*Macromolecules* **2004,** *37,* 9287−9289

# Global Analysis of the Fluorescence Decays of a Pyrene-Labeled Polymer Using a Blob Model

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*Received May 17, 2004*
*Revised Manuscript Received September 19, 2004*

The dye pyrene has been coined as being “by far the most frequently used dye in fluorescence studies of labeled polymers” (Py-POLs).[1] Using time-resolved fluorescence, steady-state fluorescence, or UV−vis absorption, information can be retrieved about the dynamics of encounter between pyrene pendants,[2] their microenvironment,[3] or whether they form aggregates of ground-state pyrenes,[1] respectively. Over the years, these experiments have provided a wealth of knowledge on the behavior of Py-POLs in solution.[1−3] However, in the specific case where quantitative information about the kinetics of pyrene−pyrene encounters is desired, such information could only be gained, until recently, from the study of monodisperse polymers end-labeled with pyrene.[4] This is because the process of excimer formation has been shown to depend strongly on the chain length spanning[2] or flanking[5] two pyrene moieties. Consequently, the encounters between pyrenes attached randomly along a polymeric backbone yield a distribution of chain lengths between two pyrenes, resulting in a distribution of rate constants for excimer formation.[6] This distribution turned out to be a major complication in the analysis of the fluorescence data obtained with polymers randomly labeled with pyrene. A solution to this deadlock was found in 1999 by noticing that a pyrene attached onto a polymer will probe a finite volume of the polymer coil during its lifetime.[7] This finite volume, called a blob, was then used to divide the polymer coil into identical blobs. Appropriately, this model was coined “the blob model”.[4,7−14] By assuming that the pyrene dyes would distribute themselves according to a Poisson distribution among the blobs, quantitative information was retrieved about the volume probed by an excited pyrene during its lifetime, the dynamics of the polymer chain, and the level of association of the pyrene pendants (i.e., the fraction of all pyrenes that are associated, $f_{agg}$).[7,9,11,12,14] As often assumed for pyrene-labeled polymers studied at room temperature,[13] the dissociation rate constant of the pyrene excimer is neglected in the derivation of the blob model.

Hydrophobically modified alkali swellable emulsion (HASE) polymers are hydrophobically modified water-soluble polymers (HMWSP) whose solutions exhibit peculiar viscoelastic properties which have found numerous applications in industrial settings.[15] They are terpolymers consisting of methacrylic acid, ethyl acrylate, and a macromonomer. The macromonomer is a poly(ethylene oxide) spacer capped at one end with a hydrophobe and connected at the other end to a methylstyrene monomer via a urethane linker. In basic aqueous solutions, the HASE polymer is soluble but associates into polymeric aggregates held together via hydrophobic aggregates. As with any other water-soluble associative thickener, the level of association ($f_{agg}$) is believed to be an important parameter to controlling the viscoelastic properties of HMWSP.[16]

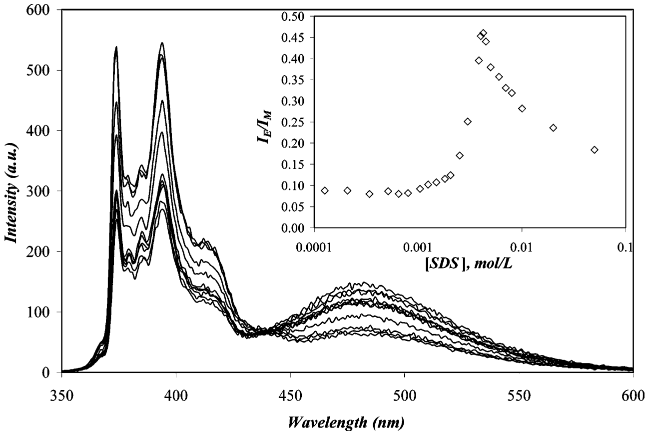

**Figure 1.** Fluorescence spectra of 1 g L$^{-1}$ Py-HASE solution in 0.01 M $Na_2CO_3$ aqueous solution at pH 9 upon addition of SDS ($\lambda_{ex}$ = 344 nm). The inset shows the ratio $I_E/I_M$ as a function of SDS concentration.

Since a surfactant can associate with the hydrophobic pendants of a HMWSP in solution, it affects the associations taking place between the hydrophobes of the HMWSP. Consequently, $f_{agg}$ is altered and so are the viscoelastic properties of the HMWSP solution.[17] To this date, the *quantitative* determination of the $f_{agg}$ parameter for a HMWSP where a surfactant is continuously added to the solution has never been undertaken. The reason for this lack of data rests with the utmost complexity of these polymeric systems. The hydrophobes of the HMWSP are present under the form of aggregates or single units, which are distributed inside polymeric aggregates. The polymeric aggregates are polydisperse in size and made of polydisperse polymer chains. Yet we report here the successful determination of $f_{agg}$ as a function of the concentration of sodium dodecyl sulfate (SDS) added to a solution of HASE whose hydrophobes have been replaced by the dye pyrene (referred to as Py-HASE).[18]

For the first time, $f_{agg}$ is obtained by fitting globally the fluorescence decays of both the pyrene monomer and excimer according to the equations derived for the blob model (see Supporting Information).[7,9,11,12] As established in numerous earlier publications,[7,9,11,12] the blob model analysis of the fluorescence decays assumes the presence of four species in solution, namely $[Py^*_{diff}]_{(t=0)}$, $[Py^*_{free}]_{(t=0)}$, $[E0^*]_{(t=0)}$, and $[D^*]_{(t=0)}$. They represent the equilibrium concentrations of the pyrenes that form excimer via diffusion, are too isolated to form excimer and fluoresce with their lifetime $\tau_M$, are preassociated and form an excimer upon direct excitation, and are preassociated and form a long-lived excimer upon absorption of a photon, respectively. The lifetimes of E0* and D* were retrieved from the analysis and are referred to as $\tau_{E0}$ and $\tau_D$, respectively.

The fluorescence spectra of Py-HASE are shown as a function of SDS concentration in Figure 1. The inset shows that the ratio of excimer to monomer ($I_E/I_M$) goes through a maximum around the cmc of SDS in the

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10.1021/ma049032n CCC: $27.50 © 2004 American Chemical Society
Published on Web 11/03/2004

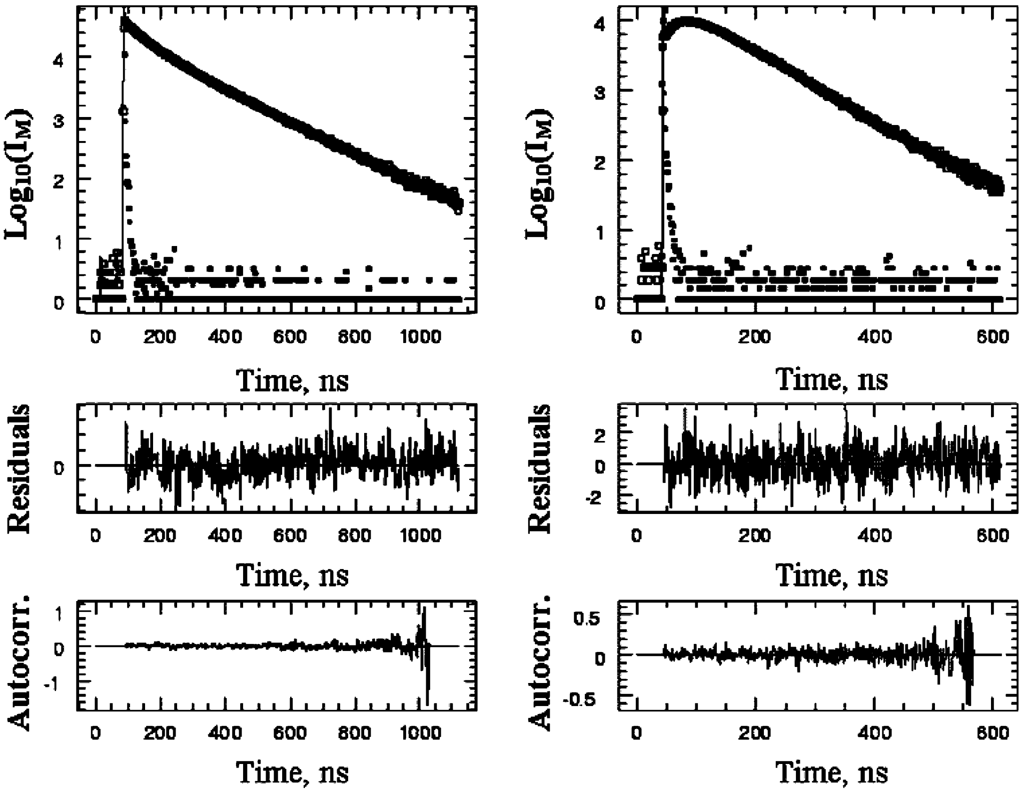

**Figure 2.** Fluorescence decays of the pyrene monomer and excimer of a 1 g L$^{-1}$ Py-HASE solution in 0.01 M $Na_2CO_3$ aqueous solution at pH 9 containing 0.0045 mmol L$^{-1}$ of SDS. The solution was excited at $\lambda_{ex} = 344$ nm. The monomer decay (left panel) was acquired at $\lambda_{em} = 375$ nm. The excimer decay (right panel) was acquired at $\lambda_{em} = 510$ nm. The $\chi^2$ obtained for this set of monomer and excimer decays equals 1.18, the worst value reported in this work.

buffer solution estimated to equal 3.5 mM from surface tension measurements.[19] At the cmc, SDS micelles form and they concentrate the pyrene pendants into their hydrophobic interiors, leading to an increase of the $I_E/I_M$ ratio. At higher SDS concentrations, more SDS micelles are present, and the average number of pyrenes per micelle decreases, resulting in a lower $I_E/I_M$ value.

The fluorescence decays of the Py-HASE solutions were acquired, and an example is shown in Figure 2. Analysis of the excimer fluorescence decays with a sum of exponentials gives information about how a pyrene excimer is formed. If the ratio of the sum of the negative preexponential factors over the sum of the positive preexponential factors ($A_{E-}/A_{E+}$) equals 0 or $-1.0$, it indicates that the excimer is produced via ground-state dimers or diffusional encounters, respectively.[7,9,11,12] As shown in Figure 3, the excimer is formed essentially via diffusional encounters for SDS concentrations larger than ~4 mM since the $A_{E-}/A_{E+}$ ratio changes from $-0.06 \pm 0.02$ to $-0.85 \pm 0.02$ for SDS concentrations below and above the cmc, respectively. For SDS concentrations larger than 4 mM, the environment probed by pyrene changes since the $I_1/I_3$ ratio drops from $1.60 \pm 0.02$ to $1.31 \pm 0.04$ for SDS concentrations below and above the cmc, respectively. These values were close to those of $1.69 \pm 0.03$ and $1.38 \pm 0.02$ obtained respectively below and above the cmc for a poly(ethylene oxide) of same molecular weight as the side chain of Py-HASE (i.e., 53 ethylene oxide units) and labeled at one end with pyrene (Py-PEO). The simultaneous drop in the $I_1/I_3$ ratio observed at the cmc for both Py-PEO and Py-HASE led us to the conclusion that the pyrene pendants of Py-HASE were more exposed to water below the cmc and were experiencing the hydrophobic environment of the SDS micelles above the cmc. The Py-PEO model compound is used to establish the environment of the pyrene pendants since the correlation between the microenvironment polarity and the $I_1/I_3$ ratio is not as clear for pyrenyl labels as for the pyrene molecule.[3] The 4 mM SDS concentration corresponds also to the maximum $I_E/I_M$ ratio. Combined together, these measurements demonstrate that the pyrene aggregates have disappeared at the cmc of SDS and that the molecularly solubilized pyrenes form excimer via diffusion for SDS concentrations larger than the cmc. Yet no information about $f_{agg}$ is retrieved from such measurements.

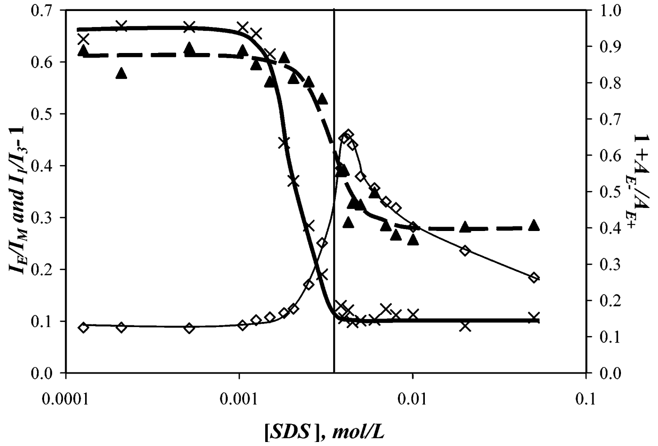

**Figure 3.** Ratios $I_E/I_M$ (◇), $I_1/I_3 - 1$ (▲), and $1 + A_{E-}/A_{E+}$ (×) for a 1 g L$^{-1}$ Py-HASE solution as a function of SDS concentration. The lines are drawn to guide the eye and have no physical meaning. All solutions were prepared in 0.01 M $Na_2CO_3$ aqueous solution at pH 9 and were excited at 344 nm. They were aerated. The vertical line drawn at an SDS concentration of 3.5 mM indicates the position of the cmc of SDS with no polymer.

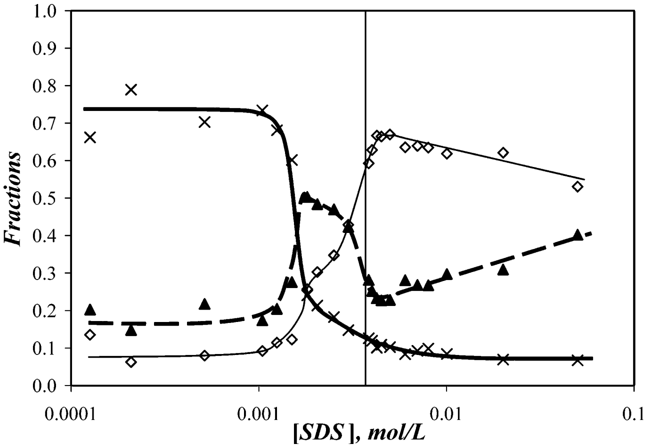

**Figure 4.** Ratios $f_{\text{diff}}$ (◇), $f_{\text{free}}$ (▲), and $f_{\text{agg}}$ (×) for a 1 g L$^{-1}$ Py-HASE solution as a function of SDS concentration. The experimental conditions are similar to those used in Figure 3.

This information is obtained by conducting a global analysis of the fluorescence decays of the pyrene monomer and excimer using the blob model equations. In the analysis, the monomer lifetime, $\tau_M$, was estimated to equal 165 ns with or without SDS, since the lifetime of Py-PEO equaled 169 ns in buffer solution and 162 ns in 0.5 M SDS. All $\chi^2$ were between 0.96 and 1.18, and the distribution of the residuals and the autocorrelation function of the residuals were randomly distributed around zero, indicating excellent fits (cf. Figure 2). The results from the global blob model analysis are listed as Supporting Information. The fractions of pyrenes present as $[\text{Py}^*_{\text{diff}}]_{(t=0)}$, $[\text{Py}^*_{\text{free}}]_{(t=0)}$, $[\text{E0}^*]_{(t=0)}$, and $[\text{D}^*]_{(t=0)}$ are referred to as $f_{\text{diff}}$, $f_{\text{free}}$, $f_E$, and $f_D$, respectively. The parameter $f_{\text{agg}}$ is obtained by summing $f_E$ and $f_D$. The results are shown in Figure 4. Starting from a value of 0.68 ± 0.07 at low SDS concentration, the $f_{\text{agg}}$ parameter decreases past a SDS concentration of 2 mM and takes a constant value of 0.08 ± 0.01 for SDS concentrations larger than 4 mM, indicating that the hydrophobes are not aggregated in this concentration range. The fraction of pyrenes forming excimer via diffusion ($f_{\text{diff}}$) increases above a SDS concentration of 2 mM and plateaus for SDS concentrations larger than 4 mM. During the crossover between 2 and 4 mM SDS, the breakup of the pyrene aggregates is accompanied by a burst of isolated pyrene monomers ($f_{\text{free}}$ goes through a maximum) which are released into the aqueous solution. Interestingly, these pyrene monomers are not hosted by the SDS micelles for SDS concentrations between 2 and 4 mM because the $I_1/I_3$ ratio remains constant and equal to that of the pyrenyl moiety of Py-PEO in water for these SDS concentrations (cf. Figure 3). As the SDS concentration is increased past 4 mM, the pyrene pendants distribute themselves into SDS micelles as indicated by the lower $I_1/I_3$ ratio and $f_{\text{free}}$ increases.

This analysis provides the first complete and quantitative description of the behavior of all the forms under which the hydrophobes of a HMWSP can be found in aqueous solution when a surfactant is added to the solution.

**Acknowledgment.** H.S. and J.D. thank NSERC, CFI, and OIT for financial support. J.D. acknowledges the financial support provided by a Tier-2 Canada Research Chair.

**Supporting Information Available:** Results from the global blob model analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

(1) Winnik, F. M. *Chem. Rev.* **1993**, *93*, 587–614.
(2) Winnik, M. A. *Acc. Chem. Res.* **1985**, *18*, 73–79.
(3) Winnik, F. M.; Regismond, S. T. A. *Colloids Surf. A: Physicochem. Eng. Asp.* **1996**, *118*, 1–39.
(4) Kanagalingam, S.; Spartalis, J.; Cao, T.-M.; Duhamel, J. *Macromolecules* **2002**, *35*, 8571–8577.
(5) Lee, S.; Winnik, M. A. *Macromolecules* **1997**, *30*, 2633–2641.
(6) Winnik, M. A.; Li, X.-B.; Guillet, J. E. *Macromolecules* **1984**, *17*, 699–702. Winnik, M. A.; Egan, L. E.; Tencer, M.; Croucher, M. D. *Polymer* **1987**, *28*, 1553–1560.
(7) Mathew, A.; Siu, H.; Duhamel, J. *Macromolecules* **1999**, *32*, 7100–7108.
(8) Kanagalingam, S.; Ngan, C. F.; Duhamel, J. *Macromolecules* **2002**, *35*, 8560–8570.
(9) Duhamel, J.; Kanagalingam, S.; O'Brien, T.; Ingratta, M. *J. Am. Chem. Soc.* **2003**, *125*, 12810–12822.
(10) Duhamel, J. *Macromolecules* **2004**, *37*, 1987–1989.
(11) Zhang, M.; Duhamel, J.; van Duin, M.; Meessen, P. *Macromolecules* **2004**, *37*, 1877–1890.
(12) Prazeres, T. J. V.; Beingessner, R.; Duhamel, J.; Olesen, K.; Shay, G.; Bassett, D. R. *Macromolecules* **2001**, *34*, 7876–7884.
(13) Piçarra, S.; Relógio, P.; Afonso, C. A. M.; Martinho, J. M. G.; Farinha, J. P. S. *Macromolecules* **2003**, *36*, 8119–8129.
(14) Picarra, S.; Duhamel, J.; Fedorov, A.; Martinho, J. M. G. *J. Phys. Chem. B* **2004**, *108*, 12009–12015.
(15) Winnik, M. A.; Yekta, A. *Curr. Opin. Colloid Interface Sci.* **1997**, *2*, 424–436.
(16) Leibler, L.; Rubinstein, M.; Colby, R. H. *Macromolecules* **1991**, *24*, 4701–4707. Annable, T.; Buscall, R.; Ettelaie, R.; Whittlestone, D. *J. Rheol.* **1993**, *37*, 695–726.
(17) Deo, P.; Jockusch, S.; Ottaviani, M. F.; Moscatelli, A.; Turro, N. J.; Somasundaran, P. *Langmuir* **2003**, *19*, 10747–10752. Magny, B.; Iliopoulos, I.; Zana, R.; Audebert, R. *Langmuir* **1994**, *10*, 3180–3187. Biggs, S.; Selb, J.; Candau, F. *Langmuir* **1992**, *8*, 838–847. Annabale, T.; Buscall, R.; Ettelaie, R.; Shepherd, P.; Whittlestone, D. *Langmuir* **1994**, *10*, 1060–1070.
(18) The Py-HASE sample contained 35 μmol of pyrene per gram of polymer. All the chemicals and instruments used in this study have been described in an earlier publication.[12] The differences reside in (1) the acquisition of the fluorescence decays which were obtained on an IBH time-resolved fluorometer using a hydrogen flashlamp and (2) the Py-HASE solutions which were not degassed for the fluorescence experiments. The monomer and excimer decays were acquired with at least 20 000 and 10 000 counts at the decay maximum, respectively. The decays were acquired over 1024 channels.
(19) A DuNouy tensiometer from the CSC Scientific Co., Inc., was used for the surface tension measurements.

MA049032N