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The Importance of Considering Nonfluorescent Pyrene Aggregates for the Study of Pyrene-Labeled Associative Thickeners by Fluorescence

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The statement made by Françoise Winnik in 1993 that pyrene *is by far the most frequently used dye in fluorescence labeled polymers*[1] still holds even in 2005 as attested by a recent review on the topic.[2] Out of all the interesting photophysical properties used in the study of pyrene-labeled polymers, the ability of pyrene to form an excimer is certainly the most applied one.[1,2] An excimer is a complex which results from the encounter between an excited pyrene and a ground-state pyrene.[3] Whereas a pyrene monomer fluoresces around 375 nm, the excimer emits around 480 nm. These two well-separated emissions allow one to use steady-state fluorescence to determine the ratio of the intensity of the excimer over that of the pyrene monomer, $(I_E/I_M)^{SS}$. A large $(I_E/I_M)^{SS}$ ratio usually indicates that the pyrenes are located close to one another, as observed when the chain length of a pyrene end-labeled polymer decreases.[4]

Pyrene, being highly hydrophobic (solubility in water is reported to be in the 0.3-0.7 μM range),[5,6] has become the chromophore of choice to study the behavior of hydrophobically modified water-soluble polymers (HMWSP) by fluorescence.[1,2] This is done by covalently attaching pyrene onto the water-soluble polymer in lieu of the hydrophobe. By monitoring the fluorescence intensity of the pyrene monomer and excimer, information about the hydrophobic pendants of the HMWSP is obtained. Yet conflicting results are sometimes obtained when the experiments are performed by using either steady-state (SS) or time-resolved (TR) fluorescence. This is illustrated in Figure 1 where the fraction of aggregated pyrenes, f_{agg}, and the $(I_E/I_M)^{SS}$ ratio of a pyrene labeled hydrophobically modified alkali swellable emulsion copolymer (Py-HASE)[7−9] are plotted as a function of the concentration of sodium dodecyl sulfate (SDS) added to the aqueous solution.[10] At low SDS concentration, the $(I_E/I_M)^{SS}$ ratio is small. As SDS is added to the solution, SDS molecules start binding onto the polymer above an onset concentration. The pyrene pendants are drawn into a few mixed micelles, and the $(I_E/I_M)^{SS}$ ratio increases. Past the cmc,[11] SDS micelles form which draw in more pyrene pendants. Excimer formation increases, and $(I_E/I_M)^{SS}$ passes through a maximum. As more SDS is added to the solution, the pyrene monomers distribute themselves among numerous micelles, excimer formation is hindered, and the $(I_E/I_M)^{SS}$ ratio decreases. On the other hand, f_{agg} equals 0.70 ± 0.06 at low SDS concentration, indicating that 70% of all pyrene pendants are aggregated. At high SDS concentration ([SDS] > 4.25 mM), f_{agg} equals 0.09 ± 0.01, demonstrating that the pyrene aggregates dissociate in the presence of SDS micelles. These results have been presented in two earlier publications.[8,9]

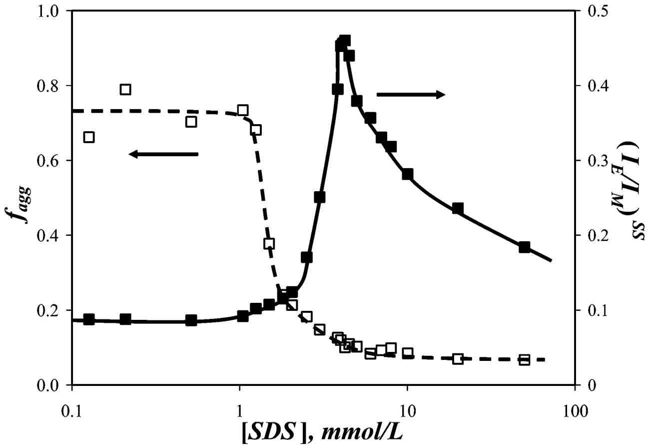

Figure 1. Fraction of aggregated pyrenes, f_{agg} (□), and $(I_E/I_M)^{SS}$ ratio (■) of Py-HASE (0.036 mmol of pyrene per gram of polymer) at a concentration of 1 g/L in 0.01 M Na_2CO_3 aqueous solution at pH 9. λ_{ex} = 344 nm.

Interestingly Figure 1 clearly highlights the incompatible trends obtained by SS and TR fluorescence. The $(I_E/I_M)^{SS}$ ratio is lowest when most pyrenes are aggregated, and it is highest when most pyrenes are dissociated. If the $(I_E/I_M)^{SS}$ ratio is to reflect the frequency of pyrene encounters,[3,4] surely aggregated pyrenes are expected to form excimer most efficiently and produce a high $(I_E/I_M)^{SS}$ ratio since the process of excimer formation is not delayed by the diffusion of the two pyrene moieties, as proved by the markedly reduced rise time in the excimer decays (cf. Figure SI.1 in the Supporting Information (SI)).[7−9] Yet the opposite is found experimentally, as shown in Figure SI.2 in SI. At low SDS concentration where the pyrenes of Py-HASE are aggregated, I_E is small. It increases and passes through a maximum at the cmc and decreases at higher SDS concentration. The combination of the results obtained by SS and TR fluorescence hints at a weakly emissive excimer when it is formed inside a pyrene aggregate, as has been inferred in earlier reports.[9,12−14]

It must be pointed out at this stage that the interactions of pyrene-labeled water-soluble polymers with surfactants has led invariably to two families of $(I_E/I_M)^{SS}$ vs [surfactant] trends. The first family exhibits a $(I_E/I_M)^{SS}$ vs [surfactant] trend similar to the one shown in Figure 1, where $(I_E/I_M)^{SS}$ passes through a maximum close to the cmc of the surfactant and decreases past the cmc.[15−17] The $(I_E/I_M)^{SS}$ vs [surfactant] trend displayed by the second family mimics the f_{agg} profile shown in Figure 1.[13,14,18−20] The $(I_E/I_M)^{SS}$ ratio is large at low surfactant concentration, and it drops to a low plateau value past a transition which occurs in the neighborhood of the cmc. Several effects can be invoked to rationalize these two trends, but among these, the extent of self-quenching experienced by an excimer inside a pyrene aggregate is the most likely one.

Self-quenching of the pyrene excimer formed inside a pyrene aggregate has been proposed in earlier reports;[9,12−14] however, the strength of the conclusions

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drawn from these earlier reports is weakened by the ignorance of f_{agg}, for in order to draw any conclusion about the emission quantum yield of any given chromophore, one must first determine how many such chromophores are present in solution. In other words, information about the emission quantum yield of a pyrene forming an excimer inside a pyrene aggregate can only be known if the number of aggregated pyrenes is known. SS fluorescence cannot yield this information because it does not distinguish whether an excimer is formed via diffusion or excitation of a pyrene aggregate. This information can only be retrieved from TR fluorescence measurements. Yet this information has remained elusive due to difficulties encountered when attempting to analyze the complex monomer and excimer fluorescence decays obtained with polymers randomly labeled with pyrene. Thanks to recent developments performed by this laboratory in the analysis of fluorescence decays obtained with polymers randomly labeled with pyrene,[7–9,21–24] the first quantitative comparison of the radiative rate constant of the excimer is being performed in the present work whether the excimer is formed by diffusional encounters or inside a pyrene aggregate. This is accomplished by noting that the fluorescence decays provide all the information required to determine the I_E/I_M ratio by TR fluorescence, namely the $(I_E/I_M)^{\mathrm{TR}}$ ratio, which must equal $(I_E/I_M)^{\mathrm{SS}}$.[25] As demonstrated in the Supporting Information (SI), the $(I_E/I_M)^{\mathrm{TR}}$ ratio can be expressed by eq 1

$$\left(\frac{I_E}{I_M}\right)^{\mathrm{TR}} = K\frac{k_{\mathrm{rad}}^{\mathrm{E0(diff)}}}{k_{\mathrm{rad}}^{\mathrm{M}}}\left(\frac{I_E}{I_M}\right)^{\mathrm{SPC}} = \left(\frac{I_E}{I_M}\right)^{\mathrm{SS}} \qquad (1)$$

where K is a constant depending on the fluorometer, $k_{\mathrm{rad}}^{\mathrm{E0(diff)}}$ is the radiative rate constant of the excimer formed by diffusion, $k_{\mathrm{rad}}^{\mathrm{M}}$ is the radiative rate constant of the pyrene monomer, and the $(I_E/I_M)^{\mathrm{SPC}}$ ratio is determined from parameters retrieved from the global analysis of the monomer and excimer fluorescence decays (cf. the SI).

Under conditions where the pyrene pendants are strongly aggregated such as for low SDS concentration, $k_{\mathrm{rad}}^{\mathrm{E0(diff)}}$ represents the radiative rate constant of an excimer formed by the diffusive encounter between an excited monomer and a ground-state pyrene aggregate. In other words, $k_{\mathrm{rad}}^{\mathrm{E0(diff)}}$ represents the radiative rate constant of an excited pyrene aggregate.[8] At high SDS concentration where the pyrene pendants are weakly associated, $k_{\mathrm{rad}}^{\mathrm{E0(diff)}}$ represents the radiative rate constant of an excimer formed by the diffusive encounter between an excited pyrene monomer and a ground-state pyrene.[8] Consequently, eq 1 provides a means to compare $k_{\mathrm{rad}}^{\mathrm{E0(diff)}}$ under conditions where the pyrene pendants are aggregated or not.

The parameters required to determine the $(I_E/I_M)^{\mathrm{SPC}}$ ratio have been reported in two earlier publications.[8,9] Using those parameters, the $(I_E/I_M)^{\mathrm{SPC}}$ ratio was determined and is plotted as a function of SDS concentration in Figure 2. At low SDS concentration where the pyrene pendants are aggregated, the $(I_E/I_M)^{\mathrm{SPC}}$ ratio takes its largest value, which is contrary to the trend exhibited by the $(I_E/I_M)^{\mathrm{SS}}$ ratio. The inset in Figure 2 shows a plot of $\Psi = K(k_{\mathrm{rad}}^{\mathrm{E0(diff)}}/k_{\mathrm{rad}}^{\mathrm{M}}) = (I_E/I_M)^{\mathrm{SS}}/(I_E/I_M)^{\mathrm{SPC}}$ vs [SDS], which indicates that at concentrations lower than 1.25×10^{-3} mol/L (where the pyrene pendants are aggregated) and larger than 8.0×10^{-3} mol/L (where the pyrene pendants are not associated) Ψ remains constant and equals 0.11 ± 0.03 and 0.89 ± 0.05, respectively.

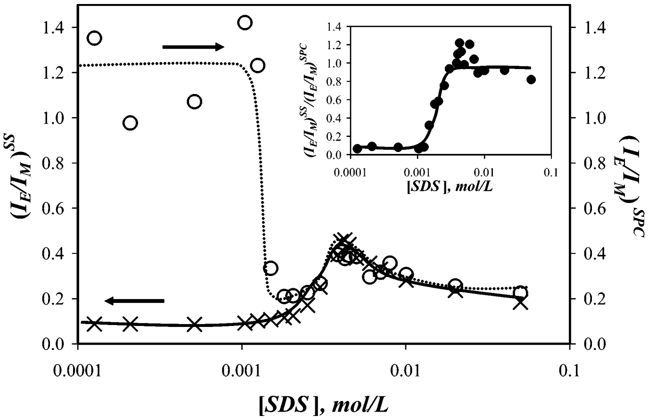

Figure 2. Comparison of the $(I_E/I_M)^{\mathrm{SS}}$ ratio (×) obtained by SS fluorescence with the $(I_E/I_M)^{\mathrm{SPC}}$ ratio (○) obtained by TR fluorescence for Py-HASE at a concentration of 1 g/L in 0.01 M Na_2CO_3 aqueous solution at pH 9. $\lambda_{\mathrm{ex}} = 344$ nm. Inset: $(I_E/I_M)^{\mathrm{SS}}/(I_E/I_M)^{\mathrm{SPC}}$ as a function of [SDS].

It is clear from the expression of Ψ that knowledge of $k_{\mathrm{rad}}^{\mathrm{M}}$ provides information about $k_{\mathrm{rad}}^{\mathrm{E0(diff)}}$. $k_{\mathrm{rad}}^{\mathrm{M}}$ was measured with a poly(ethylene oxide) chain terminated at one end with pyrene as a model compound (cf. the SI).[26] Measurements shown in Figure 2 were conducted for Py-HASE concentrations of 0.1, 1.0, and 10 g/L.[27] Using the Ψ and $k_{\mathrm{rad}}^{\mathrm{M}}$ values obtained at low and high SDS concentrations for each Py-HASE concentration, one finds that $k_{\mathrm{rad}}^{\mathrm{E0(diff)}}$ is 4.5 ± 1.4 times larger when an excimer is formed by the diffusive encounter between two pyrene monomers located inside an SDS micelle than between an excited pyrene and a pyrene aggregate. Since the excimer lifetime, τ_E, was found to equal 49 ± 4 ns regardless of SDS concentration,[8,9] the quantum yield of the Py-HASE excimer in an aggregate, $\phi_E^{\mathrm{agg}} = \tau_E k_{\mathrm{rad}}^{\mathrm{E0(diff)}}$, is also 4.5 times smaller than that of an excimer formed inside an SDS micelle, certainly due to self-quenching of the excimer formed inside a pyrene aggregate.

This result rationalizes the low $(I_E/I_M)^{\mathrm{SS}}$ value obtained in Figure 1 for small SDS concentrations. This work represents the first example where quantitative information about ϕ_E^{agg} has been obtained. This is an important result in view of the numerous examples where trends of the $(I_E/I_M)^{\mathrm{SS}}$ ratio are used to draw conclusions about the level of aggregation of pyrene labeled water-soluble macromolecules.[1,2]

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Supporting Information Available: Fluorescence decays of Py-HASE with and without SDS, plot of fluorescence intensities of Py-HASE monomer and excimer as a function of SDS concentration, experimental part, description of Py-HASE, determination of $(I_E/I_M)^{\mathrm{TR}}$, and determination of the quantum yields of the pyrene monomer of Py-HASE in aqueous solution and SDS. This material is available free of charge via the Internet at http://pubs.acs.org.

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