Characterization of the Association Level of Pyrene-Labeled HASEs by Fluorescence

Telmo J. V. Prazeres, Rachel Beingessner, and Jean Duhamel*

Institute of Polymer Research, Department of Chemistry and Biochemistry, University of Waterloo, 200 University Avenue West, Waterloo, ON N2L 3G1, Canada

Keith Olesen, Greg Shay, and David R. Bassett

The Dow Chemical Company, UCAR Emulsion Systems, 410 Gregson Drive, Cary, North Carolina 27511

Received May 29, 2001; Revised Manuscript Received August 1, 2001

ABSTRACT: The hydrophobic associations of three hydrophobically modified alkali swellable emulsion polymers (HASE) were studied by fluorescence. The chromophore pyrene was used as the hydrophobic pendant. The level of association was characterized qualitatively and quantitatively by monitoring the fluorescence behavior of the pyrene monomer and excimer. Excimer formation occurs mostly by diffusion in an organic solvent (tetrahydrofuran, THF) where the pyrene moieties are not associated. In aqueous solutions where pyrene is insoluble, excimer formation occurs mostly via direct excitation of ground-state pyrene aggregates. The level of association of these pyrene-labeled HASEs was determined quantitatively by analyzing the monomer and excimer fluorescence decays using a *blob* model. It was found that 58% of the hydrophobic pendants were associated in aqueous solutions, but that only 5% of them were associated in THF.

Introduction

In recent years, hydrophobically modified alkali swellable emulsion polymers (HASE) have become a major type of associative polymer (AP).[1] Their backbone is a terpolymer made of methacrylic acid (MAA, about 49 mol %), ethyl acrylate (EA, about 50 mol %), and a hydrophobically modified macromonomer (HMM, about 1 mol %).[2] The macromonomer is composed of a α-methylstyrene monomer and a hydrophobe connected via a poly(ethylene oxide) spacer. In basic solution (pH > 8), the methacrylic acid functions are neutralized, and solubility in aqueous solution is ensured. In a basic solution, a HASE is a polyelectrolyte and its dimensions increase due to a same-charge repulsion on the polymer backbone. Swelling of the coil promotes intermolecular associations between the hydrophobes, leading to the formation of a polymeric network and a sharp increase in viscosity. Since these interpolymeric linkages are physical and not chemical, they can be disturbed and broken under application of a stress such as shear. Under shear some linkages of the network structure are severed and the viscosity drops, a phenomenon referred to as shear thinning. This behavior is well documented and has been reported in numerous papers.[2,3,6]

HASEs' appealing rheological properties have triggered the interest of numerous research groups aiming at understanding their behavior at the molecular level. Two parameters are of particular interest for the characterization of HASEs, namely the residence time (τ_{RES}) of a hydrophobe inside a hydrophobic aggregate and the average number of hydrophobes per aggregate (N_{agg}). In the case of more easily characterized associative polymers such as hydrophobically modified ethoxylated urethane (HEUR), the parameters τ_{RES} and N_{agg} have been determined by rheology[4] and fluorescence,[5] respectively. Despite numerous efforts, these parameters have proved elusive for HASEs because this class of APs yields several relaxation times by rheology[6] (compared to a single relaxation time for HEUR)[4] and the expected polydispersity in size of the hydrophobic aggregates[6,7] makes it difficult to apply the classic Poisson protocol used by fluorescence. With time, these efforts will come to fruition.

APs interesting viscoelastic properties are due, in part, to the level of association existing between APs associating pendants. In view of the associative nature of APs, it is surprising that very few reports investigate APs' *level of association* (i.e., how many hydrophobes are associated and how many are unassociated). Considering the strong scientific interest gathering toward HASE characterization, the investigation of the HASE level of association was undertaken. In this study, the HASE hydrophobe was the pyrene chromophore instead of more conventional saturated hydrocarbons. The reason for choosing pyrene as a hydrophobic moiety resides in pyrene's ability to form excimers.[8,9]

Since an excimer results from the encounter between an excited pyrene and a ground-state pyrene, monitoring carefully the process of excimer formation yields information about the HASE coil conformation in organic and aqueous solvents. First steady-state and time-resolved fluorescence were applied to develop a qualitative understanding on how solvent conditions (organic vs aqueous solvent, effect of salt) affect HASE coil structure. Then we took advantage of a new theory based on the use of *blobs*.[10] This *blob* model accounts for the distribution of rate constants of excimer formation, which arises from the distribution of distances spanning two pyrenes randomly attached along a polymer backbone. Using this *blob* model, the fluorescence decays of the pyrene-labeled HASEs could be quantitatively analyzed to retrieve the level of association of

---

* To whom correspondence should be addressed.

10.1021/ma0109065 CCC: $20.00

Scheme 1

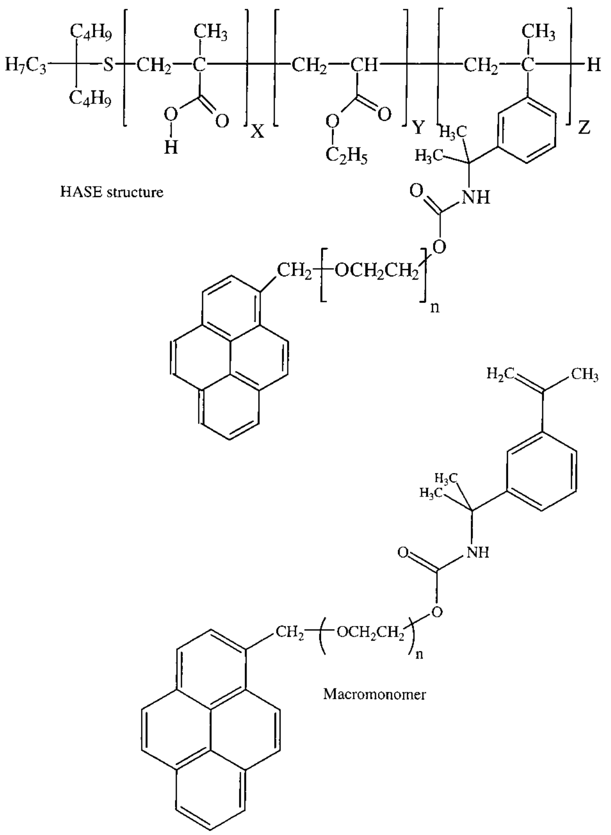


Table 1. Weight (*W*) and Molar (*X*) Fractions of the Reaction Mixtures for the Synthesis of HASE11, HASE42, and HASE65 and Pyrene Content of the Three HASEs Determined by UV−Vis Absorbance Measurements

sample	W_{MAA}; X_{MAA}	W_{EA}; X_{EA}	W_{HMM}; X_{HMM}	pyrene content (λ) (μmol of pyrene/g of polymer)
HASE65	0.35; 0.49	0.40; 0.49	0.25; 0.021	65
HASE42	0.35; 0.44	0.50; 0.55	0.15; 0.011	42
HASE11	0.35; 0.40	0. 60; 0.69	0.05; 0.0035	11

these polymers as well as determine the kinetics of association in aqueous and organic solvents.

Experimental Section

Materials. Hexanes (HPLC grade, Fischer), THF (glass-distilled, Caledon), NaOH (BDH), KCl (BDH), and sodium dodecyl sulfate (SDS, EM Science) were used as received. Nitromethane (Aldrich) was distilled. Milli-Q Water was used to prepare the aqueous solutions. The polymer solutions were dialyzed with a Spectra/Por membrane (MWCO: 3500 spectrum).

Polymer Synthesis. The terpolymers (Scheme 1) were synthesized by the Dow Chemical Corp., UCAR Emulsion Systems (DOW), via an emulsion polymerization reaction. Details of the synthesis have been described elsewhere.[2] The only difference was the introduction of 1-pyrenemethanol instead of the usual hydrophobic alcohol.

The weight and molar fractions in MAA, EA, and HMM are listed in Table 1 for all three polymers.

Polymer Purification. For fluorescence studies, it was necessary to eliminate impurities and small molecules (such as unattached pyrene dyes) present in the polymer dispersion supplied by DOW. Water was removed from the aqueous dispersion under vacuum. The recovered solid was dissolved in THF. The THF solution was centrifuged to remove any cross-linked polymer. The supernatant was precipitated with hexanes. After traces of solvent were removed under vacuum, the solid was redissolved in THF, and the precipitation cycle was repeated three times. For the studies in THF, the solid was dissolved in THF to make a stock solution, which was later diluted to the required polymer concentration. For the studies in aqueous solutions, the resulting solid was then mixed with a concentrated NaOH solution (1 mol/L). Vigorous stirring for a few hours ensured dissolution. Then, the solutions were dialyzed for a week against either water at pH 9 or a 0.05 M KCl aqueous solution at pH 9.

UV Absorbance Measurements. They were carried out on a Hewlett-Packard 8452A diode array spectrophotometer.

Pyrene Content of Polymer Samples. The pyrene content (λ, in mol of pyrene/g of polymer) was determined by measuring the absorbance of a solution prepared by careful dilution of an initial stock solution. THF is a good solvent for pyrene and few pyrene−pyrene interactions are expected to occur between the pyrene groups.[11] Consequently, the absorbance spectra should not be distorted. The pyrene concentration of a polymer solution was then estimated from the absorbance value at 344 nm and the extinction coefficient of a pyrene model compound, typically 1-pyrenemethanol (ϵ[344 nm, in THF] = 42 700 M^{-1} cm^{-1}). The pyrene content of all polymers is listed in Table 1.

Determination of Polymer Concentration in Water. In water, the hydrophobic interactions induce associations between the pyrene groups. This process is known to affect the features of the pyrene absorbance spectrum. To determine the polymer concentration in aqueous solutions and avoid pyrene−pyrene associations, aliquots of polymer solutions were mixed with a concentrated (0.5 M) SDS solution, so that all pyrene groups are located in different micelles. The pyrene concentration of the solution could then be determined from its absorbance value at 344 nm and the extinction coefficient value of 1-pyrenemethanol in 0.5 M SDS solution (ϵ [344 nm, in 0.5 M SDS solution] = 39 900 M^{-1} cm^{-1}). The polymer concentration was obtained using the pyrene content of the polymer listed in Table 1.

Steady-State Fluorescence Measurements. They were carried out on a Photon Technology International LS-100 steady-state system with a pulsed xenon flash lamp as the light source. Fluorescence spectra of HASE solutions in THF were obtained using the right angle geometry for polymer concentrations below 1 g/L and the front face geometry for polymer concentrations above 1 g/L. The samples were degassed with a gentle flow of nitrogen (for 20 min) to prevent oxygen quenching. In water, the samples were not degassed due to foam formation. The fluorescence spectra were acquired for polymer concentrations ranging from around 0.02 g/L to 18 g/L with a fluorescence microcell (Hellma, with an inner cross section of 3 × 3 mm) using the usual right angle configuration.

Emission spectra were acquired by exciting the samples at 344 nm. The fluorescence intensities of the monomer (I_M) and the excimer (I_E) were calculated by taking the integrals under the fluorescence peaks from 372 to 378 nm for the pyrene monomer and from 500 to 530 nm for the pyrene excimer.

Time-Resolved Fluorescence Spectroscopy. The fluorescence decay profiles were obtained by the time-correlated single photon counting (TCSPC) technique. More details about this instrumentation can be found in earlier references.[10]

Fluorescence Decay Analysis. A function $g(t)$ was assumed for the fluorescence decays. It was convoluted with the instrument response function to fit the experimental decay.[12] A light-scattering correction was applied to the analysis to account for residual light scattering reaching the detector.[12] Two methods were used to optimize the fit of the fluorescence decays.

The first method was based on the Marquardt−Levenberg algorithm.[13a] The function $g(t)$ was assumed for the fluorescence decays. It was either a sum of exponentials with an expression given in eq 1 where the number of exponentials n is varied from 1 to 3, or the more complicated eq 2, which is presented in the Discussion section. The index X in eq 1 is either M or E for the monomer or the excimer, respectively.

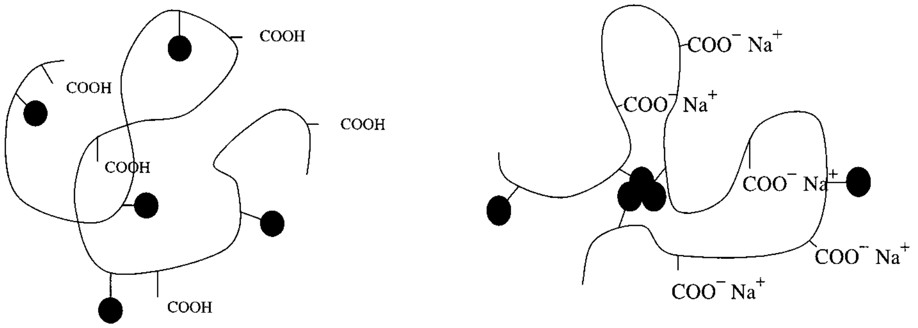
Scheme 2

⇒ **In THF**

the entire polymer is soluble and no hydrophobic interactions between pyrene groups are expected (THF is a "good" solvent for pyrene).

⇒ **In aqueous solution,**

water is a "poor" solvent for pyrenes leading to pyrene-pyrene associations



The parameters of $g(t)$ were retrieved by using a least-squares curve fitting program based on the Marquardt–Levenberg algorithm.[13a]

$$g(t) = \sum_{\substack{i=1 \\ X=M,E}}^{i=n} A_{Xi} e^{-t/\tau_{Xi}} \qquad (1)$$

The second method was applied to fit the excimer fluorescence decays with eq 3. Fixing the parameters A_2, A_3, and A_4 retrieved from eq 2, τ_{E1} and τ_{E2} in eq 3 transforms this equation into a linear sum of four functions. Equation 3 involves an infinite series, which was calculated up to the 11th term in our analysis programs. According to the experimental parameters that we retrieved from our analysis, the contribution of the higher terms is negligible. A general linear least-squares routine was used to fit the excimer fluorescence decays.[13b] The χ^2 parameter was optimized by applying a Golden section search to the lifetime τ_{E1}.[13c]

For all fluorescence decays, the quality of the fits was assessed from the χ^2 parameter ($\chi^2 < 1.3$ for a good fit) and the random distribution of the residuals and of the autocorrelation function.

Results

The process of excimer formation between the pyrenes attached onto the HASE copolymers was investigated in THF and aqueous solutions at pH 9. Scheme 2 illustrates the expected differences in behavior for the pyrene-labeled HASE. In THF, the pyrene groups do not associate. In aqueous solution at pH 9, the electrostatic repulsion between ionized acid functions induces an expansion of the polymer coil, which is couterbalanced by hydrophobic pyrene–pyrene associations. Steady-state fluorescence spectra and time-resolved fluorescence decays of the pyrene-labeled polymers were acquired and their behaviors did not depend on polymer concentration. They are shown in Figures 1 and 2 for THF and aqueous solutions at pH 9 with 0.05 M KCl. The steady-state spectra acquired in THF and aqueous solutions show similar trends, i.e., a higher pyrene content yields more encounters between the pyrene pendants and more excimer is being produced as expected by a simple Birks' scheme analysis.[8] However, very different behaviors between the THF and the aqueous solutions are observed by time-resolved fluorescence.

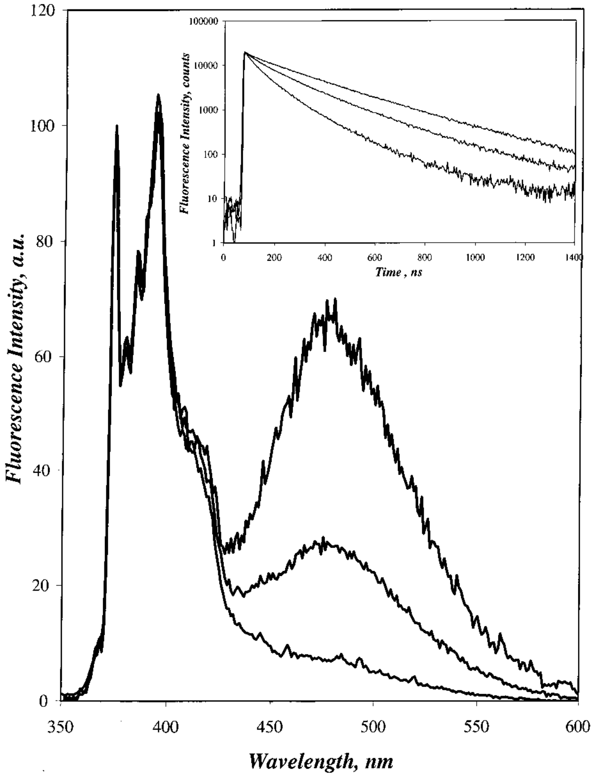


Figure 1. Steady-state fluorescence spectra of pyrene-labeled HASE in THF excited at 344 nm and normalized at 375 nm. The solutions were dilute in pyrene with pyrene concentrations ranging from 2.6×10^{-6} to 8.0×10^{-6} M. From top to bottom: HASE65 (0.04 g/L), HASE42 (0.09 g/L), and HASE 11 (0.35 g/L). Inset: Overlap of the normalized time-resolved fluorescence decays of the pyrene monomer excited at 344 nm and collected at 375 nm. The solutions are the same as for the steady-state spectra with the HASE65 and HASE11 solutions exhibiting the shortest and longest decays, respectively.

The fluorescence exhibited by the pyrene-labeled polymers is a consequence of the various fluorescence species present in solution. Information about the nature of those species is obtained by analyzing the time-resolved fluorescence decays. Scheme 3 A) describes how excimer is expected to form. A fraction of pyrene moieties (f_{agg}) is associated in the ground-state and absorbs a fraction $f_{agg} \times h\nu$ of the excitation light.

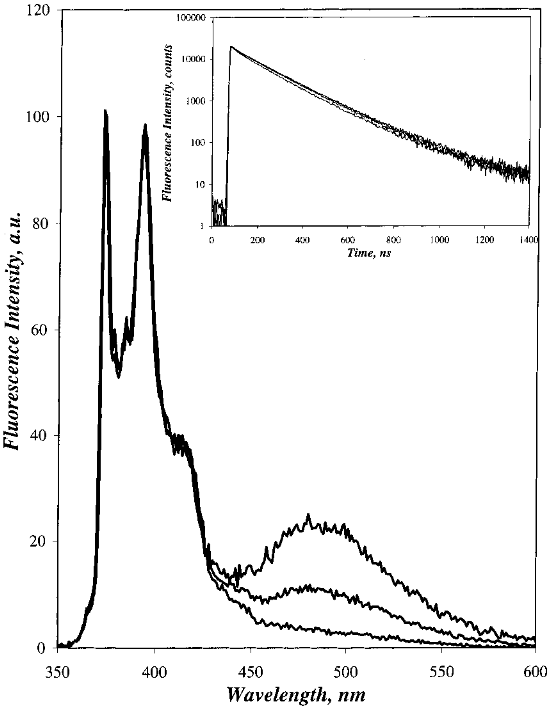
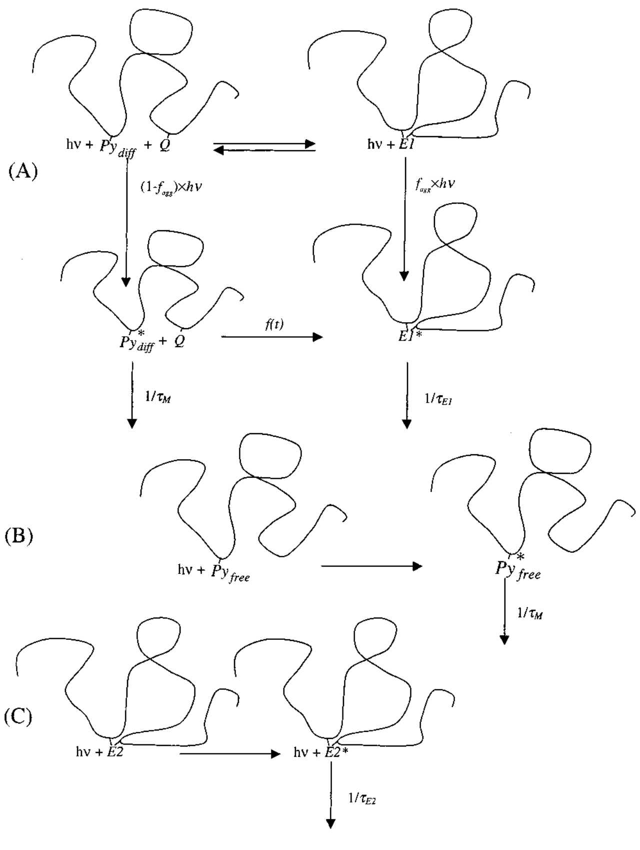


Figure 2. Steady-state fluorescence spectra of pyrene-labeled HASE in water pH 9 with 0.05 M KCl excited at 344 nm and normalized at 375 nm. From top to bottom: HASE65 (0.05 g/L), HASE42 (0.19 g/L), and HASE11 (0.50 g/L). Inset: Overlap of the normalized time-resolved fluorescence decays of the pyrene monomer excited at 344 nm and collected at 375 nm. The solutions are the same as for the steady-state spectra.

Scheme 3



Those excited pyrene aggregates form excimer instantaneously. A fraction $(1 - f_{agg}) \times h\nu$ of the light is absorbed by single pyrene moieties, which can either encounter a ground-state pyrene or a ground-state pyrene aggregate to form an excimer via diffusion, or not encounter any ground-state species and emit with their own lifetime (τ_M). In the following, the excited pyrene monomers, which form excimer via diffusion, are referred to as Py$^*_{diff}$, whereas the excited pyrenes, which emit their own fluorescence, are referred to as Py$^*_{free}$. Excimers which are formed by diffusion are created with a complicated function *f*(*t*), whose expression is given in eqs A4 and A5 in the Supporting Information. The function *f(t)* is a consequence of the distance distribution existing between pyrene groups randomly attached along the polymer backbone.

The monomer and excimer fluorescence decays were complicated and were fitted with three exponentials. For most studies, the parameters retrieved from the monomer fluorescence decays were constant for a given polymer/solvent system over the entire concentration range studied. The values reported in this study are averaged over all polymer concentrations, and the listed errors represent the standard deviations calculated over all the fluorescence decays acquired for a given polymer/solvent system. In the monomer decay analysis, the third long decay time was fixed to equal the lifetime τ_M of Py$^*_{free}$ in a given solvent. τ_M was determined from the fluorescence decay of the pyrene-labeled macromonomer and was found to equal 276 and 170 ns in THF and aerated aqueous solutions, respectively. The two shorter decay times were attributed to the process of excimer formation via diffusion. The excimer fluorescence decays were also fitted with three exponentials, where all decay times were optimized. The preexponential factors and the decay times retrieved from the monomer and excimer decay analysis are listed in Table 1a–f in the Supporting Information.

The fluorescence decays were acquired at polymer concentrations ranging from as low as 0.06 g/L up to as high as 41 g/L. In this concentration range, some general features were observed for the monomer and excimer fluorescence decays. In THF increasing the pyrene content of the polymer produces more excimer via diffusion leading to a clear reduction of the average decay time of the pyrene monomer (cf. inset in Figure 1). As the pyrene content decreases, more pyrenes are isolated and do not form excimer, less diffusional encounters take place and the fraction of the decay contribution arising from unquenched pyrene (a_{M3}) increases from 0.02 ± 0.01 for HASE65 to 0.41 ± 0.03 for HASE11. A different behavior is observed in aqueous solutions, where all fluorescence decays of the monomer seem to overlap within experimental error regardless of pyrene content (cf. Insert in Figure 2). This is due in part to the very large contribution of the unquenched pyrenes. Indeed a_{M3} ranges from 0.51 ± 0.12 for HASE65 to 0.67 ± 0.06 for HASE11, a contribution much larger than in THF. The expansion of the polymer coil following same charge repulsion between ionized MAA functions generates more isolated pyrenes in aqueous solution than in THF. Since far less pyrene monomers produce excimer via diffusion in aqueous solutions, the Py$^*_{diff}$ contribution is less visible than in THF (insert in Figure 1) and the monomer decays appear to overlap (insert in Figure 2).

In THF, the monomer decay times averaged over all polymer concentrations ($\langle\tau\rangle = \sum_{i=1}^{3} a_{Mi}\tau_{Mi}/\sum_{i=1}^{3} a_{Mi}$) were found to equal 199 ± 4, 135 ± 6, and 79 ± 3 ns for HASE11, HASE42, and HASE65, respectively. The

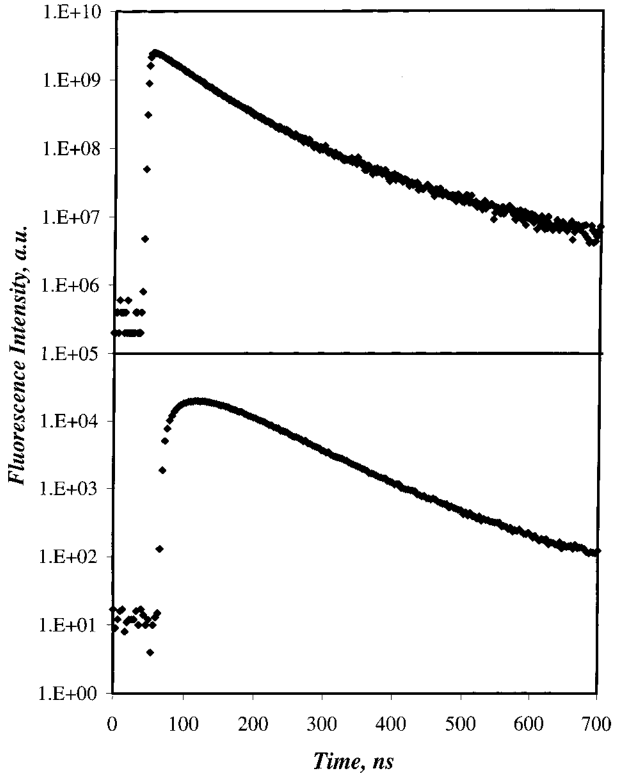


Figure 3. Time-resolved fluorescence decays of the excimer excited at 344 nm and collected at 510 nm. Top: HASE65 in water pH 9 with 0.05 M KCl (0.05 g/L). Bottom: HASE65 in THF (0.04 g/L).

average decay times for the monomer in aqueous solutions were found to equal 145 ± 4, 135 ± 5, and 122 ± 5 ns for HASE11, HASE42, and HASE65, respectively. A 6-fold increase of the pyrene content of the HASEs leads to a 2.5 decrease of $\langle\tau\rangle$ in THF. This is a consequence of the increased number of diffusional encounters inside the polymer coil resulting in a 10-fold increase in the formation of excimer. Indeed the ratios of excimer intensity over monomer intensity (I_E/I_M) averaged over all polymer concentrations in THF equal 0.05 ± 0.00, 0.24 ± 0.02, and 0.51 ± 0.02 for HASE 11, HASE42, and HASE65, respectively. On the other hand, $\langle\tau\rangle$ decreases by a factor of 1.2 in aqueous solutions when the HASE pyrene content is increased 6-folds. This indicates that increasing the pyrene content leads to a marginal increase in pyrene–pyrene diffusional encounters. Nevertheless 7.5 more excimers are formed for HASE65 than for HASE11 in aqueous solutions, whether the solution contains salt or not. Thus, aqueous solutions promote the formation of "nondiffusional excimers". Those so-called "nondiffusional excimers" are ground-state pyrene dimers.

That pyrene aggregates are present in aqueous solutions and not in THF was confirmed from the observed broadening of the absorbance peaks of pyrene when switching the solvent from THF (no association) to water (association).[11] Further validation of this fact was obtained by monitoring the excimer fluorescence decays. Figure 3 shows the excimer fluorescence decays in THF (bottom) and water at pH 9 with 0.05 M KCl (top), respectively. For all concentrations studied, all excimer decays exhibited a rise time in THF. Hardly any rise time could be detected in aqueous solutions. The excimer decays were fitted with three exponentials. When the excimer decays exhibit a rise time, the first preexponential factor a_{E1} is smaller than zero and the preexponential factors a_{E2} and a_{E3} are positive. If no ground-state dimer is present, the ratio $\xi = a_{E1}/(a_{E2} + a_{E3})$ equals −1.00. If ground-state dimers are present, the ratio ξ is "more positive" than −1.00.[14] The data listed in Table 1d–f in the Supporting Information yield ξ ratios of −0.83 ± 0.01, −0.90 ± 0.03, and −0.94 ± 0.01 in THF for the excimer decays of HASE11, HASE42, and HASE65, respectively. ξ ratios of 0.00, −0.08 ± 0.03, and −0.12 ± 0.02 were retrieved in aqueous solutions for the excimer decays of HASE11, HASE42, and HASE65, respectively. Since the ξ ratios are very close to −1.00 for all HASEs in THF, very few ground-state dimers are present in THF and excimer is formed essentially by diffusion. Because the ξ ratio is much more positive than −1.00 in aqueous solutions, ground-state dimers are present.

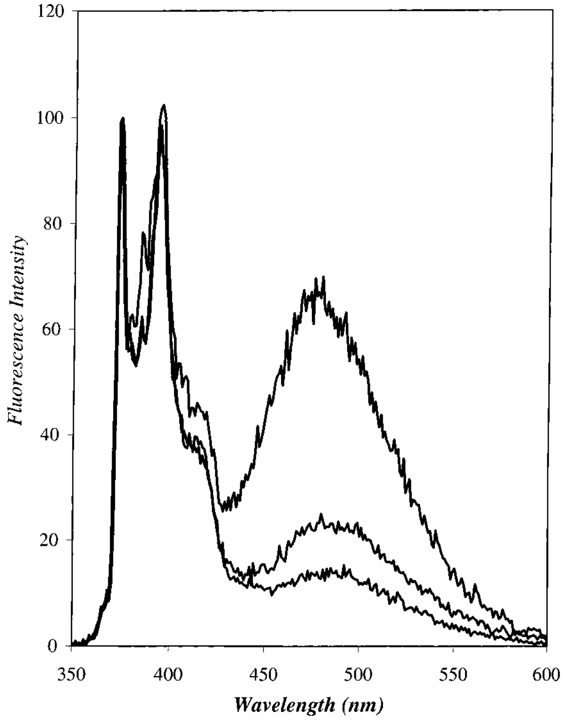


Figure 4. Steady-state fluorescence spectra of HASE65 excited at 344 nm and normalized at 375 nm. From top to bottom: in THF (0.04 g/L), in water pH 9 with 0.05 M KCl (0.05 g/L) and in water pH 9 (0.06 g/L).

Whether the aqueous solutions contain salt or not, the excimer fluorescence decays exhibit very little variations in aqueous solutions. For all excimer fluorescence decays in aqueous solutions regardless of the pyrene and salt contents, the decay times τ_{E2} and τ_{E3} equal 53 ± 2 and 135 ± 9 ns, respectively. The value recovered for the decay time τ_{E2} is typical of the pyrene excimer.[8] The longer decay time τ_{E3} is attributed to either the slow process of excimer formation via diffusion, or long-lived low-energy pyrene dimers. Long-lived pyrene dimers could be generated when an aggregate of pyrene monomers, held together by hydrophobic forces, is excited by UV light. In these aggregates, not all pyrenes have the perfect π–π stacking required to form an excimer, leading to the formation of long-lived low-energy pyrene dimers.[15] In the following, excimers and long-lived low-energy pyrene dimers are referred to as E1* and E2*, respectively.

The steady-state fluorescence spectra showed some solvent-sensitive variations. Figure 4 demonstrates that less excimer is formed in water than in THF by comparing the steady-state fluorescence spectra acquired in THF and aqueous solutions at pH 9. This result is contrary to the reported trends obtained with noncharged water-soluble polymers labeled with pyrene. When a noncharged water-soluble polymer like poly(ethylene oxide)[16] or poly(*N*,*N*-dimethylacrylamide)[10b] is hydrophobically modified with pyrene and is switched from an organic solvent to an aqueous solution, a large increase in excimer formation is observed. It is because entropically driven attractive hydrophobic forces induce the formation of pyrene aggregates in water, which form excimer on a fast time scale. In an organic solvent, pyrene groups are present as monomers only and excimers are formed by diffusion, a much slower process. In the case of a polyelectrolyte such as HASE65 shown in Figure 4, switching from THF to water at pH 9 results in a lower excimer formation. This is due to the repulsive electrostatic forces generated by the negative charges distributed along the polymer backbone, which counteract the attractive hydrophobic forces. Adding a salt to the aqueous solution screens the charges and lowers the electrostatic repulsion, which tilts the balance of forces toward the attractive hydrophobic force.[17] It results in an increase in the formation of excimer as observed in Figure 4.

At this stage, several facts have been established about the pyrene-labeled HASEs in aqueous solutions. The charges along the backbone extend the polymer coil, isolating pyrene moieties, which cannot form excimer by diffusion. Whereas excimer formation is essentially diffusion-controlled in THF, it is not the case in aqueous solutions, where most excimers are generated via ground-state hydrophobic associations between pyrene moieties. Adding salt to the solution shrinks the polymer coil and more excimers are formed, certainly via increased hydrophobic associations, since within experimental error the fraction of pyrene monomers forming excimer by diffusion ($1 - a_{\mathrm{M3}}$) is not affected by the addition of salt. This qualitative understanding of the HASE system would be strengthened if a quantitative analysis of the fluorescence data could be performed. Ideally this analysis would provide information about the fraction of pyrenes that are associated and the association dynamics. Despite the numerous studies dealing with water-soluble polymers labeled with pyrene,[11] very few such investigations present a quantitative analysis of the fluorescence data.

One such report uses fluorescence quenching to determine the fraction (f_{agg}) of associated pyrene groups for a monodisperse poly(ethylene oxide) labeled at both ends with pyrene (Py−PEO−Py).[18] In water, diffusional encounters produce excimer over time, whereas direct excitation of a ground-state dimer produces excimer instantaneously. By adding a quencher to the solution, only the diffusional process of excimer formation is affected. By carefully quantifying the kinetics for the quenching of Py−PEO−Py by iodide ions, Duhamel et al. found that 8% of the pyrene moieties of Py−PEO−Py were associated in water.[18] They also predicted that the ratio I_E/I_{M} would not be affected by the quencher if the quencher does not inhibit the excimer emission itself and if no ground-state pyrene associations were present, but that this ratio would increase as a second-order polynomial if preassociations are taking place.

The same procedure was applied to HASE65 using nitromethane as a neutral water-soluble quencher. Similar trends as those observed by Duhamel et al. for Py−PEO−Py quenched by iodide[18] were obtained, confirming the presence of pyrene ground-state associations in aqueous solutions and their absence in THF. Unfortunately the mathematical treatment derived by Duhamel et al. to determine the fraction of preassociated pyrenes in aqueous solutions can only be applied to polymeric systems which form excimer via diffusion with a single rate constant. The rate constant of excimer formation via diffusion depends strongly on the chain length spanning two pyrene groups.[9] Since Py−PEO−Py is monodisperse in the Duhamel et al.'s study, the diffusional process of excimer formation is described by a single rate constant.[18] On the other hand, pyrene-labeled macromonomers are randomly distributed along the polymer backbone of HASE65. Consequently HASE65 exhibits a distribution of excimer formation rate constants, which forbids any quantitative analysis of the quenching data.

As usual with polymers randomly labeled with pyrenes, the distribution of excimer formation rate constants must be taken into account before any quantitative analysis can be undertaken. Over the past few years, our laboratory has shown that a *blob* model could accomplish the complete analysis of the process of excimer formation between pyrenes randomly distributed along a polymer backbone.[10] This *blob*-based analysis is applied to the HASEs in the following section.

Discussion

Several recent studies have established that the coil of a polymer randomly labeled with pyrene can be divided into *blobs* among which the pyrene pendants distribute themselves randomly according to a Poisson distribution.[10] A *blob* is defined as the volume equal to or larger than the space probed by an excited pyrene during its lifetime. According to this definition, the excited pyrene cannot leave a blob, but quenchers (Q) can move in and out of a blob with a rate constant k_{e}[blob]. Quenchers can be either single ground-state pyrenes, or ground-state pyrene aggregates if pyrene associations take place. Inside a blob diffusional encounters take place with a rate constant k_{diff}. The average number of quenchers per blob is $\langle n \rangle$.

The framework of the blob model is depicted in Scheme 3. Excimer E1* can be formed via diffusional encounters between $\mathrm{Py}^*_{\mathrm{diff}}$ and a quencher Q with a complicated function $f(t)$ due to the distribution of distances existing between an excited pyrene group and various quenchers. Some excimers are formed by direct excitation of a pyrene aggregate and their initial concentration is given by $[\mathrm{E1}^*]_{(t=0)}$. Pyrene groups can be isolated in the polymer coil ($\mathrm{Py}^*_{\mathrm{free}}$) and fluoresce with their own lifetime τ_{M} (Scheme 3B). Low-energy long-lived pyrene dimers (E2*) fluoresce with their own lifetime τ_{E2} (Scheme 3C). The fluorescence decays of the pyrene monomers were fitted by eq 2[10a−b]

$$[\mathrm{Py}^*]_{(t)} = [\mathrm{Py}^*_{\mathrm{diff}}]_{(t=0)} \times \exp\left[-\left(A_2 + \frac{1}{\tau_M}\right)t - A_3(1 - \exp(-A_4 t))\right] + [\mathrm{Py}^*_{\mathrm{free}}]_{(t=0)} \exp(-t/\tau_{\mathrm{M}}) \quad (2)$$

where the expressions of the parameters A_2, A_3, and A_4 are functions of k_{e}[blob], k_{diff}, and $\langle n \rangle$ and have been given in earlier reports.[10] The excimer fluorescence decays were fitted by eq 3.

$$[E^*] = -[\mathrm{Py}^*_{\mathrm{diff}}]_{(t=0)} e^{-A_3} \sum_{i=0}^{\infty} \frac{{A_3}^i}{i!} \times \frac{\frac{1}{\tau_M} + A_2 + iA_4}{\frac{1}{\tau_M} - \frac{1}{\tau_{\mathrm{E1}}} + A_2 + iA_4} \exp\left(-\left(\frac{1}{\tau_M} + A_2 + iA_4\right)t\right) + \left([\mathrm{E1}^*]_{(t=0)} + [\mathrm{Py}^*_{\mathrm{diff}}]_{(t=0)} e^{-A_3} \sum_{i=0}^{\infty} \frac{{A_3}^i}{i!} \frac{\frac{1}{\tau_M} + A_2 + iA_4}{\frac{1}{\tau_M} - \frac{1}{\tau_{\mathrm{E1}}} + A_2 + iA_4}\right) \times e^{-t/\tau_{\mathrm{E1}}} + [\mathrm{E2}^*]_{(t=0)} e^{-t/\tau_{\mathrm{E2}}} \quad (3)$$

A detailed derivation of eq 3 is given in the Supporting Information. The fluorescence decays of the pyrene monomer for HASE solutions in THF, water at pH 9, and water at pH 9 with 0.05 M KCl were fitted with eq 2 and the parameters retrieved from the analysis are listed in Table 2a−c in the Supporting Information. The fractions of pyrene monomers that form excimer via diffusion or cannot form excimer are denoted as f_{Mdiff} and f_{Mfree}, respectively. Their expressions are given in eq 4, parts a and b:

$$f_{\mathrm{Mdiff}} = \frac{[\mathrm{Py}^*_{\mathrm{diff}}]_{(t=0)}}{[\mathrm{Py}^*_{\mathrm{diff}}]_{(t=0)} + [\mathrm{Py}^*_{\mathrm{free}}]_{(t=0)}} \quad (4a)$$

$$f_{\mathrm{Mfree}} = \frac{[\mathrm{Py}^*_{\mathrm{free}}]_{(t=0)}}{[\mathrm{Py}^*_{\mathrm{diff}}]_{(t=0)} + [\mathrm{Py}^*_{\mathrm{free}}]_{(t=0)}} \quad (4b)$$

The fraction f_{Mfree} shows the same trends as were observed by the triexponential fits. It is very sensitive to the pyrene content in THF, where f_{Mfree} increases from 0.03 ± 0.00 to 0.41 ± 0.03 between HASE65 and HASE11. On the other hand, f_{Mfree} is not much affected by the pyrene content in aqueous solutions, since it varies between 0.47 ± 0.02 to 0.69 ± 0.08 for all HASE/aqueous solution systems tried. The fact that so many pyrenes are not forming excimer (f_{Mfree} is larger than 0.10 for all polymer/solvent system but HASE65 in THF) leads to large uncertainties for the parameters k_{diff}, $\langle n \rangle$, and k_{e}[blob] retrieved from the analysis of the monomer fluorescence decays with eq 2 (cf. Table 2a−c in the Supporting Information).

To analyze the excimer fluorescence decays with eq 3, the parameters k_{diff}, $\langle n \rangle$, and k_{e}[blob], which were retrieved from the analysis of the monomer decays, are fixed in eq 3. For a given excimer lifetime τ_{E1}, eq 3 becomes a linear sum of three functions. Taking advantage of this fact, the excimer decays were analyzed by optimizing the lifetime τ_{E1} with a Golden section search routine[13c] and using a general linear least-squares[13b] routine to determine the parameters f_{Ediff}, f_{EE1} and f_{EE2}, which expressions are given in eq 5:

$$f_{\mathrm{Ediff}} = \frac{[\mathrm{Py}^*_{\mathrm{diff}}]_{(t=0)}}{[\mathrm{Py}^*_{\mathrm{diff}}]_{(t=0)} + [\mathrm{E1}^*]_{(t=0)} + [\mathrm{E2}^*]_{(t=0)}} \quad (5a)$$

$$f_{\mathrm{EE1}} = \frac{[\mathrm{E1}^*]_{(t=0)}}{[\mathrm{Py}^*_{\mathrm{diff}}]_{(t=0)} + [\mathrm{E1}^*]_{(t=0)} + [\mathrm{E2}^*]_{(t=0)}} \quad (5b)$$

$$f_{\mathrm{EE2}} = \frac{[\mathrm{E2}^*]_{(t=0)}}{[\mathrm{Py}^*_{\mathrm{diff}}]_{(t=0)} + [\mathrm{E1}^*]_{(t=0)} + [\mathrm{E2}^*]_{(t=0)}} \quad (5c)$$

Since the parameters k_{diff}, $\langle n \rangle$, and k_{e}[blob] describe the process of excimer formation via diffusion, a large uncertainty in these parameters matters mostly for those excimer fluorescence decays, where the excimer is essentially formed via diffusion. In THF, this is the case for HASE11 and to a lesser extent HASE42. Since f_{Mfree} equals 0.03 ± 0.00 for HASE65 in THF, the parameters k_{diff}, $\langle n \rangle$, and k_{e}[blob] are recovered with good accuracy (standard deviation < 6%) and the excimer decays are expected to be properly fitted. In aqueous solutions, where excimer is formed mostly via ground-state preassociations, uncertainty on the parameters k_{diff}, $\langle n \rangle$, and k_{e}[blob] is expected to have a smaller effect on the analysis. Nevertheless HASE11 forms so little excimer in THF and aqueous solutions (cf. Figures 1 and 2), that the uncertainty associated with the parameters k_{diff}, $\langle n \rangle$, and k_{e}[blob] is too large. Indeed fitting the excimer decays with the retrieved parameters k_{diff}, $\langle n \rangle$, and k_{e}[blob] led, in some cases, to negative fractions f_{Ediff}, which is impossible. Thus, only the excimer decays of HASE65 and HASE42 were thoroughly analyzed with eq 3.

Analysis of the excimer decays with eq 3 was initiated by assuming that no long-lived pyrene dimers were present ($[\mathrm{E2}^*]_{(t=0)} = 0$). This analysis implies that all excimers emit with a lifetime τ_{E1} whether they are formed by diffusion or direct excitation of a pyrene aggregate. In each case, the χ^2 values were much larger than 1.00, and the residuals indicated that the fits were very poor at longer times. This led to the conclusion that a long-lived species emitting at 510 nm was contributing to the emission of the excimers. This contribution was assigned to long-lived low-energy pyrene dimers. The triexponential fits of the excimer decays in THF and aqueous solutions yielded a long decay time τ_{E3}, which could be as large as 293 and 156 ns, respectively (cf. Table 1d−f in Supporting Information). Since the pre-exponential factor a_{E3} was small, the long decay time τ_{E3} is obtained with little accuracy. It was arbitrarily fixed to equal 250 ns in THF and 140 ns in aqueous solutions. When the contribution from the long-lived low-energy pyrene dimers E2* was taken into account, analysis of the excimer fluorescence decays with eq 3 yielded good χ^2, which were smaller than 1.30 for all but two decays. The fractions f_{Ediff}, f_{EE1}, and f_{EE2} are listed in Table 2d−f in the Supporting Information. In THF, f_{Ediff} equals 0.95 ± 0.01, which confirms that the excimer is formed essentially via diffusion. In aqueous solutions, f_{Ediff} equals 0.26 ± 0.08, which indicates that much less excimer is formed by diffusion. The contribution from the long-lived low-energy pyrene dimers is very small in THF ($f_{\mathrm{EE2}} = 0.01 \pm 0.01$) and small in aqueous solutions where it equals 0.08 ± 0.02. The excimer lifetime τ_{E1} in aqueous solutions equals 51 ± 2 ns, which is typical for the pyrene excimer.[8]

The contributions from all four species $[\mathrm{Py}^*_{\mathrm{free}}]_{(t=0)}$, $[\mathrm{Py}^*_{\mathrm{diff}}]_{(t=0)}$, $[\mathrm{E1}^*]_{(t=0)}$, and $[\mathrm{E2}^*]_{(t=0)}$ was determined in eq 6, parts a−d:

$$f_{\mathrm{diff}} = \frac{[\mathrm{Py}^*_{\mathrm{diff}}]_{(t=0)}}{[\mathrm{Py}^*_{\mathrm{diff}}]_{(t=0)} + [\mathrm{Py}^*_{\mathrm{free}}]_{(t=0)} + [\mathrm{E1}^*]_{(t=0)} + [\mathrm{E2}^*]_{(t=0)}} = \left(1 + \frac{f_{\mathrm{Mfree}}}{f_{\mathrm{Mdiff}}} + \frac{f_{\mathrm{EE1}}}{f_{\mathrm{Ediff}}} + \frac{f_{\mathrm{EE2}}}{f_{\mathrm{Ediff}}}\right)^{-1} \quad (6a)$$

$$f_{\mathrm{free}} = \frac{[\mathrm{Py}^*_{\mathrm{free}}]_{(t=0)}}{[\mathrm{Py}^*_{\mathrm{diff}}]_{(t=0)} + [\mathrm{Py}^*_{\mathrm{free}}]_{(t=0)} + [\mathrm{E1}^*]_{(t=0)} + [\mathrm{E2}^*]_{(t=0)}} = f_{\mathrm{diff}}\frac{f_{\mathrm{Mfree}}}{f_{\mathrm{Mdiff}}} \quad (6b)$$

$$f_{\mathrm{E1}} = \frac{[\mathrm{E1}^*]_{(t=0)}}{[\mathrm{Py}^*_{\mathrm{diff}}]_{(t=0)} + [\mathrm{Py}^*_{\mathrm{free}}]_{(t=0)} + [\mathrm{E1}^*]_{(t=0)} + [\mathrm{E2}^*]_{(t=0)}} = f_{\mathrm{diff}}\frac{f_{\mathrm{EE1}}}{f_{\mathrm{Ediff}}} \quad (6c)$$

$$f_{\mathrm{E2}} = \frac{[\mathrm{E2}^*]_{(t=0)}}{[\mathrm{Py}^*_{\mathrm{diff}}]_{(t=0)} + [\mathrm{Py}^*_{\mathrm{free}}]_{(t=0)} + [\mathrm{E1}^*]_{(t=0)} + [\mathrm{E2}^*]_{(t=0)}} = f_{\mathrm{diff}}\frac{f_{\mathrm{EE2}}}{f_{\mathrm{Ediff}}} \quad (6d)$$

The values of f_{diff}, f_{free}, f_{E1}, and f_{E2} for all polymer concentrations are listed in Table 3a–c in the Supporting Information, and their average values are given in Table 2. The fraction of associated pendants f_{agg} equals the sum of f_{E1} and f_{E2}. It is shown as a function of polymer concentration in THF and aqueous solutions in Figure 5. Although the f_{agg} values recovered from aqueous solutions do exhibit some scattering, it is clear from Figure 5 that pyrenes are more associated in water than in THF. f_{agg} equals 0.05 ± 0.01 and 0.58 ± 0.09 in THF and aqueous solutions, respectively. That 95% of all pendants are unassociated in THF was expected. The unexpected result however is that not all pendants are associated in aqueous solutions. Actually the fraction of pyrene groups which never form excimer during the lifetime of the excited monomer (f_{free}) equals 0.21 ± 0.07 in aqueous solutions. This is interesting because the determination of the aggregation number of the hydrophobic domains generated by hydrophobically modified polymers is usually based on the assumption that all pendants are associated. This is clearly not the case with this system. Of course, the HASE's hydrophobic pendant in this study is pyrene. Its structure is different from the linear alkyl chains typically used as hydrophobic pendants for HASEs.[6] Certainly, pyrene's ability to associate is not as good as other hydrophobic pendants, so that many pyrenes are not involved inside the hydrophobic aggregates. It is nevertheless worthwhile pointing out that not all hydrophobic pendants yield 100% association. Furthermore, our analysis method to determine the level of association of a hydrophobe by fluorescence could certainly be extended to the characterization of HASEs displaying other hydrophobes, as long as these hydrophobes be terminated with a pyrene group.

Table 2. Fractions f_{diff}, f_{free}, f_{E1}, and f_{E2} for HASE42 and HASE65 in THF and Aqueous Solutions

sample	solution	f_{diff}	f_{free}	f_{E1}	f_{E2}
HASE42	THF	0.83 ± 0.01	0.12 ± 0.03	0.04 ± 0.01	0.00 ± 0.00
	pH 9	0.14 ± 0.04	0.20 ± 0.04	0.60 ± 0.06	0.06 ± 0.02
	pH 9, 0.05 M KCl	0.19 ± 0.06	0.18 ± 0.04	0.55 ± 0.06	0.08 ± 0.02
HASE65	THF	0.93 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.00 ± 0.00
	pH 9	0.20 ± 0.03	0.28 ± 0.07	0.47 ± 0.06	0.05 ± 0.01
	pH 9, 0.05 M KCl	0.27 ± 0.05	0.22 ± 0.05	0.47 ± 0.04	0.05 ± 0.01

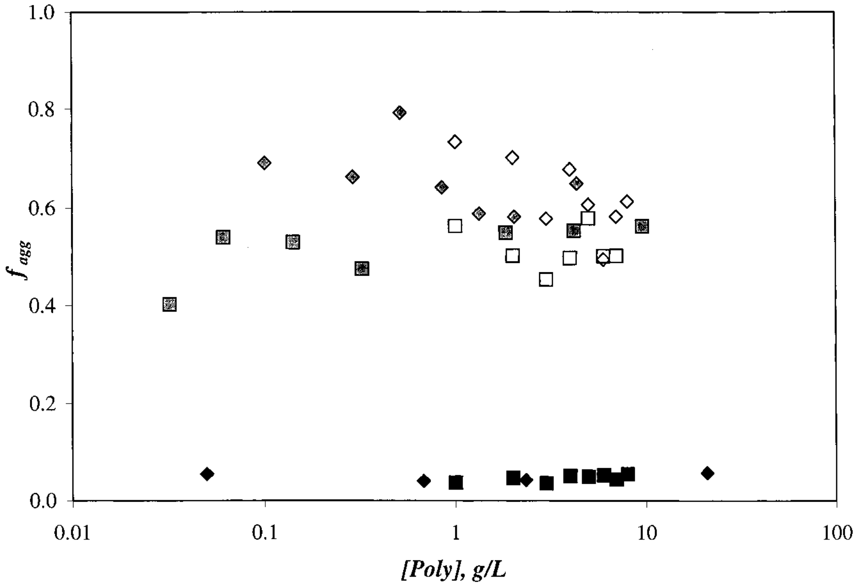


Figure 5. Fraction of associated pyrenes as a function of polymer concentration: in THF, HASE42 (■) and HASE65 (◆); in water pH 9, HASE42 (shaded box) and HASE65 (shaded tilted square); in water pH 9 with 0.05 M KCl, HASE42 (□) and HASE65 (◇).

Conclusion

A series of three pyrene-labeled HASEs has been investigated by steady-state and time-resolved fluorescence. The effect of solvent on the level of association between the pyrene pendants could be qualitatively described in terms of hydrophobic and electrostatic forces. In an organic solvent such as THF where pyrene groups are soluble, little ground-state associations between pyrene groups could be detected and excimer formation occurred mostly via diffusion. In aqueous solutions, ground-state pyrene aggregates are mostly responsible for excimer formation. In water at pH 9, electrostatic repulsive forces between neutralized carboxylic acids induce an expansion of the polymer coil, which results in the presence of many pyrene pendants, which are not involved into excimer formation. When 0.05 M KCl is added to the aqueous solution, the screening effect shields the charges and the polymer coil shrinks, resulting in a larger formation of excimer.

To investigate quantitatively the level of association of these associative polymers, pyrene−pyrene ground-state associations were characterized with a blob model. The fluorescence behavior of the pyrene monomer and excimer could be described by assuming the presence of four species, namely $Py^*_{\rm free}$, $Py^*_{\rm diff}$, E1*, and E2*. With this model, it was found that 5% of the pyrene pendants were associated in THF, but 58% of them were associated in aqueous solutions.

Although water induces more hydrophobic associations between the pyrene groups than THF does, not all pyrene groups are associated in water. The coil expansion of HASE42 and HASE65 in basic aqueous solution resulted in 21 ± 6% of all pyrene pendants being isolated and not involved in the formation of excimer. This fraction was larger than in THF, a solvent, which does not promote coil expansion to the level induced by basic aqueous solutions. In THF, the fraction of isolated pyrene equaled 12 ± 3% and 2 ± 1% for HASE42 and HASE65, respectively.

Acknowledgment. T.J.V.P., R.B., and J.D. thank the NSERC and the Dow Chemical Corp. for financial support.

Supporting Information Available: Text giving the derivation of the equations used to fit the monomer and excimer decays and tables listing the parameters retrieved from these fits. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Shay, G.; Bassett, D.; Rex, J. D. *J. Oil Colour Chem. Assoc.* **1993**, *Nov*.

(2) Jenkins, R. D.; DeLong, L. M.; Bassett, D. R. In *Hydrophobic Polymers. Performance with Environmental Acceptability*, Glass, J. E., Ed.; Advances in Chemistry 248; American Chemical Society: Washington, DC, 1996.

(3) Tam, K. C.; Guo, L.; Jenkins, R. D.; Bassett, D. R. *Polymer* **1999**, *40*, 6369−6379. English, R. J.; Gulati, H. S.; Jenkins, R. D.; Khan, S. A. *J. Rheol.* **1997**, *41*, 427−444.

(4) Annabale, T.; Buscall, R.; Ettelaie, R.; Whittlestone, D. *J. Rheol.* **1993**, *37*, 695−725.

(5) Alami, E.; Almgren, M.; Brown, W.; Francois, J. *Macromolecules* **1996**, *29*, 2229−22443. Yekta, A.; Xu, B.; Duhamel, J.; Adiwidjaja, H.; Winnik, M. A. *Macromolecules* **1995**, *28*, 956−966.

(6) Tirtaatmadja, V.; Tam, K. C.; Jenkins, R. D. *Macromolecules* **1997**, *30*, 1426−1433.

(7) Horiuchi, K.; Rharbi, Y.; Spiro, J. G.; Yekta, A.; Winnik, M. A.; Jenkins, R. D.; Bassett, D. R. *Langmuir* **1999**, *15*, 1644−1650.

(8) Birks, J. B. *Photophysics of Aromatic Molecules*; Wiley: New York, 1970; pp 301−371.

(9) Winnik, M. A. *Acc. Chem. Res.* **1985**, *18*, 73−79. Zachariasse, K. A.; Maçanita, A. L.; Kühnle, W. *J. Phys. Chem. B* **1999**, *103*, 9356−9365.

(10) (a) Mathew, A. K.; Siu, H.; Duhamel, J. *Macromolecules* **1999**, *32*, 7100−7108. (b) Kanagalingam, S.; Ngan, C. F.; Duhamel, J. *In preparation* 2001. (c) Vangani, V.; Duhamel, J.; Nemeth, S.; Jao, C.-T. *Macromolecules* **1999**, *32*, 2845−2854.

(11) Winnik, F. M. *Chem. Rev.* **1993**, *93*, 587−614.

(12) Demas, J. N. *Excited-State Lifetime Measurements*; Academic Press: New York, 1983; pp 102−111.

(13) Press, W. H.; Flannery, B. P.; Teukolsky, S. A.; Vetterling, W. T. *Numerical Receipes. The Art of Scientific Computing (Fortran Version)*; Cambridge University Press: Cambridge, England, 1992: (a) pp 523−528; (b) pp 509−513; (c) p 282.

(14) Mathew, A. K.; Duhamel, J.; Gao, J. *Macromolecules* **2001**, *34*, 1454−1469. Jones, A. S.; Dickson, T. J.; Wilson, B. E.; Duhamel, J. *Macromolecules* **1999**, *32*, 2956−2961.

(15) Piçarra, S.; Gomes, P. T.; Martinho, J. M. G. *Macromolecules* **2000**, *33*, 3947−3950. Vangani, V.; Drage, J.; Mehta, J.; Mathew, A. K.; Duhamel, J. *J. Phys. Chem. B* **2001**, *105*, 0000.

(16) Lee, S.; Duhamel, J. *Macromolecules* **1998**, *31*, 9193−9200.

(17) Guo, L.; Tam, K. C.; Jenkins, R. D. *Macromol. Chem. Phys.* **1998**, *199*, 1175−1184.

(18) Duhamel, J.; Yekta, A.; Hu, Y.; Winnik, M. A. *Macromolecules* **1992**, *25*, 7024−7030.

MA0109065