Fluorescence Studies of Hydrophobic Association of Fluorocarbon-Modified Poly(*N*-isopropylacrylamide)

Mei Li and Ming Jiang*

Institute of Macromolecular Science and Laboratories of Molecular Engineering of Polymers, Fudan University, Shanghai 200433, China

Yun-xiang Zhang and Qin Fang

Shanghai Institute of Organic Chemistry, Academica Sinica, Shanghai 200032, China

Received July 3, 1996; Revised Manuscript Received November 18, 1996[⊗]

ABSTRACT: A series of hydrophobically associating poly(*N*-isopropylacryamide) (PNIPAM) containing low amounts (0.06−0.88 mol %) of fluorocarbon ($CF_3(CF_2)_7$−) was prepared by copolymerization of NIPAM and 2-(*N*-ethylperfluorooctanesulfonamido)ethyl methacrylate. Hydrophobic association of the copolymers in an aqueous medium was investigated by fluorospectroscopy in which both pyrene (Py) and fluorocarbon-substituted pyrene ($PyCOR_f$) were used as probes. In comparison with unmodified pyrene, using $PyCOR_f$ with the same fluorocarbon substituent as in the PNIPAM copolymers proved to be more informative for monitoring hydrophobic associations of the copolymers due to its affinity for the microdomains of the fluorocarbon chains. Both the monomer emission intensity, I_{375}, and the monomer to excimer ratio of intensities, I_{375}/I_{550}, of $PyCOR_f$ were good indicators of the dependence of the association on the fluorocarbon content of the copolymers and their concentration.

Introduction

Hydrophobically modified water-soluble polymers have received increasing attention in recent years due to their unique rheological properties and industrial importance.[1,2] The polymers are composed of a hydrophilic main chain and a few hydrophobic substituents, which may be randomly attached to the main chain or appended at one or both ends of the chain. When they are dissolved in an aqueous medium, the hydrophobic gropus undergo intermolecular association leading to efficient viscosification. The solution viscosity usually begins to show a dramatic increase as the concentration reaches a critical range of about 0.1−0.5 wt %, and the viscosity exhibits large shear dependence. In some cases, both shear thickening and shear thinning may occur in different ranges of the shear rate. These properties enable such associating polymers to have wide applications as an "associative thickener" (AT).

Obviously, there is a need for understanding the association at the molecular level. In recent years, a sizable body of publications focused on molecular level studies of the hydrophobically associating polymers with emphasis on characterizing the hydrophobic microdomains and establishing the relationship between the hydrophobic microstructure and macroscopic properties.[3−16] The range of experimental techniques used includes laser light scattering (LLS),[15,16] nuclear magnetic resonance (NMR) spectroscopy,[11−13] and fluorescence spectroscopy.[3−10,12,14,17] Among the techniques, fluorospectroscopy is no doubt the most extensively used and the most informative. This can be attributed to the high sensitivity of fluorescence measurement and the strong dependence of the fluorescent behavior of probe molecules on the microenvironment.

In the literature concerning fluorescent investigations of hydrophobic associations, mainly three kinds of polymers have been studied, *i.e.*, modified polymers based on cellulose, poly(ethylene oxide), and polyacrylamides.[18] In the case of cellulose,[19−21] the label of 1-pyrenylbutyl was attached through hydrolytically stable ether linkages to the main chain of hydroxylpropyl cellulose. In water solutions of the pyrene-labeled polymers, the fluorescence intensity ratio of excimer (I_e) to monomer (I_m) was used as an indicator of association between the hydrophobic labels. A value of I_e/I_m of about 1.4 at extremely low polymer concentration (0.2 ppm) indicated intramolecular excimer formation. An increase in I_e/I_m was observed as the polymer concentration was increased to 200 ppm, consistent with interpolymer association. Dramatic changes of I_m and I_e/I_m around 40 °C were also observed, which can be attributed to the abrupt solubility variation of the polymers in water around LCST.

Studies on poly(ethylene oxide) (PEO)-based hydrophobically associating polymers have been reported by Frank's group[22] and Winnik's group.[3] Frank *et al.*[22] employed pyrene end-labeled PEO of various molecular weights. In the log−log plot of I_e/I_m versus concentration of the polymers in water, the curve is initially flat indicating intramolecular excimer formation and then rises due to intermolecular excimer formation. Here pyrene plays the role of both probe and modifier. Wang and Winnik[3] reported fluorescence results of PEO polymers end-capped with hexadecyl groups (AT) in water utilizing free pyrene as the fluorescence probe. The ratio of the intensity of the first and third bands (I_1/I_3) of pyrene emission was used to monitor the local environment of the pyrene molecules. In the plot of I_1/I_3 against polymer concentration, it was found that pyrene was solubilized in an environment close to that of water at low polymer concentration but in a hydrophobic environment of micelles composed of the hydrocarbon chain ends at high polymer concentration. This variation indicated the aggregation, but the "association transition" was broad.

In recent years, fluorescence decay of pyrene and excimer emission of bis(1-pyrenylmethyl) ether (dipyme) were studied which are sensitive to the local rigidity.[4−6] On the basis of the results of AT of different molecular parameters, Winnik *et al.*[7] proposed a new model, in which the AT polymers first form primary aggregates

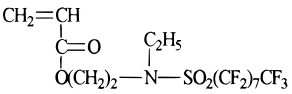
[⊗] Abstract published in *Advance ACS Abstracts,* January 15, 1997.

S0024-9297(96)00966-7 CCC: $14.00

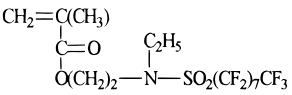
by forming micelles or micelle-like clusters with *ca.* 20 chain ends, and then at higher concentrations, they form secondary aggregates through open association of the primary clusters with bridging chains. Both hydrocarbon-modified polyacrylamide (PAM) and poly(*N*-isopropylacrylamide) (PNIPAM) have been extensively studied, especially by fluorospectroscopy. In the case of PAM copolymers, Flynn and Goodwin[23] used free pyrene as a photophysical probe to monitor association of copolymers of acrylamide and 0.2 and 0.4 mol % of the hydrophobic comonomer dodecyl methacrylate. They found that I_1/I_3 as a function of polymer concentration (1−10 g/L) indicated that the probe was solubilized in an increasingly hydrophobic environment with an increasing polymer concentration. At high polymer concentration, I_1/I_3 reaches about 1.08, similar to the value of pyrene in common micelles. From the plot of I_1/I_3 versus concentration, a continuous decrease of I_1/I_3 was observed so that no critical micelle concentration (CMC) could be estimated. This might have been because of the relatively high hydrophobic contents and the narrow concentration range used in the study. McCormick *et al.*[17] reported pyrene-labeled acrylamide copolymers containing a new comonomer, 1-(*β*-amidoethanesulfonamido)pyrene acrylamide. This pyrene-containing unit served as both a label and a hydrophobic substituent. Correlation of fluorescence data with rheology was reported, *i.e.*, plots of both I_e/I_m and reduced viscosity versus logarithm of polymer concentration show an upward swing at almost the same concentration, indicative of intermolecular association.

PNIPAM has been one of the most extensively studied water-soluble polymers because of its LCST behavior. The peculiar thermal response of PNIPAM in an aqueous medium has been exploited for wide practical applications. Synthesis of hydrocarbon-modified PNIPAM was reported by Ringsdorf *et al.*[9] and Schild *et al.*[23,24] The fluorescence techniques[23,25,26] have been used to monitor the variation of the solubility of PNIPAM with temperature and the corresponding mechanism of phase separation and shrinking of molecular coils in water. Association and LCST behavior of hydrocarbon-substituted PNIPAM were investigated by Winnik *et al.*[9,10] I_1/I_3 of both pyrene and dipyme and I_e/I_m of dipyme as functions of polymer concentration and temperature reveal the existence of multimolecular micelles below LCST in solutions of a concentration exceeding *ca.* 0.1 g/L. At LCST the micelles collapse and aggregate leading to macroscopic phase separation. This probe technique indicated some similarities and differences between the end-substituted and randomly substituted copolymers. It demonstrated the effectiveness of the free probe technique in monitoring hydrophobic associations in hydrocarbon-substituted water-soluble polymers. Nonradiative energy transfer (NRET) measurements[8] between the attached naphthalene and pyrene revealed the existence of interpolymeric micelles in solutions with polymer concentrations as low as 4 ppm. Aggregation occurred at temperatures well below the LCST, in contrast to the PNIPAM homopolymer, which does not self-aggregate in water below its LCST. The copolymers used in the study were carefully designed, *i.e.*, a chromophore and a hydrophobic substituent $C_{18}H_{37}$ were attached chemically to the same amide N atom of the copolymer chain. This close spatial proximity between chromophore and $C_{18}H_{37}$ chains ensures that the probe is selectively located in the aggregated area of long alkyl substituent.

Chart 1. Structure of Comonomers



RFA 2(N-ethylperfluorooctane sulfonamido) ethyl acrylate



RFM 2(N-ethylperfluorooctane sulfonamido) ethyl methacrylate

It is known that perfluoroalkyls are more hydrophobic than their hydrocarbon analogues.[27] Because of this, Zhang *et al.*[28−30] studied copolymers based on polyacrylamide with a small amount of an acrylate or methacrylate with a fluorocarbon-containing group. Such polymers exhibited more pronounced hydrophobic association than the corresponding hydrocarbon-substituted copolymers. For instance, in a copolymer containing as little as 0.006 mol % of 2-(ethylperfluorooctanesulfonamido)-ethyl acrylate (RFA; Chart 1), a viscosity increase by 1 order of magnitude was observed. Recently, Hogen-Esch *et al.*[31] introduced hydrophilic spacers of different length between the PAM main chain and the pendent fluorocarbon groups and found that the hydrophilic spacers promote the hydrophobic association. Although a remarkable viscosification due to intermolecular association has been observed and confirmed for series of copolymers containing fluorocarbon comonomers with different structures, characterizations and association mechanisms based on molecular level studies have not been reported, except for laser light scattering studies.[15,16] The copolymers containing the comonomer units in the range of 0.007−0.7 mol % were found to form large aggregates even at concentrations as low as 10 ppm. At various concentrations, several diffusive relaxations which are identified with aggregates, single chains, and even gel modes were observed. Quite recently, Wu *et al.*[16] studied the same copolymer systems by a combination of static and dynamic light scattering, the critical aggregation concentration (CAC) was estimated, as low as 1.34×10^{-4} g/mL for the copolymer containing 0.227 mol % of RFA, and on average, depending on concentration, each aggregate contains 5−9 individual polymer chains.

In this paper a series of a new type of RFM copolymers were prepared by solution copolymerization in dioxane. The rheological properties and LCST behavior of the copolymers will be reported in forthcoming papers. The main interest in the present paper focuses on characterization of association by fluorospectroscopy with particular attention to the proper use of probe techniques.

Experimental Section

Materials. *N*-Isopropylacrylamide (Eastman Kodak) was purified by twice repeated recrystallization from hexane/benzene (v/v, 65/35). Fluorine-containing comonomer RFM, obtained from the 3M Company, and the initiator 1,1′-azobis-(isobutyronitrile), analytical grade, were also recrystallized twice from methanol. Pyrene (Aldrich, 99%), perfluorooctanoic acid, and PCl_5 were purchased commercially and used as received. Aluminum chloride was sublimated two times under vacuum at 180 °C. All solvents used in the reactions were analytical grade and redistilled after being dried.

Table 1. Characterization Data of PNIPAN and Copolymers

polymer	comonomer (mol %) in feed	comonomer (mol %) found	conversion of comonomer	$[\eta]$ (dL/g)	M_w (10^4 g/mol)
PNIPAM homopolymer					6.2
Co-1	0.078	0.060	76.3	0.61	7.3
Co-2	0.156	0.104	65.3	0.42	6.0
Co-3	0.317	0.208	65.6	0.53	6.0
Co-4	0.622	0.414	67.0	1.23	6.0
Co-5	1.240	0.878	70.8	0.57	6.0

Polymerization. The poly(*N*-isopropylacrylamide) copolymers were prepared using dispersion of 2.30 g (0.02 mol) of *N*-isopropylacrylamide and varying amounts of the comonomer RFM in a 50 mL capped round-bottom flask containing 40 mL of 1,4-dioxane. After the solution was purged with pure nitrogen for 35 min, the mixture was heated, and then polymerization was continued for 24 h at 60.0 ± 0.5 °C. The polymerization was stopped by cooling to room temperature, and the product was precipitated in 500 mL of diethyl ether and filtered. The dried copolymer was dissolved in 20 mL of acetone and precipitated in 500 mL of diethyl ether. The white precipitates were vacuum-dried at 40 °C for 24 h. The intrinsic viscosities of the copolymer in THF and the contents of the fluorocarbon chains measured by anionic chromatography are listed in Table 1.

Synthesis of Perfluorooctanoyl Chloride. Perfluorooctanoic acid (10 g, 0.023 mol) in a 100 mL two-neck round-bottom flask was heated to 50 °C; PCl_5 (7 g, 0.034 mol) was added to the flask with stirring. After the reaction quieted down, the mixture was refluxed for 1 h at 75 °C followed by distillation, and the middle fraction was collected.

Synthesis of 1-(Perfluorooctanoyl)Pyrene.[32,33] A fluorocarbon-containing fluorescence probe ($PyCOR_f$) was prepared by Friedel−Crafts reaction of pyrene with perfluorooctanoyl chloride in 1,2-dichloroethane. The procedure was as follows: Perfluorooctanoyl chloride 10 g (0.023 mol) was mixed with 20 mL of 1,2-dichloroethane in a 100 mL flask in an ice bath, 3.4 g (0.025 mol) of aluminum chloride was added, and then the pyrene solution (3 g, 0.015 mol, pyrene dissolved in 30 mL of 1,2-dichloroethane) was dropped into the above reaction mixture with magnetic stirring under a steady N_2 stream in 30 min. The reaction mixture was kept at 0 °C for 12 h. The dark solution was carefully poured into 6 M HCl ice solution, stirred, and extracted with diethyl ether; the extract solution containing $PyCOR_f$ was washed by saturated $NaHCO_3$ aqueous solution and deionized water and dried overnight in the presence of $MgSO_4$. The yellow product obtained after removing the solvent was purified using column chromatography using petroleum ether and diethyl ether (v/v, 98/2) as eluent and recrystallized twice from methanol (mp 108−110 °C). Calcd: C, 48.16; H, 1.51; F, 47.66. Found: C, 48.78; H, 1.48; F, 48.31. FTIR (KBr): 1697 cm^{-1} (s, C═O), 1238, 1201, and 1146 (vs, C−F), 3049 (w, aromatic C═C). NMR ($CDCl_3$): ^1H 8.15−8.80 ppm (H on pyrene ring in $CDCl_3$); ^{19}F CF_3COOH (0), CF_3 4.85 ppm, CF_2 35.28−50.23 (multipeak). MS: *m*/*z* 598 (M^+) (13.43), 229 ($PyCO^+$) (100), 201 (Py^+) (59.16).

Characterization and Fluorescence Measurements. 1. Light Scattering Measurements. A modified commercial LLS spectrometer (ALV/SP-125) equipped with a solid state laser (532 nm) was used. All measurements for the polymer solutions in tetrahydrofuran were carried out at 25.0 ± 0.1 °C. The refractive index increment of PNIPAM in THF was determined to be 0.09 mL/g by a novel and precise differential refractometer.[34] The molecular weights of PNIPAM and the copolymers were calculated from the Zimm plots and are listed in Table 1.

2. Sample Preparation for Fluorospectroscopy. Polymers were mixed with deionized water followed by continuous magnetic stirring for 2−5 days to get a homogeneous solution of 2−5 wt %. The solutions were progressively diluted with deionized water to the required concentration. A small amount of a probe solution in acetone was added into each sample with a microinjector giving a final concentration of either 6×10^{-7} M for pyrene or 2×10^{-6} M for $PyCOR_f$ in solution. Acetone was not removed, and its final content was 0.1%, v/v. All samples were ultrasonicated for 15 min and left for 24 h at room temperature before measurement.

3. Steady-State Fluorescence Measurements. Steady-state fluorescence spectra were recorded with a Perkin-Elmer luminescence spectrometer LS 50 with the right angle geometry (90° collecting optics) with a slit of 5 nm for excitation and 3 nm for emission. For emission spectra, $\lambda_{ex} = 333$ nm; for excitation spectra, $\lambda_{em} = 390$ nm. For the fluorocarbon-substituted pyrene, the excitation wavelength was 340 nm. All spectra were run on air-saturated samples.

Results and Discussion

Hydrophobic Association Probed by Free Pyrene. In studies of normal hydrocarbon micelles containing hydrophobic modified water-soluble polymers[3−10] and polysoaps[14] as well as block copolymers,[35,36] pyrene is a widely used fluorescent probe because of its unique photophysical characteristics. It is strongly hydrophobic, and its solubility in water is very low (7×10^{-7} M). In the presence of a hydrophobic region or microphase in aqueous media, pyrene is preferentially solubilized into the interior of the hydrophobic regions. This change in the microenvironment of the probes is reflected by variations in both its emission and excitation spectra. There is an increase of the lifetime of the excited state and a consequent increase of fluorescence quantum yield, the vibration fine structure changes, as characterized by the intensity ratio of the first band to the third band of the pyrene emission spectrum, I_1/I_3,[37,38] and the (0,0) band maximum in the excitation spectrum is red-shifted by a few nanometers.[6,35]

The I_1/I_3 ratio is probably the most widely used indicator of the polarity of the pyrene environment. As an example used in hydrophobic associating polymers, Wang *et al.*[3] reported that the plot of I_1/I_3 versus concentration of PEO end-capped with hydrocarbon in water has a sigmoidal shape. It changes a little at the value of 1.7 followed by a sizable decrease as the concentration reaches about 0.01 g/L, indicating the partitioning of pyrene, and finally settles down to a low plateau of about 1.2, characteristic of a nonpolar medium. However, using pyrene as a photophysical probe for hydrophobic microdomains composed of fluorocarbon chains has encountered some difficulties. Kalyanasundaram[39] reported the results of micellization of a perfluoro surfactant probed by free pyrene. The I_1/I_3 value of pyrene in the fluorocarbon surfactant micelles in water was found to be 1.5−1.6, apparently larger than in normal hydrocarbon surfactants (1.2) and quite different from those observed for pyrene in neat fluorocarbon solvents (1.00). The author suggested that the high value of I_1/I_3 observed in perfluorocarbon micelles reflected a more polar solubilization site, presumably in the outer layer of the micelle. The fluorescence lifetime in the range 260−300 ns observed is also slightly lower, indicating some quenching of the pyrene singlet excited state by fluorocarbon molecules or even

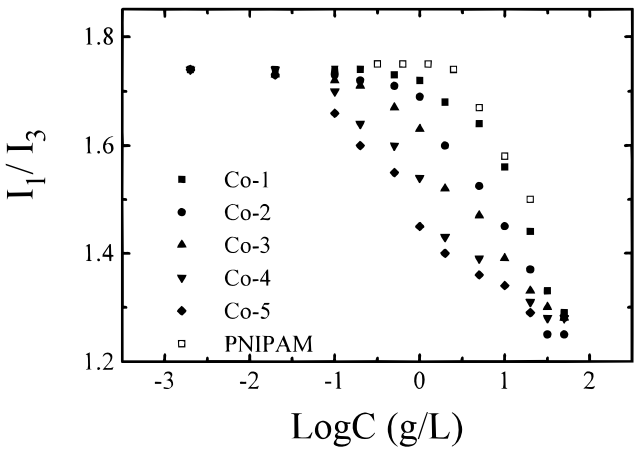


Figure 1. Plot of the fluorescence intensity ratio I_1/I_3 as a function of polymer concentration.

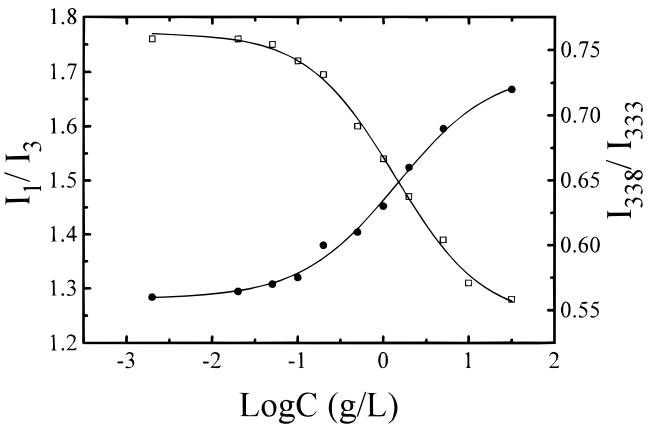


Figure 2. Plots of the fluorescence intensity ratios I_1/I_3 and I_{338}/I_{333} as functions of copolymer Co-4 concentration.

by some water present nearby. In short, pyrene was found to have a limited solubility in fluorocarbon micelles. This conclusion has found support from excimer studies. It is well known that pyrene molecules in the excited state may form excimers if the pyrene concentration reaches a certain value. The dynamics of pyrene excimer formation in normal hydrocarbon micelles quantify compartmentalization effects of micellar aggregates and allow the micellar aggregation number to be deduced. However, in estimating the aggregation number of the perfluorocarbon micelles, Kalyanasundaram[39] found that, unlike in normal hydrocarbon micelles, an excimer-like emission of pyrene appeared only when the pyrene concentration reached 10^{-4} M. He suggested that the emission actually came from pyrene in the microcrystalline state rather than solvated molecules. Based on these results, pyrene itself may not be able to serve as an effective probe for associations caused by fluorocarbon chains.

In this study, both emission and excitation spectra of pyrene in PNIPAM homopolymer and the copolymers containing fluorocarbon chains in water were measured. In the homopolymer solutions, as the polymer concentration increases, the monomer emission increases slightly while I_1/I_3 decreases from 1.75, a typical value in aqueous media when the polymer concentration is low, to 1.45 at high (20 g/L) polymer concentration. This may be because PNIPAM is relatively hydrophobic compared to other water soluble polymers such as PEO and PVP (poly(vinylpyrrolidone)) as evidenced by the lower value of LCST.[40] It is therefore understandable that the bulk hydrophobicity of the solution is increased substantially when a certain amount of PNIPAM is added.[40] This result is different from that reported by Ringsdorf *et al.*[8] They found that the intensity ratio exhibits a value close to that of pyrene in water even at high PNIPAM concentrations, but they did not mention how high the concentration was.

For the copolymer aqueous solutions, the data of monomer emission intensity versus polymer concentration are rather scattered. However, I_1/I_3 shows regular variations with both copolymer concentration and fluorocarbon content in the copolymers. In Figure 1, all the curves for the copolymers show that at low polymer concentrations, $I_1/I_3 = 1.75$, characteristic of pyrene in water (*ca.* 1.8). It decreases then with the increasing polymer concentration. At the high concentration range of 10−50 g/L, the values of I_1/I_3 in all copolymer systems are around 1.25, lower than that of pyrene in a perfluorocarbon surfactant.[39] In comparison with the curve of PNIPAM, those for the copolymers show a more pronounced decrease in I_1/I_3 and the decrease starts at a much lower concentration. The higher the fluorocarbon chain content, the smaller the polymer concentration corresponding to the turning point of I_1/I_3. For the highest and lowest fluorocarbon-containing samples, Co-5 and Co-1, the turning points are at concentrations of *ca.* 0.05 and 0.6 g/L, respectively. In addition, for the higher fluorocarbon-containing copolymers, a detectable decrease of I_1/I_3 appears at a concentration as low as 0.01 g/L, leading to the very broad association transition that spans almost 3 decades in polymer concentration, much broader than in the alkyl-substituted polymers.[10] This can be attributed to intramolecular association at very low concentration. At modest concentrations there exist both intra- and intermolecular associations, and the latter becomes dominant at higher concentrations. As to the low fluorocarbon-containing PNIPAM system, it only possesses intermolecular association in a higher concentration range, so its transition concentration is higher and the transition range is not so broad. Although evidence of pyrene transferred from water to a less polar environment has been obtained in all the systems studied, we cannot conclude that hydrophobic fluorocarbon chains form micelle-like clusters in a similar manner as end-alkylated and randomly alkylated PNIPAM.[10] The reasons are as follows: It is remarkable that the (0,0) band of pyrene is only 0.5−1 nm red-shifted when the ratio of I_1/I_3 changes from 1.75 to 1.25. In well-characterized micellar media, this shift is typically 2−5 nm relative to that in pure water.[6,35] This small red shift may raise doubts about the existence of micellar domains in our systems.

As shown by Wilhelm *et al.*[35] and Eisenberg *et al.*,[36] the ratio of I_{338}/I_{333} obtained from the (0,0) bands of pyrene excitation spectra can also show onset of micellization and provide a quantitative method of CMC determination. As an example, the plots of the intensity ratios of I_1/I_3 and I_{338}/I_{333} of pyrene in Co-4 as functions of polymer concentration are shown in Figure 2. At low concentrations the ratio I_{338}/I_{333} takes the value of about 0.56, slightly higher than that of pyrene entirely in a hydrophilic environment (0.3−0.4).[35,36] The ratio rises at concentration of 0.05 g/L, the same value where the ratio I_1/I_3 starts to decrease, indicating the onset of aggregation. At high concentrations, I_{338}/I_{333} only reaches 0.72, much smaller than the value of pyrene in a micellar structure (2.1−2.4).[35,36] That the (0,0) band of the excitation spectra shows only a little shift and the intensity ratio I_{338}/I_{333} is relatively low even when the polymer concentration is quite high imply that most of

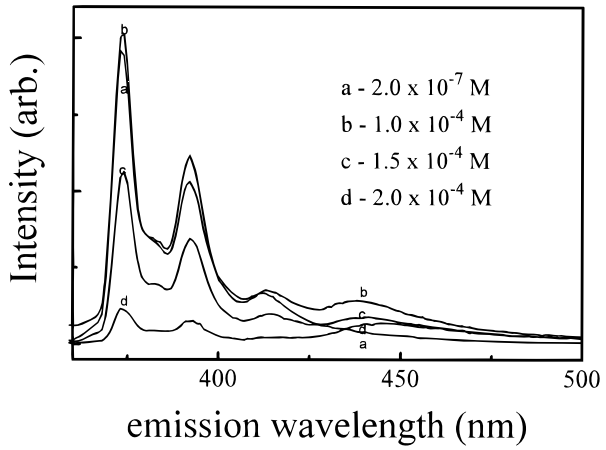


Figure 3. Fluorescence emission spectra of $PyCOR_f$ in methanol at different concentrations.

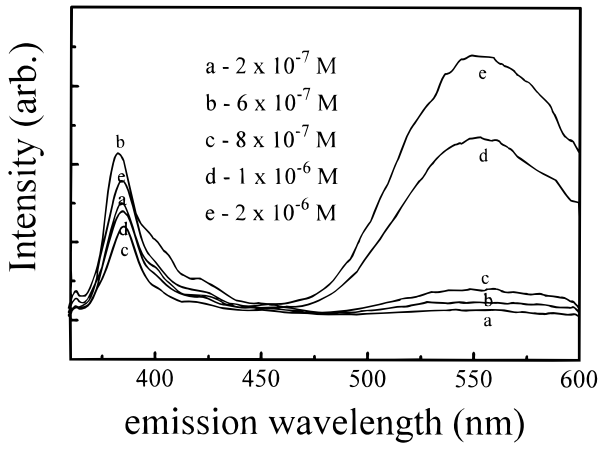


Figure 4. Fluorescence emission spectra of $PyCOR_f$ in water at different concentrations.

the pyrene has probably not moved from the aqueous bulk to the micelles. However, the variation of I_1/I_3 does indicate such transport. This apparent discrepancy may be due to the following reasons.

First, in the water solutions, as the polymer concentration increases, the hydrophobicity of the system increases, causing I_1/I_3 to decrease, as evidenced in the solutions of PNIPAM homopolymer which could not cause any micelle-like association. This effect makes a substantial contribution to the decrease in I_1/I_3 of the copolymer solutions as well. Second, as mentioned before, since pyrene is not completely miscible with fluorocarbon chains, it may not be able to penetrate into the interior of the micelle but is located at their outer layer. It will cause a relatively small I_{338}/I_{333} and little (0,0) band shift. Moreover, no excimer emission in all copolymer systems was found, probably meaning that the number of pyrene molecules in the hydrophobic microdomains is too small to lead to detectable excimer emission. We may conclude that pyrene provides some indication of hydrophobic association of fluorocarbon-substituted PNIPAM but no compelling evidences.

Fluorescence of $PyCOR_f$. As mentioned above, due to the poor affinity of pyrene to fluorocarbon chains, it cannot serve as an effective probe for association or micellization of the fluorocarbon chains. In order to improve its solubility in the fluorocarbon microdomains and its power of discrimination therein, we synthesized and employed a pyrene derivative ($PyCOR_f$) with a fluorocarbon chain identical with the substituent in PNIPAM copolymers. For the purpose of proper understanding of the photophysical behavior of $PyCOR_f$ in solutions of fluorine-containing PNIPAM copolymers, the fluorescent properties of the probe alone in different environments were first investigated. The steady-state fluorescence spectrum of $PyCOR_f$ in methanol covering a broad range of concentrations (2×10^{-7}–2×10^{-4} M) is shown in Figure 3. At the low $PyCOR_f$ concentration range (10^{-7}–10^{-5} M), three maxima at 374, 394, and 415 nm, characteristic of the monomer emission, are present. When the concentration is increased to 10^{-4} M, a new structureless peak centered at 445 nm appears, which can be attributed to excimer emission. A further increase of concentration causes a decrease of both monomer and excimer intensity, while the intensity ratio I_e/I_m increases continuously. This concentration of $PyCOR_f$ is not only high enough to form excimer but could also result in concentration quenching.

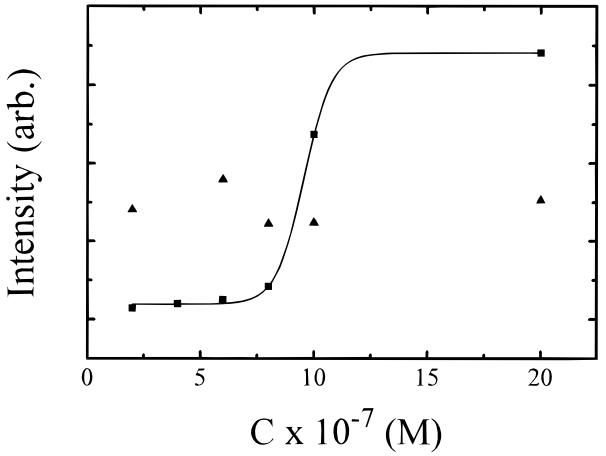


Figure 5. Fluorescence emission at 550 nm (I_e, ■) and 385 nm (I_m, ▲) of $PyCOR_f$ in water as a function of concentration. The curves are drawn as a guide for the eye.

$PyCOR_f$ displays a very particular fluorescent character in water as shown in Figure 4. There is no fine-structure monomer emission at very low concentrations; instead, a single peak at about 385 nm is present. The monomer intensity varies slightly as the probe concentration is increased. However, a new broad band with a maximum at 550 nm appears and increases substantially with concentrations above 10^{-6} M. This is more clearly seen in Figure 5, showing the monomer intensity I_m and excimer intensity I_e as functions of probe concentration. I_m varies a little with the concentration, but I_e shows an abrupt rise when the concentration is increased to 1×10^{-6} M. This can be regarded as an indication of self-aggregation of the probe at this concentration.

The self-aggregation behavior of organic small molecules containing long alkyl chains in water−organic mixed solvents has been extensively investigated by organic chemists and photochemists.[33,41−43] For example, Tung *et al.*[33,43] reported the aggregation of long alkyl-substituted pyrene and long fluorocarbon chain-substituted naphthalene and anthracene in poor solvents as well as its effect on the photophysical properties. They observed the fluorescence behavior of alkyl-substituted pyrene in different organic solvents as well as in DMSO−water mixtures and found that the location and shape of the monomer and excimer peak changed, obviously due to self-aggregation of long alkyl chains. By examining these changes, one can obtain much information on the polarity of the environment

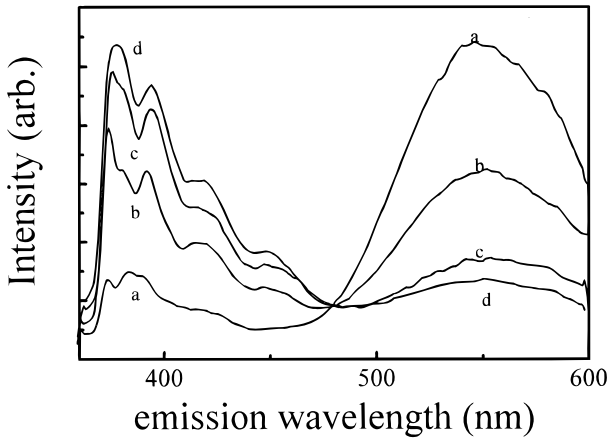


Figure 6. Fluorescence emission spectra of $PyCOR_f$ (5×10^{-5} M) in water solutions of fluorocarbon surfactant (5×10^{-2} M) after different standing times and treatment conditions: (a) ultrasonicated for 0.5 h, (b) heated at 70 °C for 3 h, (c) heated at 70 °C for 12 h, and (d) heated at 70 °C for 12 h and then stood at room temperature for 12 h.

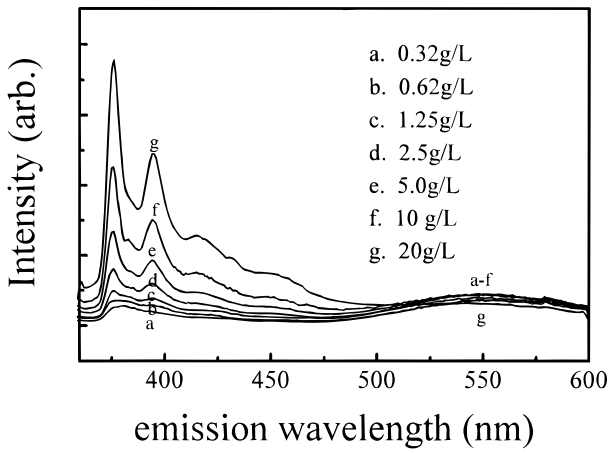


Figure 7. Fluorescence emission spectra of $PyCOR_f$ (2×10^{-6} M) in copolymer Co-1 solutions with different concentrations in water.

in which the dye is located. In our case, water is an extremely poor solvent for $PyCOR_f$. Therefore, it is understandable that $PyCOR_f$ self-aggregates in water at very low concentrations, leading to an excimer emission with a red shift of almost 100 nm relative to the emission in methanol.

In order to know whether $PyCOR_f$ is sensitive to microdomain or to a micelle constituted by fluorocarbon chains, the fluorescent behavior of $PyCOR_f$ in water solutions of a fluorocarbon surfactant was investigated. Figure 6 shows the emission spectra of $PyCOR_f$ in a surfactant ($C_7F_{15}COOK$) aqueous solution at a concentration of 5×10^{-5} M for different standing times and treatment conditions. The surfactant concentration of 5×10^{-2} M used is much higher than its CMC (2.7×10^{-2} M) for ensuring the formation of micelles. The emission spectrum of $PyCOR_f$ displays a strong excimer emission centered at 550 nm and a weak monomer emission with fine structure after the solution was ultrasonicated for 0.5 h. When the solution was heated and left standing for some time, the intensity of monomer emission increased gradually, while the excimer emission eventually disappeared. These show that the probe formed self-aggregates as soon as it was added to the aqueous solution. Only a small amount of the probe diffused into the micelle after the solution was ultrasonicated for 0.5 h. This led to the typical excimer emission in water and a weak monomer emission with the fine structure characteristic of a lower polarity environment. When heated and left standing for a long time, the aggregates of the probe in water gradually disappeared and more and more of the probe molecules diffused into the micelles. Since the micelles composed of the surfactant molecules have an anionic outer layer, $PyCOR_f$ needs to overcome a considerable energy barrier to enter into the cores of the micelles. Such a diffusion process should be slow and temperature-dependent. It is worth noting that $PyCOR_f$ in fluorocarbon chain domains has fluorescence properties similar to those in the organic solvents. The monomer emission has a fine structure, and the location of the peaks is the same as in methanol. In addition, it could be seen from the spectra that with more and more probe molecules diffusing into the micelles, a new broad peak centered about 450 nm appears, somewhat similar to the excimer emission of $PyCOR_f$ in methanol. The increase of the peak at 450 nm coincides with the decrease of the excimer of emission in water; therefore, the emission around 450 nm may be attributed to excimer emission of $PyCOR_f$ in the hydrophobic environment of micelles. On the basis of all these results, we have confirmed that $PyCOR_f$ is sufficiently sensitive enough to explore fluorocarbon microdomains.

Hydrophobic Association Probed by Free $PyCOR_f$. In the experiments with $PyCOR_f$, in polymer solutions, a relatively high concentration, *i.e.*, 2×10^{-6} M, of the probe in water is assumed. At this concentration, $PyCOR_f$ itself shows apparent excimer emission, which may undergo some variation when the medium becomes more hydrophobic as PNIPAM copolymer is dissolved. Figures 7 and 8 present the emission spectra of $PyCOR_f$ in the solutions of copolymers with the minimum and maximum fluorocarbon contents, *i.e.*, Co-1 and Co-5, respectively. At low polymer concentrations, the probe shows the spectrum characteristic of self-aggregates of $PyCOR_f$ in water, *i.e.*, a weak monomer band with a peak at 385 nm without fine structure and a strong broad band around 550 nm associated with excimer emission. As the concentration of the polymer is increased, the monomer emission strengthens considerably, accompanied by the appearance of fine structure. Meanwhile, the excimer emission gradually disappears. This reflects the transfer of $PyCOR_f$ probe molecules from the bulk aqueous phase to the microregion with high hydrophobility leading to an increase of quantum efficiency and enhancement of fluorescent lifetime.

Figure 8 for the copolymer Co-5 with the maximum fluorocarbon content presents the same trend of variation and two new features. First, the fine structure of the monomer emission appears even for the solutions of low concentrations. This means that the copolymer with higher fluorocarbon content possesses a greater ability to associate at low concentrations, and part of $PyCOR_f$ has moved to the interior of the hydrophobic microregion while part remains in the water. Second, at the high concentration range, the emission intensity band around 550 nm relative to the monomer emission becomes negligible, which indicates that most $PyCOR_f$ molecules have transferred from the water phase to the hydrophobic microdomains. This feature can be more clearly seen in Figure 9, which shows the emission spectra of $PyCOR_f$ normalized at I_{375} in Co-4 solutions of different concentrations. It is interesting to note that for the cases of Co-4 (Figure 9) and Co-5 (Figure 8) solutions, as the copolymer concentration increases, a

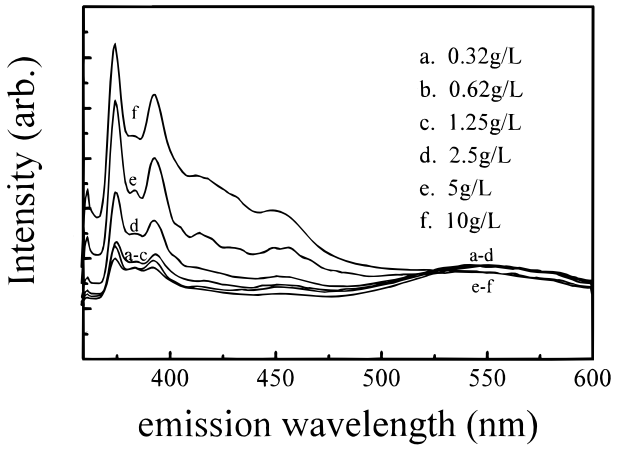


Figure 8. Fluorescence emission spectra of $PyCOR_f$ (2×10^{-6} M) in solutions of copolymer Co-5 with different concentrations in water.

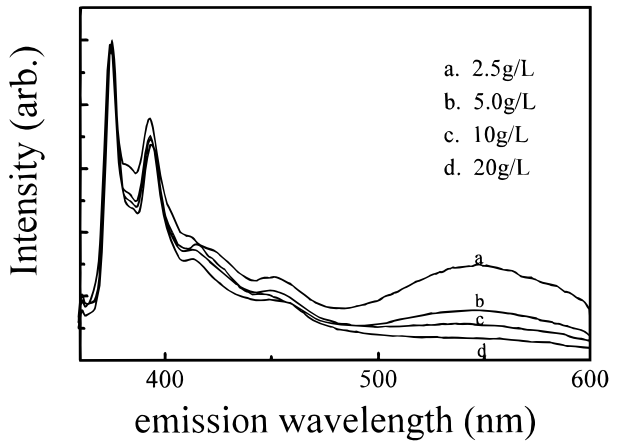


Figure 9. Fluorescence emission spectra of $PyCOR_f$ (2×10^{-6} M) normalized at I_{375} in solutions of copolymer Co-4 with different concentrations in water.

new emission peak around 450 nm appears, and its intensity increases with increasing concentration. Since this appearance of the band only occurs for the copolymers with higher fluorocarbon content at higher concentrations and the location of the peak is quite similar to that of $PyCOR_f$ in methanol at high concentrations (Figure 3) and in perfluoro surfactant solution (Figure 6), this peak is probably associated with the excimer emission of $PyCOR_f$ in nonhydrophilic microenvironments. In addition, the higher the content of fluorocarbon chains in the copolymer is, the more apparent the two features will be. All these facts show that $PyCOR_f$, the probe with the fluorocarbon substituent, possesses a much greater affinity to fluorocarbon chains than pyrene itself.

Figure 10 summarizes the results of emission spectra of the homopolymer and copolymers with different fluorocarbon contents by plotting the monomer emission intensity at 375 nm versus polymer concentration. In the case of homopolymer, I_m shows a slight increase as the concentration increases. This reflects the weak tendency of hydrophilicity decrease of the solutions as the incorporation of the homopolymer. However, for the copolymers, the emission intensity remains fairly constant or varies only slightly when the concentration is below a critical value; above this value, it shows an upswing. For the three copolymers with low fluorocarbon contents, the combined data give a critical concentration $C \sim 4$ g/L. This concentration shifts to around

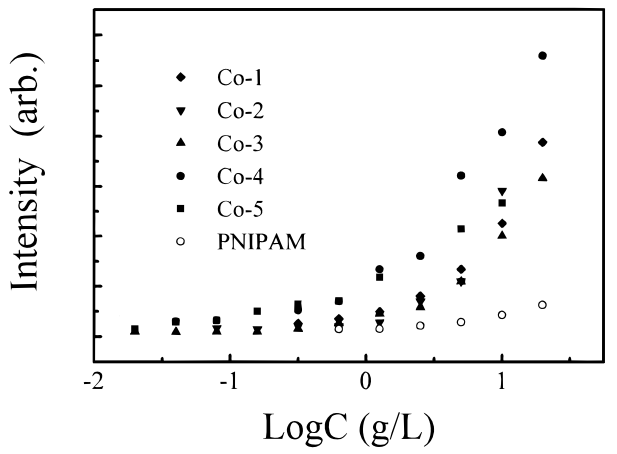


Figure 10. Emission intensity I_{375} of $PyCOR_f$ (2×10^{-6} M) as a function of polymer concentration in water.

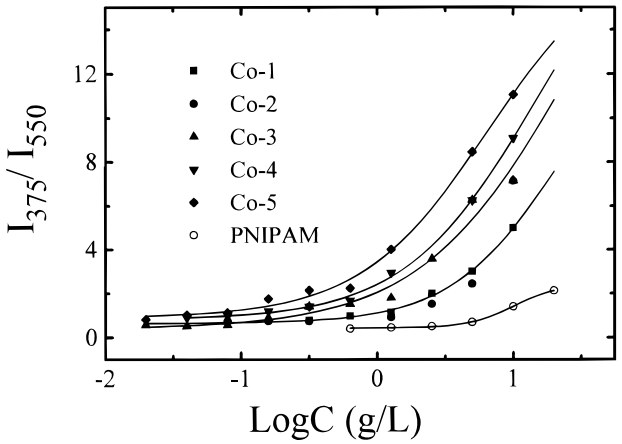


Figure 11. Plot of intensity ratio I_{375}/I_{550} of $PyCOR_f$ in water solutions as a function of polymer concentration.

$C \sim 1.6$ g/L for the copolymer with the high fluorocarbon content. The relatively large scatter of the data points and the broad range of concentration of transition make it difficult to pinpoint the onset of association. However, the dependence of the association on the fluorocarbon contents can be clearly seen. For example, for Co-1 and Co-2, a detectable increase of the intensity cannot be found until the polymer concentration reaches $C \sim 0.3$ g/L, while for Co-4 and Co-5, a detectable increase in the intensity can be seen even at a concentration much less than 0.1 g/L. As mentioned above, the decrease of excimer emission in bulk water phase also reflects the partitioning of $PyCOR_f$ between the aqueous and hydrophobic regions.

We tried to use the intensity ratio I_{375}/I_{550} as another indicator of the process. The results are shown in Figure 11. In the homopolymer solution, the intensity ratio remains almost constant until the concentration reaches about 3−5 g/L, where a detectable increases appears, reflecting the slight change of hydrophobicity of the medium. In the cases of copolymers, this intensity ratio increase starts at much lower concentrations and grows much more rapidly as the concentration increases further. For the copolymers with a low fluorocarbon content, there is a concentration range at which the intensity changes little until the concentration reaches about 0.6 g/L. However, for the copolymers with a high fluorocarbon substituent such as Co-4 and Co-5, a detectable increase of the intensity ratio already appears at the much lower value of 0.2 g/L. These results are in accordance with those of monomer emis-

sion (Figure 10). They imply that the emission intensity ratio of monomer to excimer in water can be used as another indicator of association of the hydrophobic species in water. Besides, since the intensity ratio is a fairly smooth function of the concentration, it is possible to use it to estimate the critical concentrations, identifying it by the intersection of two straight line segments: one, almost horizontal, through the points at the low-concentration range and one through the points in the rapidly rising part. Thus, the critical association concentrations for Co-1, Co-2, Co-3, Co-4, and Co-5 were found to be 3.1, 3.1, 1.8, 1.3, and 0.8 g/L, respectively.

Free Probe, Chemically Bound Probe, and Free Substituted Probe. Fluorescence studies on hydrophobic association of polymers rely on the use of a dye, either free or chemically bound. Using a free probe, which is usually introduced into polymer solutions by injection of a small amount of dye solution in a water-miscible solvent, is convenient. However, as pointed out by Winnik,[44] because the probes necessarily have a very low solubility in water, microcrystals of the probes almost always form when they are introduced into water solutions of the polymers. The microcrystals disappear slowly as the probe dissolves in the hydrophobic domains. In addition, in the case of using a free probe, the location of the probe is not known with certainty because it is free to diffuse through the entire sample although it may be preferentially solubilized in the hydrophobic environment.[26] In exploring association due to fluorocarbon chains, using aromatic probes such as pyrene encounters another problem. Because of the poor affinity of pyrene with fluorocarbon chains, as evidenced in the study of perfluoro surfactants,[39] dissolution of the pyrene microcrystals becomes very difficult. That is why we could not find a regular increase of the intensity of monomer emission of free pyrene, only scattered data, as the polymer concentration was increased.

The difficulties caused by the use of a free probe in exploring hydrophobic associations can be overcome by attaching the chromophore chemically to the polymers, especially to a position near the hydrophobic species. Using covalently bound probes can remove doubts concerning the solubilization site.[45] Therefore, the dye labeling technique has been widely used in the study of hydrophobic associations. However, there is an obvious disadvantage to this technique, *i.e.*, the chromophores introduced are always by nature hydrophobic, and so, the labeled polymers may be viewed as hydrophobically modified even without hydrocarbon or fluorocarbon chains present. To minimize the effect, less than one fluoroprobe per polymer chain was suggested.[21,46] Schild[40] investigated pyrene-labeled PNIPAM with less than one dye molecule per chain. Even at such a low label content, they observed a slight depression of the LCST and a broadening in the transition. Winnik *et al.*,[19−21] labeling HPC at a level similar to that of Schild's sample or sometimes even 1 order of magnitude more heavily, found a double LCST for one of the samples.[20] Chu *et al.*[46] observed differences in the conformational transition of poly(methacrylic acid) with positioning the pyrene unit at polymer ends versus randomly along the chain. Obviously, heavy labeling may cause associations, but the low-level labels may only provide limited information. For example, excimer emission was not observed in studying complexation of PNIPAM with surfactant SDS, when low-level pyrene labels were employed;[40] therefore, the only indicator that could be used was the absolute intensity of the monomer emission, which is more sensitive to environment hydrophobicity. However, Winnik *et al.*[47] and Turro *et al.*[48] succeeded in studying polymer−surfactant complexation using the intensity ratio of I_e/I_m as an indicator. This success is due to the use of the heavy level labeling in the samples studied.

In this study we concentrated on using free fluorocarbon-substituted pyrene as a probe in order to overcome the difficulties caused by low solubility of pyrene in fluorocarbon microdomains. Obviously, this kind of probe itself will not cause association of polymers but has a relatively large solubility in the fluorocarbon microdomains. This good affinity between the probe and the hydrophobic domain enables it to always be located preferentially in the hydrophobic area. This is evidenced by the regularity and reproducibility of the data of $PyCOR_f$ in the solutions of PNIPAM and its fluorocarbon-substituted copolymers. We can regard this fluorocarbon-substituted pyrene as a target probe for fluorocarbon-constituted microdomains. The results of this study show that this target probe provides more reliable information than the corresponding free unmodified probe and avoids the additional contribution to association caused by chemically bound probes.

Conclusions

1. Fluorocarbon-modified PNIPAM in aqueous solution shows pronounced hydrophobic association as revealed by fluorospectroscopy. All the copolymers studied with fluorocarbon contents ranging from 0.06 to 0.88 mol % display hydrophobic associations as judged by abrupt changes in the I_1/I_3 of pyrene and the I_{375} and I_{375}/I_{550} of fluorocarbon-substituted pyrene ($PyCOR_f$) with polymer concentration. The critical association concentrations for the PNIPAM copolymers with the highest (0.88 mol %) and the lowest (0.06 mol %) fluorocarbon contents were 0.8 and 3.1 g/L, respectively. The results confirmed that hydrophobic association is much more pronounced in fluorocarbon-substituted water-soluble polymers than in corresponding hydrocarbon-substituted ones.

2. In the fluorescence study using unmodified pyrene as the probe, the intensity ratio I_1/I_3 varies with both the copolymer concentration and composition, *i.e.*, I_1/I_3 decreases from 1.75, a value characteristic of pyrene in water, to 1.2 indicative of hydrophobic domains, when the copolymer concentration is increased to 10−50 g/L. This change might be regarded as an indication of hydrophobic association. However, this decrease includes a contribution from the PNIPAM backbone which is more hydrophobic than other water-soluble polymers. Also, corresponding changes in the excitation spectrum were not convincingly detected, namely, the (0,0) band of the excitation spectrum shows only a small red shift and the intensity ratio I_{338}/I_{332} reaches only 0.72, much smaller than for pyrene in a micellar structure, even when the polymer concentration is quite high. This implies that the main part of pyrene has probably not moved from the aqueous bulk to the hydrophobic microdomains in which the solubility of pyrene is limited. Therefore, we conclude that free pyrene provides some indication of hydrophobic association of the fluorocarbon-substituted PNIPAM, but the evidence is not compelling.

3. $PyCOR_f$, the fluorocarbon-modified pyrene in which the fluorocarbon chain is identical with that in PNIPAM copolymers, was found to possess a much

higher solubility in fluorocarbon micelles and displays a strong tendency of self-aggregation in an aqueous medium. This property enables the modified pyrene to always be located preferentially in the hydrophobic area once the domains form. Therefore, it can serve as a target probe for the association of fluorocarbon chains. The association is evidenced by a regular increase in the intensity of the monomer emission (375 nm) and by a decrease of the excimer emission at 550 nm. As the polymer concentration is increased to a critical value, which depends on the fluorocarbon content in the copolymers, plots of I_{375} and I_{375}/I_{550} versus copolymer concentration rise sharply. This means that the probe is being transferred from the aqueous bulk to the hydrophobic microdomains. The plots reveal the dependence of the hydrophobic associations on both polymer concentration and fluorocarbon contents. The results show that our fluorocarbon-modified pyrene provides more reliable information than the original pyrene dose. Furthermore, our modified pyrene, being a free probe, retains the advantages of the original pyrene: it is simple to use in practice and makes no contribution to polymer association, the last is almost unavoidable when using covalently bound fluorescent probes.

Acknowledgment. This work was supported by the National Natural Science Foundation of China and by the National Basic Research Project—Macromolecular Condensed State. The light scattering experiments were carried out in Dr. Chi Wu's laboratory in The Chinese University of Hong Kong. The authors are very grateful to Prof. M. Winnik for helpful discussion during his visit to Shanghai in 1994.

References and Notes

(1) *Macromolecular Complexes in Chemistry and Biology*; Dubin, P., Bock, J., *et al*., Eds.; Springer-Verlag: New York, 1994; Part 1.
(2) *Water Soluble Polymers*; Shalaby, S., McCormick, C., Butler, G., Eds.; ACS Symposium Series 467; American Chemical Society: Washington, DC, 1991.
(3) Wang, Y.; Winnik, M. *Langmuir* **1990**, *6*, 1437.
(4) Duhamel, J.; Yekta, A.; Hu, Y.; Winnik, M. *Langmuir* **1993**, *9*, 881.
(5) Duhamel, J.; Yekta, A.; Hu, Y.; Winnik, M. *Macromolecules* **1992**, *25*, 7024.
(6) Yekta, A.; Duhamel, J.; Brochard, P.; Adiwidjaja, H.; Winnik, M. *Macromolecules* **1993**, *26*, 1829.
(7) Yekta, A.; Xu, B.; Buhamel, J.; Adiwidjaja, H.; Winnik, M. *Macromolecules* **1995**, *28*, 956.
(8) Ringsdorf, H.; Simon, J.; Winnik, F. *Macromolecules* **1992**, *25*, 5353.
(9) Ringsdorf, H.; Venzmer, J.; Winnik, F. *Macromolecules* **1991**, *24*, 1678.
(10) Winnik, F.; Dovidson, A.; Hamer, G.; Kitano, H. *Macromolecules* **1992**, *25*, 1876.
(11) Rao, B.; Vemura, Y.; Dyke, L.; Macdonald, P. *Macromolecules* **1995**, *28*, 531.
(12) Uemura, Y.; McNulty, J.; Macdonald, P. *Macromolecules* **1995**, *28*, 4150.
(13) Persson, K.; Adramsen, S.; Stilbs, P.; Hansen, F.; Walderhaug, H. *Colloid Polym. Sci.* **1992**, *270*, 465.
(14) McCormick, C.; Hoyle, C.; Clark, M. *Polymer* **1992**, *33*, 243.
(15) Seery, T.; Yassini, M.; Hogen-Esch, T.; Amis, E. *Macromolecules* **1992**, *25*, 4784.
(16) Zhang, Y. B.; Wu, C.; Zhang, Y. X. *Macromolecules* **1996**, *29*, 2494.
(17) Ezzell, S.; McCormick, C. In ref 2, Chapter 8.
(18) Varadaraj, R.; Branham, K.; McCormick, C.; Bock, J. In ref 1, Chapter 2.
(19) Winnik, F.; Winnik, M.; Tazuke, S.; Ober, C. *Macromolecules* **1987**, *20*, 38.
(20) Winnik, F. *Macromolecules* **1987**, *20*, 2745.
(21) Winnik, F. *Macromolecules* **1989**, *22*, 734.
(22) Char, R.; Frank, C.; Gast, A.; Tang, W. *Macromolecules* **1987**, *20*, 1833.
(23) Schild, H. G. In ref 1, Chapter 16, p 249.
(24) Schild, H. G.; Tirrell, D. *Langmuir* **1991**, *7*, 1317.
(25) Winnik, F. *Polymer* **1990**, *31*, 2125.
(26) Winnik, F. *Macromolecules* **1990**, *23*, 233.
(27) Myers, D. *Surfactant Science and Technology*; VCH Publishers: New York, 1988; Chapter 3.
(28) Zhang, Y.-X.; Da, A.-H.; Hogen-Esch, T. E.; Butler, G. In ref 2, Chapter 10, p 159.
(29) Zhang, Y.-X.; Huang, F.; Hogen-Esch, T. In ref 1, Chapter 7, p 95.
(30) Zhang, Y.-X.; Da, A.-H.; Butler, G.; Hogen-Esch, T. *J. Polym. Sci., Part A: Polym. Chem.* **1992**, *30*, 1383.
(31) Hwang, F.; Hogen-Esch, T. *Macromolecules* **1995**, *28*, 3328.
(32) *Friedel-Crafts and Related Reactions*; Olah, G., Ed.; Wiley Interscience: New York, 1963; Vol. 3, p 78.
(33) Tung, C.; Ouyiang, X.; Liu, Y. *Photograph. Sci. Photochem.* (Chinese) **1988**, No. 4, 17.
(34) Wu, C.; Xia, K.-Q. *Rev. Sci. Instrum.* **1994**, *65*, 587.
(35) Wilhelm, M.; Zhao, C.; Wang, Y.; Xu, R.; Winnik, M.; Mura, J.; Reiss, G.; Croucher, M. *Macromolecules* **1991**, *24*, 1033.
(36) Astafieva, I.; Zhong, X.; Eisenberg, A. *Macromolecules* **1993**, *26*, 7339.
(37) Kalyanasundaram, K.; Thomas, J. *J. Am. Chem. Soc.* **1977**, *99*, 2039.
(38) Nakajima, A. *Photochem. Photobiol.* **1977**, *25*, 593.
(39) Kalyanasundaram, K. *Langmuir* **1988**, *41*, 942.
(40) Schild, H. In ref 2, Chapter 17, p 262.
(41) Jiang, X. K. *Acc. Chem. Res.* **1988**, *21*, 362.
(42) Zhang, J. T.; Cui, X. L.; Ji, G. Z.; Jiang, X. K. *Chinese Chem. Lett.* **1993**, *4*, 703.
(43) Tung, C. H.; Ji, H. F. *Chinese Chem. Lett.* **1995**, *6*, 31.
(44) Winnik, F. In *Interactions of Surfactants and Polymers and Proteins*; Goddard, E., Ananthapadmanabham, K., Eds.; CRC Press: London, 1992; Chapter 9.
(45) McGlade, M.; Olufs, J. *Macromolecules* **1988**, *21*, 2346.
(46) Chu, D.; Thomas, J. *Macromolecules* **1984**, *17*, 2142.
(47) Winnik, F.; Winnik, M.; Tazuke, S. *J. Phys. Chem.* **1987**, *91*, 594.
(48) Chandar, P.; Somasundaran, P.; Turro, N. *Macromolecules* **1988**, *21*, 950.

MA960966O