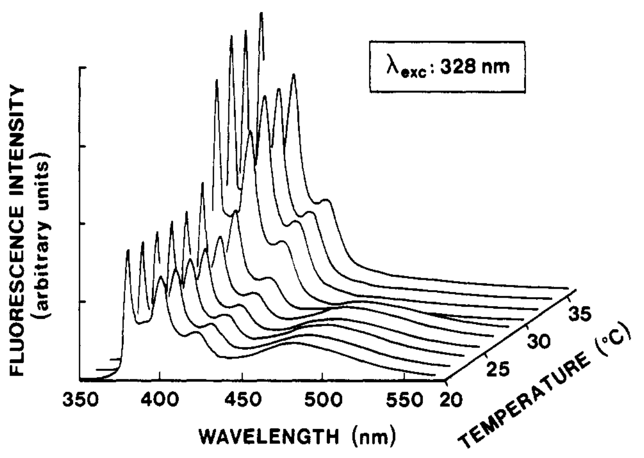

Figure 4. Fluorescence spectra of a solution in water of PNIPAM–C_{18}Na/200 and PNIPAM–C_{18}Py/200 (prepared from aqueous stock solutions of each copolymer) at several temperatures between 20 and 35 °C; λ_{exc} = 330 nm.

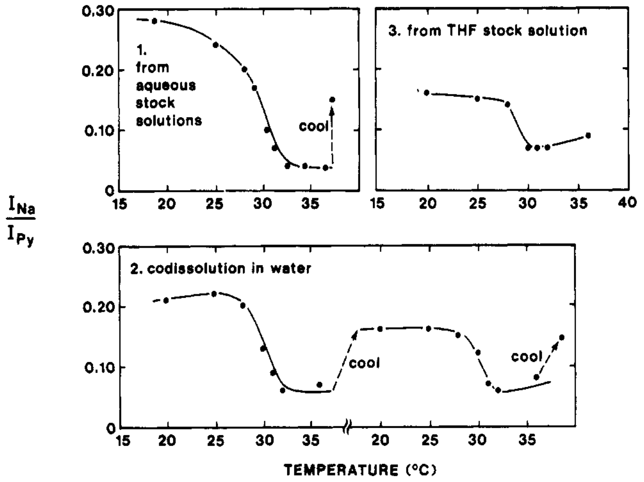

Figure 5. Changes as a function of temperature of the ratio (I_{Na}/I_{Py}) of the intensity of naphthalene emission to the intensity of pyrene emission due to NRET (λ_{exc} = 290 nm) for mixtures in water of PNIPAM–C_{18}Na/200 and PNIPAM–C_{18}Py/200: (1) solution prepared from aqueous stock solutions; (2) solution prepared from THF stock solution; (3) solution prepared by codissolution in water (two consecutive heating/cooling cycles).

Terpolymer Solutions. In contrast to the mixed polymer solution a terpolymer solution remained rather unaffected as it was heated through its LCST. The only notable change was an increase in the total fluorescence intensity (ca.) between 29 and 31 °C, when the sample was excited at 290 nm. This increase cannot be attributed to an increase in energy transfer efficiency, but rather may reflect changes in the fluorescence lifetimes of the chromophores as they are transferred from the hydrophobic environment of the polymeric micelles in cold water into

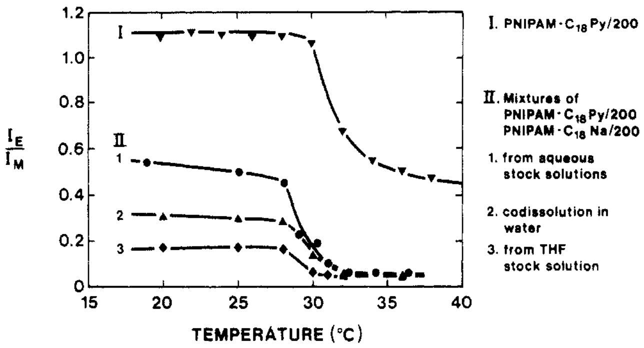

Figure 6. Changes as a function of increasing temperature of the ratio I_E/I_M of pyrene excimer emission intensity to pyrene monomer intensity (λ_{exc} = 330 nm) for (I) a solution in water of PNIPAM–C_{18}Py/200 and (II) solutions in water of PNIPAM–C_{18}Na/200 and PNIPAM–C_{18}Py/200.

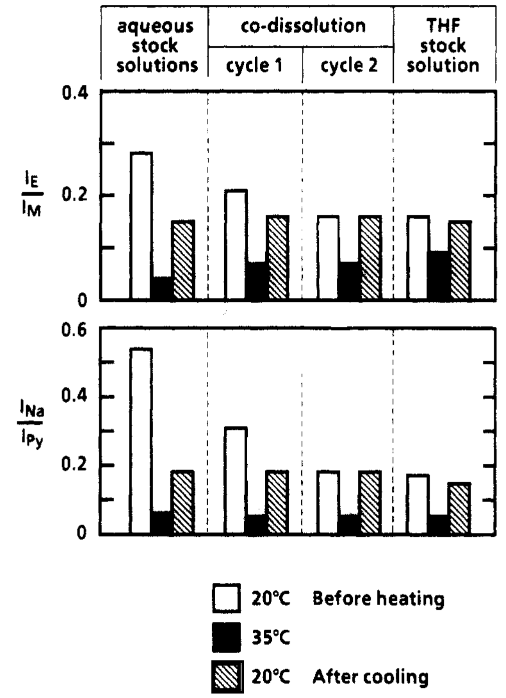

Figure 7. Effects of sample preparation and thermal history on the ratios I_{Na}/I_{Py} (bottom) of the intensity of naphthalene emission to the intensity of pyrene emission (λ_{exc} = 290 nm) and I_E/I_M (top) of the pyrene excimer to monomer emission intensities (λ_{exc} = 330 nm).

the phase-separated medium. The pyrene emission due to direct excitation also exhibited a corresponding increase in total intensity.

Effects of Sample Preparation and Thermal History. Three sample preparation methods were used (Table II): (1) mixing of aqueous stock solutions of each copolymer, the technique described in the preceding section, (2) codissolution in water of freeze-dried polymer powders, and (3) dilution into water of a concentrated mixed polymer solution in THF. Care was taken to follow as closely as possible the conditions described in the preceding section, in terms of polymer concentrations and relative ratios, and of heating/cooling cycles. In Figures 5–7 are shown salient features of the data collected during the fluorescence measurements, expressed as changes in the ratios I_E/I_M (direct pyrene excitation) and I_{Na}/I_{Py} (NRET). Note that a high value of I_E/I_M expresses a large contribution of pyrene excimer emission to the total Py* emission; conversely a low I_{Na}/I_{Py} value corresponds to situations where the efficiency of NRET is high.

The effect of sample preparation on the mixed solution properties is best appreciated in a comparison of the I_E/I_M and I_{Na}/I_{Py} values recorded at 20 °C before heat treatment (Figure 7). Among the three samples the solution prepared from aqueous stock solutions exhibits the highest I_E/I_M and I_{Na}/I_{Py} values. Hence, in this solution significant Py–Py interactions take place and there are few Py–Na contacts, within the distance scale probed by excimer formation and under NRET conditions, respectively. The lowest I_E/I_M and I_{Na}/I_{Py} ratios are measured in the solution prepared from a THF stock solution. They reflect a more intimate mixing of the naphthalene and pyrene labels.

Focusing next on the changes triggered by heating each sample above its LCST, we observe that in all cases the efficiency of NRET increases abruptly at the LCST (Figure 5) and that the pyrene excimer emission is all but precluded in the phase-separated environment (Figure 6). However, noteworthy differences exist among the three samples as they are cooled below their LCST (Figure 7). Only the solution prepared from THF recovered its initial photophysical parameters. Solutions prepared either by codissolution in water or from aqueous stock solutions showed lower I_E/I_M and I_{Na}/I_{Py} values after the heat treatment. Quite striking, however, is the fact that these new values are identical to those exhibited by the solution prepared from THF. Moreover, any solution subjected to a second (or third) heating/cooling cycle exhibited perfect reversibility in its spectroscopic features (see for example Figures 5c and 7).

Discussion

In cold water hydrophobically-modified PNIPAM copolymers form discrete microdomains consisting of several polymer chains "stuck" together through their hydrophobic substituents. As a working hypothesis based on our fluorescence results[6] and on the description proposed by Schild and Tirrell for related copolymers,[10] these entities are depicted here as multipolymeric aggregates consisting of a hydrophobic core which brings together the octadecyl groups and the chromophores, surrounded by a shell made up of poly(*N*-isopropylacrylamide) chains. They range in size between 40 and 60 nm for polymers of comparable molecular weights. The type and properties of polymeric micelles formed in aqueous solutions of neutral amphiphilic copolymers are controlled by a delicate balance between opposing forces. The driving force for the formation of micelles is the reduction in free energy when the hydrophobic side chains lose contact with water. Several forces oppose structure formation. Unfavorable interfacial forces are created through the residual contacts between water molecules in the shell region of the structures and the surface of the hydrophobic core. Bringing several polymer chains into close proximity also requires severe free energy deficit, as a result of loss of configurational entropy due to confinement and orientational restriction. When several polymer chains associate, they also lose translational energy, a factor which minimizes the number of multipolymeric micelles.[20] In the case of NIPAM copolymers the interactions between the polymer main chain and water molecules are controlled predominantly by the formation of hydrogen bonds between the amide groups and water.[21] The chains adopt an expanded conformation surrounded by a layer of "organized, iceberg-like" water molecules. No interpolymeric aggregation occurs in solutions of PNIPAM, emphasizing the strength of the forces opposed to bringing the chains in close proximity.

Dependence of the Microdomain Architecture on Sample History. Overall features of the microdomains,

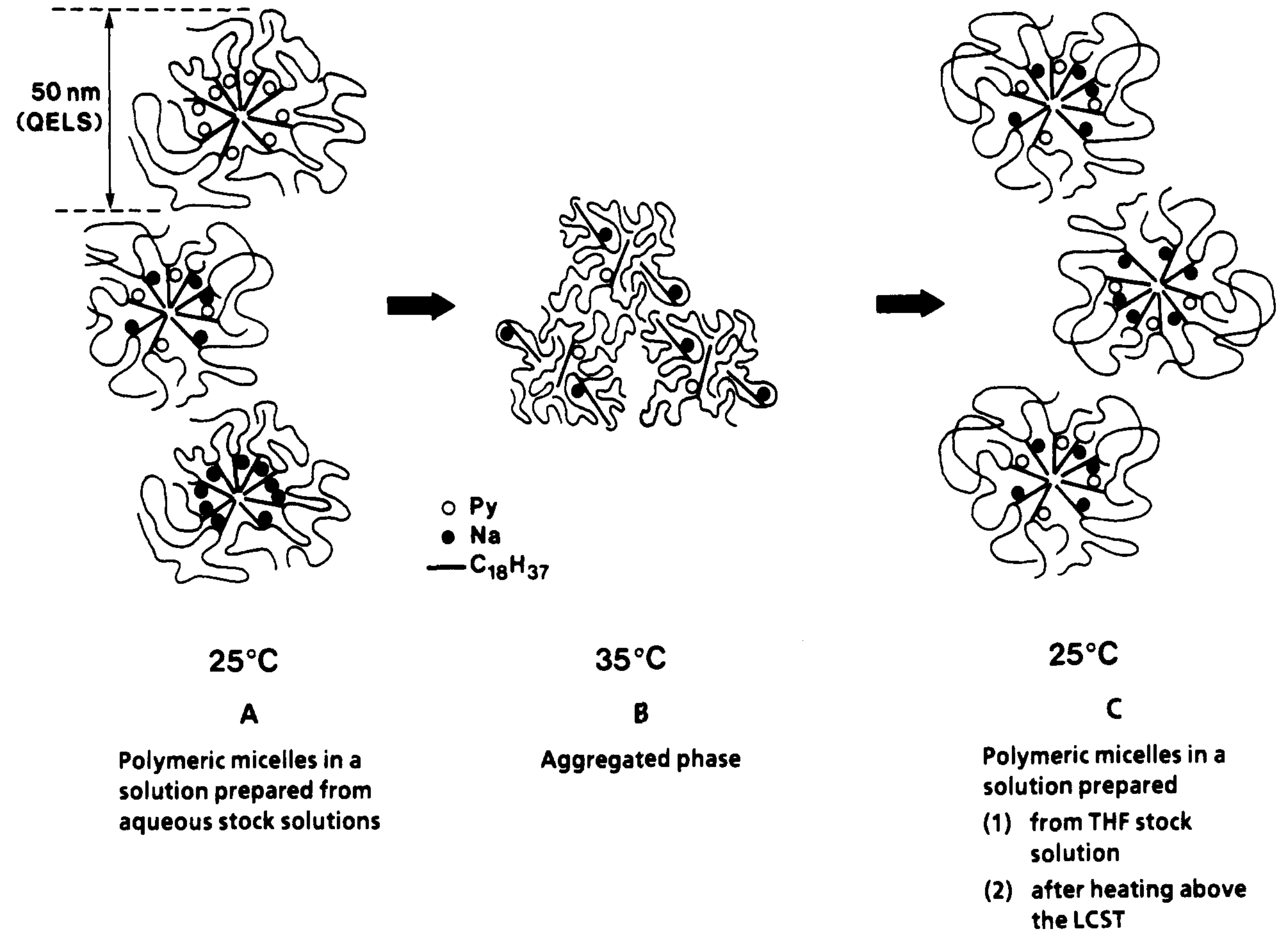

Figure 8. Idealized representation of the polymeric micellar structures in water at 25 °C before (A) and after (C) heating above the LCST (B).

such as their size and hence the number of polymer chains aggregated in an individual micelle, do not seem to be sensitive to sample preparation, as indicated by the QELS data. The important aspects of the results reported here are those related to the aging and intermixing of preformed micelles. When the naphthyl- and pyrenyl-labeled polymers are thoroughly mixed prior to micelle formation, as in the case of a solution prepared from a THF stock, the polymers intermix at random within each micelle. In contrast when micellar structures of each type of polymer exist prior to mixing, only little intermixing takes place. This limited interchange is observed when mixtures are prepared from two aqueous stock solutions or by codissolution of polymers, although to a lesser extent in the latter case. The codissolution results are revealing since they highlight that the polymeric micelles "remember" to some extent the structures present in the solid phase formed by freeze-drying from water. In both instances though, mixed polymeric micelles form. Whether they result from association of previously isolated polymer chains or whether they form through collision and intermixing of multipolymeric structures is an issue that remains to be resolved. The stability of solution structure with time argues against facile interchange of polymer chains from one polymeric micelle to another, in contrast to the well-known dynamic exchange between micelles of (low molecular weight) surfactants.[22]

Effects of Heat Treatment (Figure 8). In the polymer-rich phase that separates from solutions at temperatures above the LCST, hydrophobic microdomains may still exist, but their structure is quite different: the octadecyl groups are mostly separated from each other, although a few pairs may remain in close proximity. The collapsed polymer chains divested of the layers of structured water molecules provide a nonpolar environment in which hydrophobic groups are dissolved at random and kept protected from surrounding water (Figure 8B).

Upon cooling below the LCST, the collapsed polymer main chains recover their expanded conformation. To minimize contact with water, the hydrophobic side chains reassociate. This triggers the formation of hydrophobic microdomains with a gross architecture identical to the original structures, as judged by their size. However, the detailed morphology of the hydrophobic sectors has been modified (Figure 8C). This "memory loss" is most clearly revealed by the experiments performed with mixtures of aqueous stock solutions of pyrene- and naphthalene-labeled copolymers (Figure 7). In the cooled solutions the number of pyrene–naphthalene pairs within the hydrophobic domains has increased at the expense of pyrene–pyrene pairs. Scrambling of the chromophores has taken place.

Most intriguing is the observation that the same level of intermixing exists, in the cooled solutions, independently of the original mode of solution preparation. Further heating/cooling treatments also are ineffective in achieving any changes in intermixing. Hence, above the LCST the separated and still swollen polymer phase provides a fluid medium in which polymer chains are free to move and intermix at random. This observation may argue in favor of a transition from spherical micellar structures in cold water into longer cylindrical structures above the LCST, a mechanism proposed in the case of poly(ethylene oxide) derivatives heated above their LCST.[23]

Conclusions

The energy transfer efficiency between donor and acceptor chromophores has been measured as a function of temperature and sample preparation technique in aqueous solutions of hydrophobically-modified NIPAM copolymers labeled with naphthalene (donor), or pyrene (acceptor), or both chromophores. The detailed morphology of the microdomains formed by these polymers in water depends on the method of sample preparation and on the thermal history of a solution. Exchange of polymer chains among individual polymeric micelles is slow in cold water. Above the LCST, efficient and rapid intermixing of the polymers occurs. The copolymers are distributed at random in the polymeric micelles formed in solutions brought from 35 °C to room temperature. Identical photophysical properties are exhibited by all solutions after they were subjected to one or several heating/cooling cycles.

The results reported here contribute to a better understanding of the intricate mechanisms in play in solutions of industrially important rheological modifiers, known as associative thickeners.[24] Techniques probing macroscopic solution properties, such as viscosity measurements and rheology studies, have been instrumental in highlighting gross effects related to the incorporation of low levels of hydrophobic substituents along the backbone of water-soluble polymers. Few analytical tools probing molecular interactions are useful in the study of such polymers. NMR and IR spectroscopies are not sensitive enough to monitor changes in polymer interactions because of the low level of alkyl group incorporation. Photophysical techniques, by contrast, are proving extremely successful in this area, as evidenced in the experiments reported here as well as those performed with modified polyacrylamides[25] and with modified cellulose ethers and polyethylene glycols.[26]

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References and Notes

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Registry No. (PNIPAM)(C_{18}Py/200) (copolymer), 129674-16-4; (PNIPAM)(C_{18}Na/200) (copolymer), 143123-55-1; (PNIPAM)(C_{18}Na/200)(C_{18}Py/200) (copolymer), 143123-56-2.