Time-Resolved and Steady-State Fluorescence Studies of Hydrophobically Modified Water-Soluble Polymers

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A comprehensive study of the photophysical behavior of poly(acrylic acids) randomly labeled with pyrene using steady-state and time-resolved fluorescence spectroscopy is presented. The influence of external factors, such as different solvents and pH in the aqueous solution, on the polymer photophysics has been investigated. These factors induce major changes in the polymer conformation, which are reflected in the fluorescence experiments. The random introduction of the hydrophobic pyrene groups along the macromolecule favors the coexistence of static (preformed dimers) and dynamic (excimers) monomer quenching phenomena together with a fraction of isolated monomers (not able to form excimer), as revealed by time-resolved and steady-state fluorescence data. In dioxane and methanol solvents, the global analysis of the fluorescence time profiles shows a rise time of ∼21−32 ns followed by a decay of ∼90−138 ns with an additional longer decay component with a low preexponential factor. This is consistent with the fact that in organic solvents such as methanol and dioxane, which are considered to be good solvents, the fraction of preassociated and isolated chromophores is highly reduced and excimer formation is essentially due to a dynamic mechanism. A kinetic scheme involving two types of monomers (M_A and M_B) and one excimer (E) is proposed. From the fluorescence decays it was possible to extract quantitatively the percentage of ground-state preformed dimers along with the percentage of isolated chromophores at room temperature. In addition, it is shown that the fraction of associated ground-state chromophores that can be excited is always larger than that of the isolated chromophores. The rate constants for excimer formation (k_a), dissociation (k_d), and deactivation (k_E) were determined considering the absence and presence of preformed dimers. Additional photophysical and spectroscopic data consisting of wavelength shifts, peak-to-valley ratios and differences (obtained both from absorption and from excitation spectra collected at the monomer and excimer emission region), and the vibronic I_1/I_3 ratio in the pyrene monomer emission were found to be pH-dependent for the polymers in aqueous solution. Hence, by combining the results from steady-state fluorescence measurements with time-resolved fluorescence data, information is provided on how the chain conformation of the labeled PAA polymers changes depending on the solvent (water at different pH values or organic solvents). In water, the conformation changes from compact at low pH to an open polymer coil at high pH, whereas the polymers are in an extended state when in dioxane and methanol.

Introduction

The use of fluorescent probes to study conformational changes in polymers, polymer−surfactant interactions, micelle formation, the aggregation number of surfactants, and so forth has been widely reported in the literature. See, for example, refs 1 and 2 and references therein for a recent review.

The study of copolymers, which incorporate chromophores of interest in polymer photophysics, has the major advantage of varying the level of grafted chromophore (intramolecular chromophore concentration), allowing the possibility to obtain photophysical parameters in ideal situations of "zero intramolecular concentration" and remove the concentration dependence on rate constants for excimer formation. This is so because the pyrene concentration, needed to promote intermolecular excimer formation, is usually found to be 10^{-3} M [3], whereas with intramolecular excimer formation the effective pyrene concentration can be lower than 10^{-6} M. In polymer photophysics, the implications of the above are obvious, consisting of the absence of an inter-polymeric association (use of low polymer concentration), and at the same time, because the pyrene absorbing probe is also found in low concentrations, situations such as reabsorption,[3,4] which may have a strong influence on the shape of the monomer band,[5] can be easily avoided.

Water-soluble polymers covalently labeled with fluorescent hydrophobic dyes are self-organizing and a major focus of interest in polymer photophysics. Among the most studied cases within this group of polymers are the polyelectrolytes, poly(acrylic acid) (PAA),[6−9] and the cellulose-containing[10−12] polymers with aromatic fluorophores (e.g., pyrene or naphthalene) randomly attached. By employing photophysically active groups as hydrophobes in hydrophobically modified polymers, a direct molecular-level study of the association and, consequently, molecular conformation can be made.

Associating polymers have important applications for surface modification, structuring and, in particular, rheology control. Hydrophobically modified water-soluble polymers, developed

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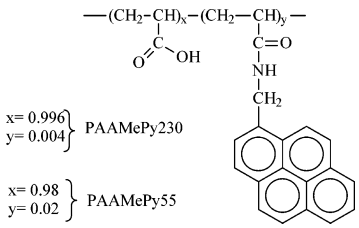
during the last 10−20 years, can now be found in all modern water-based paints and have gained increasing importance in pharmaceutical, cosmetic, and other formulations. The very important thickening effect results from the formation of a 3D physically cross-linked network at concentrations above the overlap concentration (0.1−1 wt %). The viscoelastic effects are related to both the number of cross links and their lifetimes. Recent research on mixed systems of water-soluble polymers with hydrophobic grafts and surfactants has shown that whereas the number of cross links remain unchanged on addition of surfactant there is a dramatic increase in the cross-link lifetimes.[13,14] Because the way to control the rheological effects is to control the lifetimes, it is important to be able to monitor these directly. This is the background to our attempt to study the dynamics of such systems directly on a molecular level. Time-resolved fluorescence then appears as a technique of choice: it has adequate time resolution and monitors molecular events. No probe needs to be added because the fluorescent hydrophobic groups give rise to the desired rheological effects; important commercial thickeners also contain aromatic groups. Fluorescent studies are performed well below the overlap concentration. However, this appears not to be a serious limitation because the intramolecular associations at low concentrations are very much the same as the intermolecular ones at high concentrations.[15,16] Steady-state and time-resolved fluorescence measurements, based on excimer formation/decay kinetics, are usually used as tools to follow the self-association of these polymers in aqueous solution.[1] The steady-state fluorescence method has been extensively used as a direct technique to follow excimer formation. However, it is well understood that time-resolved fluorescence measurements are needed in order to have more reliable insight into the association behavior. The final aim is to obtain and understand the lifetimes of associations and then to be able to predict and control rheological properties in solutions of hydrophobically modified polymers with and without cosolutes.

In this work, in addition to steady-state measurements, time-resolved fluorescence spectroscopy has been employed to study pyrene-labeled PAA. It is shown that the latter method is an important technique to use in understanding the complex photophysical behavior of this type of hydrophobically modified polymer. Recent work reported photophysical studies of poly(acrylic acid) (PAA), randomly grafted with pyrene[7,17−19] or naphthalene[6,7] in aqueous or organic solvents. However, with the lack of complete and detailed time-resolved data, a full photophysical picture of the system could not be obtained until now.

Unlike their fluorescent probe units in solution, grafted polymers frequently show complex luminescence behavior with multiexponential decay laws as the rule and not the exception. Consequently, fluorescence techniques and in particular monomer−excimer time-resolved emission are useful tools for the study of polymer dynamics. In the 1980s, a revision of the monomer−excimer kinetics for polymers was proposed.[20−25] It was then suggested that the kinetic scheme for excimer formation and decay after Birks[3] could not be applied to fluorescent polymers and that only a sum of three exponentials could properly fit the fluorescence decays.[21] In the large majority of the proposed models, the three species that were involved were one monomer and two excimers[26−28] or two monomers and one excimer.[22,24]

The existence of free or isolated chromophores as the essential motive for deviations from Birk's kinetics was first proposed by Holden et al.,[20] and the additional lifetime identification was made by comparison with model compounds.

SCHEME 1



Recently, the presence of isolated monomers, coexisting with chromophore conformations that can proceed to the excimer configuration, was verified in other copolymer systems, where a kinetic model similar to the one found for the PAAMePy polymers studied here was presented.[29,30] More recently, Duhamel and co-workers[31,32] have developed an alternative model to interpret excimer formation kinetics with different kinds of pyrene-labeled polymers. In the so-called "blob model", different regions of the polymer are set in compartments related to different kinds of pyrene stages (ground-state, excited, excimer-forming, symmetric and nonsymmetric ground-state dimer), thus allowing the characterization of the diffusion-controlled excimer formation rate constant within a blob, the average number of ground-state pyrenes per blob, and the rate constant at which pyrene groups exchange from blob to blob.[31−34] The model was then used to quantify the degree of association of a pyrene-labeled polyelectrolyte; this was found to equal ∼60 mol % in aqueous solvent and decrease to less than 5 mol % in THF (good solvent).[34] Although versatile, the blob model cannot be applied in situations where there is excimer-to-monomer reversibility, as in the present case. In this work, we use an alternative and more classic approach to solve the excited-state kinetics presented by such systems.

Experimental Section

A 25% aqueous solution of PAA with nominal molecular weight of 150 000 g/mol was purchased from Wako Chemicals, Japan. Before labeling with 1-pyrenyl methylamine, the solution was freeze dried to give solid PAA, which was used in the postmodification step. The polymers were kindly provided by Dr. Dan Anghel, and the synthesis followed the same route as previously described.[6,7] Two pyrene (Py)-labeled PAA samples were prepared, one with a higher and one with a lower number of pyrene groups. All chemicals used in the syntheses were reagent grade. 1-Pyrenylmethylaminehydrochloride, 1,3-dicyclohexylcarbodiimide, and anhydrous 1-methylpyrrolidone were received from Aldrich Chemicals. Spectrum Medical Industries supplied the dialysis membranes with a molecular weight cutoff of 12 000−14 000 g/mol. The amounts of pyrene were determined both by UV absorption measurements using 1-pyrenylmethylamine hydrochloride as a model compound and by ^{1}H NMR measurements in deuterium oxide. The pyrene content of the low-labeled PAA polymer was determined to be $[Py]_{UV}$ = 0.43 mol % (or 6.0×10^{-5} mol/g polymer) and $[Py]_{NMR}$ = 0.44 mol % (or 6.1×10^{-5} mol/g polymer). These correspond to 230 (UV) or 227 (NMR) unimer units per chromophore or 9 chromophores per polymer chain. This sample is denoted PAAMePy230; see Scheme 1. The pyrene content obtained from the second labeled PAA polymer was $[Py]_{UV}$ = 1.82 mol % (2.5×10^{-4} mol/g polymer) and $[Py]_{NMR}$ = 2.05 mol % (2.9×10^{-4} mol/g polymer), which corresponds to 55/49 unimer units per chromophore or 38/43 chromophores per polymer chain,

respectively. This sample is denoted PAAMePy55; see Scheme 1.

The solvents used in the polymer solutions were of spectroscopic or equivalent grade. Water was twice distilled and passed through a Millipore apparatus. The measured pH values were obtained with a Crison micropH 2000, and adjustments of the hydrogen ion concentration of the solutions were made with diluted HCl and NaOH solutions. The chromophore concentration of the aqueous PAAMePy solutions used in all fluorescence experiments ranged from 1×10^{-5} to 1×10^{-6} M. Prior to experiments, they were deoxygenated by bubbling with N_2 or Ar and sealed. This procedure was utilized for both time-resolved and steady-state experiments. For this molecular weight, the polymer concentration in the different solutions was 0.05 g/L, which is well below the critical value for coil overlap, c^*, and intermolecular chain contacts are therefore improbable in homogeneous solutions. However, the low optical density of the samples prevents self-absorption or inner filter effects. Methanol was dried over CaO, and 1,4-dioxane was purified by the procedure described in ref 35. These solvents were used in the preparation of the organic solutions of the PAAMePy polymers.

Absorption and fluorescence spectra were recorded on Shimadzu UV-2100, Olis-Cary 14, and Horiba Jobin-Ivon SPEX Fluorog 3-22 spectrometers, respectively. All of the fluorescence spectra were corrected for the wavelength response of the system.

Fluorescence decays were measured using a home-built[36] TCSPC apparatus with an N_2-filled IBH 5000 coaxial flashlamp as the excitation source, two Jobin-Ivon monochromators, a Philips XP2020Q photomultiplier, and Canberra TAC and MCA instruments. Alternate measurements (1000 cpc) of the pulse profile at 316, 335, or 355 nm and the sample emission were performed until $(1-2) \times 10^4$ counts at the maximum were reached. The fluorescence decays were analyzed using the modulating functions method of Striker.[37]

Temperature control was achieved using a home-built system based on cooled nitrogen and electric heating, which is automatically controlled by the difference between the input temperature value and the sample's real temperature, determined with a PT100 thermometer.

The results from steady-state fluorescence experiments are presented as emission and excitation spectra but often also in terms of the ratios I_E/I_M (ratio of the excimer to monomer bands) and I_1/I_3. The I_E/I_M ratio results from the decomposed area under the monomer and excimer bands, whereas $I_1[S_0(\upsilon = 0) \leftarrow S_1(\upsilon = 0)]$ and $I_3[S_0(\upsilon = 1) \leftarrow S_1(\upsilon = 0)]$ denote the intensities of the first and third vibronic bands of the monomer emission of pyrene, respectively. The ratio I_1/I_3 exhibits sensitivity to changes in the polarity of the local environment of the chromophore. The general procedure to obtain those values consisted of matching the emission spectra of pyrene in dioxane (with a concentration lower than 10^{-5} M) with the monomer band of the polymer. Because energetic differences exist between spectra of pyrene and the polymer, the match of the two implies a red shift of the pyrene monomer; after this procedure, the relative intensities and vibronic band progression is almost identical for both compounds (polymer and pyrene). The resulting differential spectrum is the excimer emission band. In this procedure, the xx′ scale must be used in energetic units (wavenumbers).

Results

The polymers studied result, as shown in the Experimental Section, from the random labeling of pyrene groups to the backbone chain of the poly(acrylic acid) (PAA). Because the polymers are randomly labeled, this means that we can obviously have more densely labeled pyrene areas coexisting with others, which are not as densely labeled, in the same polymer chain. This could possibly generate different local environments within the same polymer and have repercussions on the observed photophysical behavior. However, as will be shown, the two polymers display fairly identical decay times that are independent of the pH and level of grafting, excluding in this way the existence of strong inhomogeneous regions in the same polymer chain. With the more densely pyrene-labeled polymer, the relative amount of excimer is obviously greater than that found with the less-grafted polymer.

Absorption Spectra. All photophysical studies were performed with low polymer concentrations (0.05 g/L) to avoid intermolecular interactions in solution.

The absorption spectra of PAAMePy55 and PAAMePy230 show the characteristic pyrene vibronically resolved spectra (Figure 1). There is a significant red shift of ca. 8−10 nm in the polymer spectra relative to the spectra of pyrene, with maxima at ∼342−344 nm (polymers) versus 336 nm (pyrene). In water, the absorption wavelength maximum (λ_{abs}^{max}) is pH-dependent for both polymers. A blue shift in the wavelength maximum is obtained in methanol ($\lambda_{abs}^{max} = 342$ nm) relative to dioxane ($\lambda_{abs}^{max} = 344$ nm). However, the most important consideration to retain from Figure 1 is the progressive decrease in the difference between the wavelength maximum of the two polymers ($\Delta\lambda_1$) with a pH increase (also shown in Table 1). Additional information, resulting from the absorption spectra, is revealed by the peak-to-valley ratio, P_A, and peak-to-valley difference, ΔP_A; see Table 1 and Figure 2 respectively, where P_A results from the ratio between the values at the absorption maximum and at the valley and ΔP_A results from the difference between these two values. In water, for low-labeled polymer PAAMePy230, the ratio P_A remains practically constant all over the pH range. However, for the PAAMePy55 polymer, this ratio increases with pH and possesses a maximum value in the organic solvents dioxane and methanol. If instead ΔP_A is now considered, a different behavior is found (Figure 2). In water, a total overlap between the absorption bands of the two compounds is never observed, even at high pH values, thus revealing the presence of preformed aggregates under all circumstances. This contribution is obviously more significant in the case of the more labeled polymer. In methanol (lower right-hand panel in Figure 1) and dioxane (figure not shown), practically no difference is observed between the absorption spectra of the two polymers. This is a consequence of a lower degree of association in the ground state.

Steady-State Fluorescence. The fluorescence emission spectra of PAAMePy55 and PAAMePy230 in diluted water solutions, at different pH values, show the characteristic vibronically resolved monomer (with maxima ∼374 nm) and the structureless excimer (with maxima centered at ∼480 nm) bands (Figure 3). The ratio of the excimer (I_E) to the monomer band (I_M), I_E/I_M, changes both with pH and the excitation wavelength (Figure 3). The dependence of the I_E/I_M ratio on the excitation wavelength is direct evidence of the presence of preformed aggregates.[38,39] For both polymers, it can be seen that excitation in the red edge of the absorption leads to an increase in the intensity of the excimer band (Figure 3). Also for both polymers but more noticeably for PAAMePy55, there is a progressive decrease in the I_E/I_M ratio as the pH increases. As will be discussed later, this is a consequence of the progressive ionization of the carboxylic (COOH) groups. The electrostatic

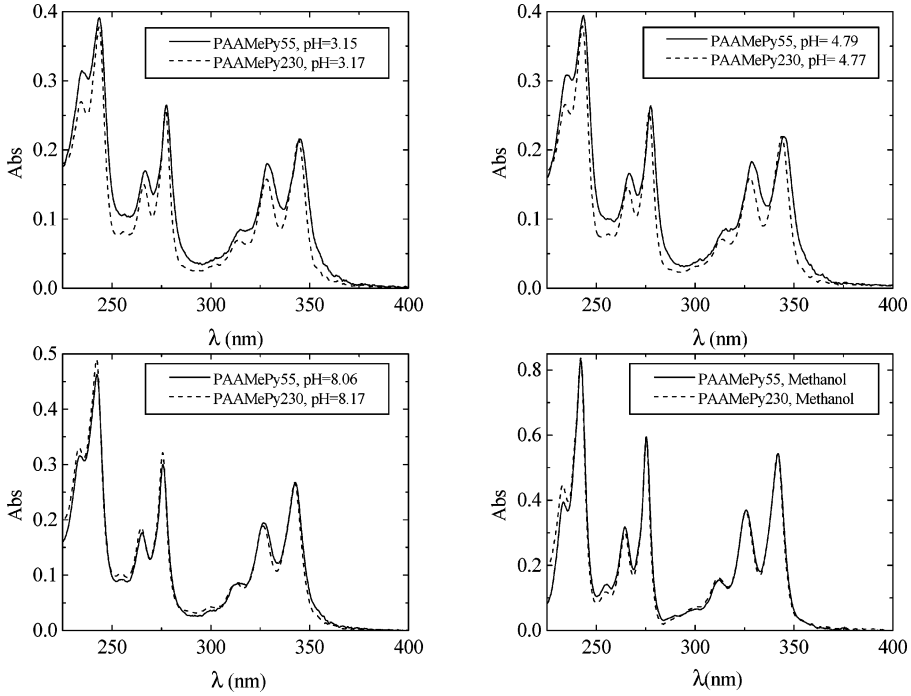


Figure 1. Absorption spectra of PAAMePy55 and PAAMePy230 polymers in water at three different pH values and in methanol, $T = 293$ K.

TABLE 1: Photophysical Data at 293 K, Taken from the Absorption and Fluorescence Excitation Spectra for PAAMePy55 and PAAMePy230[a] in Water[b], Methanol, and Dioxane[c]

pH	3.7	4.7	7.4	10.2	methanol	dioxane
$\Delta\lambda_1$	0.81	1.0	0.2	≅0	0	0.6
$\Delta\lambda_2$	2.0	3.0	1.0	2	0	0.5
	(1.3)	(1.0)	(1.5)	(1.5)	(0.5)	(1.0)
P_A	1.98	1.86	2.35	2.20	3.02	3.16
	(2.65)	(2.54)	(2.64)	(2.58)	(3.20)	(2.9)
P_M	2.41	2.42	2.36	2.23	1.96	1.91
	(2.10)	(2.19)	(2.15)	(2.15)	(1.86)	(2.10)
P_E	1.43	1.38	1.29	1.17	1.80	2.0
	(1.52)	(1.43)	(1.27)	(1.23)	(1.73)	(1.99)
$(P_M - P_E)$	0.98	1.04	1.07	1.06	0.16	≅0
	(0.58)	(0.76)	(0.88)	(0.92)	(0.13)	(0.11)

[a] In parentheses. [b] At different pH values. [c] See text for symbols.

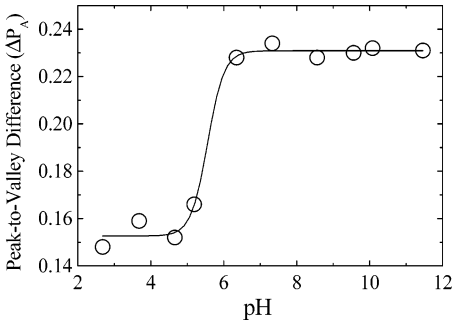


Figure 2. Peak-to-valley difference for PAAMePy55 as a function of pH. (See the text for more details). The line is a guide for the eye.

repulsion between the carboxylate (COO^-) groups will expand the polymer chain, which will affect the distance between adjacent pyrene groups; therefore, fewer excimers are created at higher pH. In dioxane, the dependence of the I_E/I_M ratio on the excitation wavelength is also strongly reduced but does not completely vanish (see Figure 3 in particular for the PAAMePy55 polymer), thus suggesting that a small, but significant, ground-state association is present in the two polymers. The degree of association in the organic solvents will be further discussed with the help of the time-resolved data.

Differences in the excitation spectra, when collected in the monomer and excimer emission regions, are frequently used as evidence for associative ground-state dimers.[38] These differences can consist of a more or less pronounced shift in the wavelength maximum of the two spectra ($\Delta\lambda_2$)[38,40,41] and differences in the peak-to-valley ratio relative to the (0,1) transition observed in the monomer (P_M) and excimer (P_E) excitation spectra, respectively, and also to a significant broadening of the $S_0 \rightarrow S_1$ absorption band, which leads to a more pronounced absorption in the red part of the spectra. All of these differences, as well as those observed in the absorption spectra, are based on the idea that a more or less intense absorption band due to ground-state aggregates is buried underneath the intense monomer band. With the gradual disappearance of the aggregates promoted by the increase in the pH or other external stimuli, for example, the addition of surfactants,[6] all of the mentioned features tend to disappear. In that limit, the excitation spectra collected at any region of the emission spectra will be identical. The data obtained for the two PAAMePy polymers in different solvents—methanol, dioxane, and water at different pH—are summarized in Table 1.

Figure 4 presents the excitation spectra, collected in the monomer and excimer regions, for PAAMePy55 and PAAMePy230 at three different pH values together with those obtained in methanol and dioxane. As may be noticed, the excitation spectra collected in the monomer and excimer emission regions display different maxima and shapes with the excimer spectrum being red-shifted. The shift of the spectra ($\Delta\lambda_2$) and the difference between the ratio of the peak intensity maximum and the intensity at the valley viewed at the monomer (P_M) and excimer (P_E) emission[38] ($P_M - P_E$) is more pronounced for PAAMePy55 than for PAAMePy230. This points out the existence of a higher percentage of ground-state aggregation in this polymer system. For identical pH values, $\Delta\lambda_2$ is larger for PAAMePy55. This is valid until pH reaches ca. 8. From there on, comparison between the two polymers reveals nonsignificant differences in their $\Delta\lambda_2$ values and peak-to-valley ratios. At

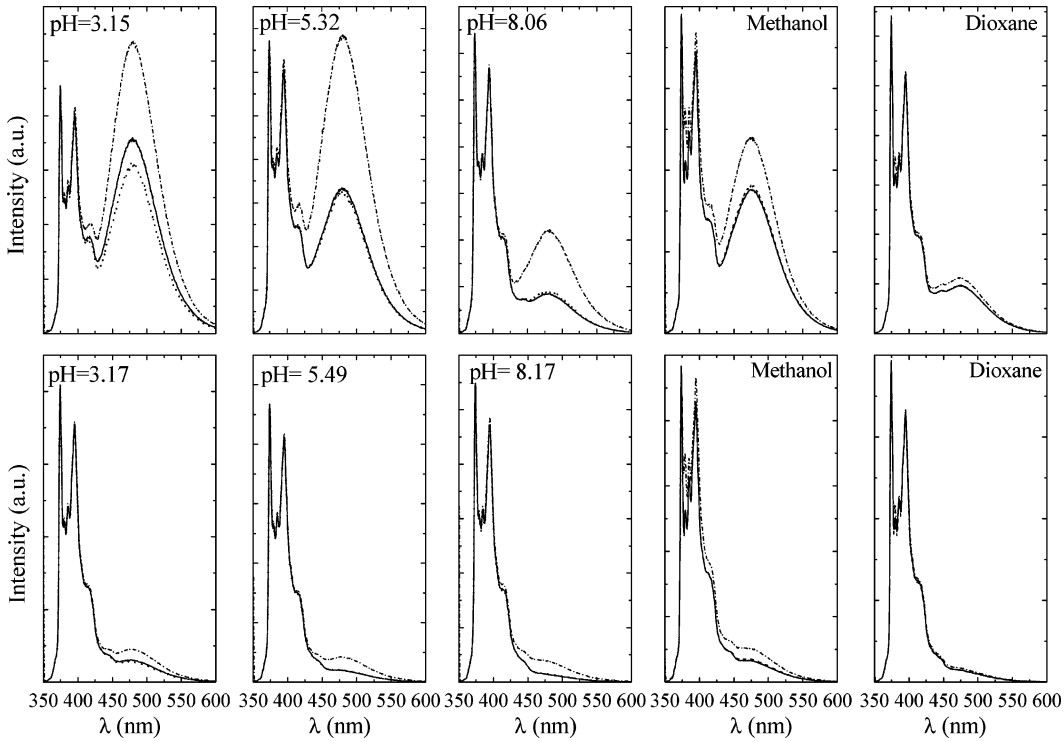


Figure 3. Fluorescence spectra of PAAMePy55 (top panels) and PAAMePy230 (bottom panels) polymers obtained in water, at various pH values, in methanol, and in dioxane with excitation wavelengths of 316 (···), 335 (—) and 350 (—·—·—), $T = 293$ K.

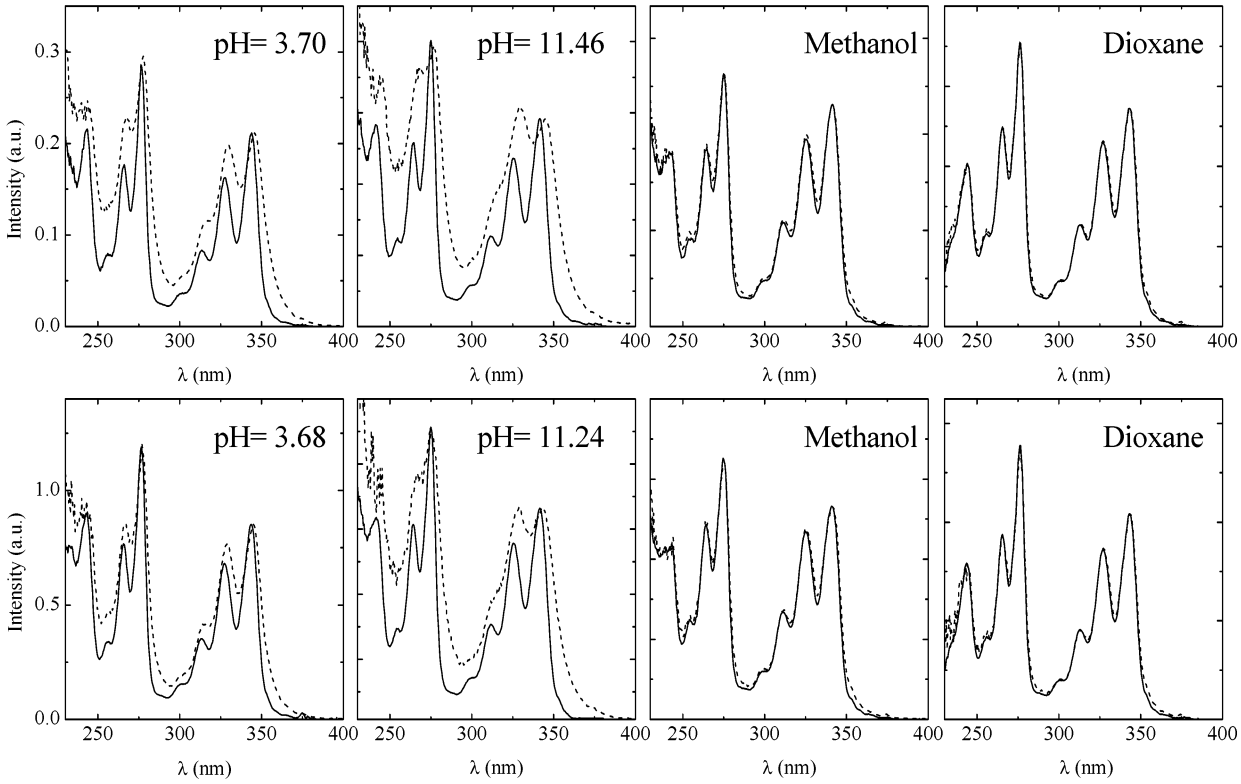


Figure 4. Fluorescence excitation spectra obtained in the monomer (—) and excimer (···) emission regions for PAAMePy55 (top panels) and PAAMePy230 (bottom panels) in water (at different pH values), methanol, and dioxane, $T = 293$ K.

higher pH values, the differences in the excitation spectra are still discernible. Still, shifts and small differences between the peak-to-valley ratios are obtained in addition to a red-edge absorption/excitation band (Figure 4).

The emission spectra of PAAMePy55 and PAAMePy230 change with temperature (data not shown). The total intensity decreases upon increasing temperature without showing iso-emissive points, albeit this might be pH-dependent.[42] This indicates that monomer and/or excimer decay competes with the direct and backward reactions and that the excited-state equilibrium cannot be reached before emission (of both the monomer and the excimer). The detailed analysis of the temperature dependence of the present systems will be the aim of a forthcoming publication.[42] Contrary to this behavior, the

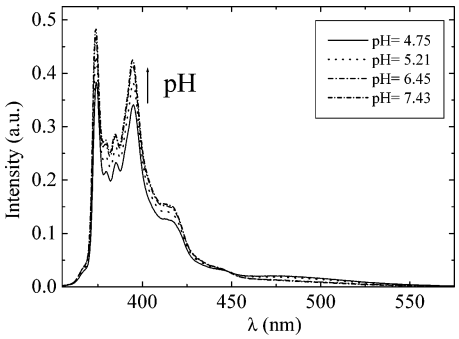


Figure 5. Fluorescence spectra of PAAMePy230 obtained at different pH values, $T = 293$ K.

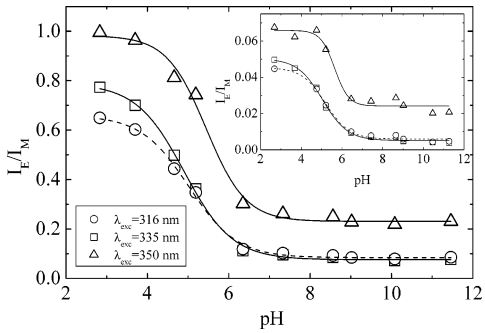


Figure 6. Dependence of the excimer-to-monomer (I_E/I_M) ratio on pH obtained at three different excitation wavelengths for PAAMePy55 and PAAMePy230 (inset). The lines are guides for the eye.

emission spectra of both polymers show an isoemissive point (~450 nm) when studied as a function of pH (Figure 5), and a gradual decrease in the intensity of the excimer emission (I_E) is accompanied by an increase in the monomer intensity (I_M) (Figure 6). Hence, the relative fraction of isolated chromophores increases with pH.

Time-Dependent Aspects. The fluorescence decays of both polymers were found to be multiexponential in the monomer (triple exponential) and excimer (double exponential) emission regions. With global analysis (simultaneous analysis) of the fluorescence curves, two of the decay times were found to be identical in both emissive regions. An additional longer component exists in the monomer region. In the wavelength region of monomer emission (decays collected in the 370−380 nm range), two of the exponential components represent the fluorescence emission from isolated excited chromophores or "free" monomers (a component with a long decay time τ_3) and the emission from the monomers that are able to give rise to the excimer, abbreviated MAGRE (a component with a short decay time τ_2). The third component (τ_1 and the associated a_{i1} prefactors in Tables 1 and 2) originates from the back reaction of an excimer to a monomer (reversibility). As mentioned above, the different degrees of pyrene labeling along the PAA backbone can lead to pyrene microdomains on the polymer chain, which may therefore induce excimer formation with different rates. In the proposed model, these rates are found in a monomodal distribution with a single k_a value. Within the alternative blob model, the existence of several exponentials in the monomer decay will lead to a distribution of excimer-formation rate constants. Using independent analysis, the decays collected in the excimer emission region (decays collected in the 520−580 nm range) are generally reduced to double-exponential functions with a rising component (the monomer decay, τ_2) and a decaying component (the excimer decay, τ_1) (Table 2). In fact, as can be seen in Figure 7 in the simultaneous analysis of the decays obtained with different excitation wavelengths, the longer component (mainly found in the decays collected in the monomer emission region) is not recovered in the excimer parameters, although sometimes an additional longer-lived component is needed to fit the decays properly. The presence of this third component with a much longer decay time (≥500 ns and with negligible preexponential) in the excimer decay could usually be considered to be a baseline correction.[43] As a consequence of the above, the independent analysis of the decays at the monomer (380−390 nm) and excimer (520−580 nm) will be used here instead of the alternative global analysis[37,43] procedure.

Excitation with a λ_{exc} in the blue region (315 nm), at the maximum (≈335 nm), and in the red region (≈350 nm) of the monomer band results in a progressive decrease of the already negative value of the preexponential factor when observed at the excimer emission (Figure 7 and Table 2). This is direct evidence for the existence of preformed dimers.

Different methods and strategies of analysis have been discussed to interpret the data obtained from time-resolved experiments.[31,32,43−45] In a Birks'-type kinetic scheme, the simultaneous analysis of the decays, collected in the monomer and excimer emission regions, should be the most appropriate method of decay analysis because the two decay times are identical over all of the spectra and the only varying parameters are the preexponential factors. When additional components are present, this strategy of analysis may no longer be the most appropriate. Individual analysis of the monomer and excimer emission regions together with the global analysis should give additional information, as others have observed with poly(phenylsiloxane) polymers.[46] In the present work, the independent analysis of the decays was used to avoid erroneous interpretations of the longer component at the excimer emission wavelength, which would obviously be present if a global analysis of the decays was made.

Different time scales were used to extract possible subnanosecond components. The simultaneous analysis of the decays gives good statistics and seems to be an adequate procedure for decay analysis.[37,43] In general, the values of the decay times are roughly constant with pH but not with solvent. The individual analysis at each wavelength gave somewhat different values for the shorter component (see Discussion). The account of this behavior, namely, the possibility of energy transfer between adjacent chromophores, will be further explored in the Discussion section.

Discussion

Spectral Features and Excimer−Monomer Relation, ΔP_A and I_E/I_M. As mentioned in the Results section, the peak-to-valley ratio taken from the absorption spectra (P_A) strongly indicates that preassociated dimers exist.[38] This essentially lies in the assumption that the displayed absorption spectrum results from the sum of two bands: the monomer and the dimer. It is known that dimer bands are devoid of vibrational resolution; consequently, the P_A ratio will change dramatically with the fraction of ground-state association. In the case of pyrene-labeled poly(*N*,*N*-dimethylacrylamide)s, others[31] have found that P_A values are close to 3.0, indicating the absence of ground-state association, and that the lowering of that value will indicate the presence of those aggregates. Similar conclusions can be drawn for the presented systems, where values close to and larger than 3 are found with both of the PAAMePy polymers in organic solvents dioxane and methanol (Table 1). At acidic pH values, where ground-state association is expected to be

TABLE 2: Fluorescence Decay Times (τ_i), Normalized Amplitudes (a_{i1}, a_{i2}, and a_{i3} with $i = 1, 2$) and Chi-Squared ($\chi 2$) Values) for the Two PAAMePy Polymers Obtained at Different pH Values and with Three Different Excitation Wavelengths

polymer	pH	λ_{exc}	λ_{em}	τ_2/ns	τ_1/ns	τ_3/ns	a_{i2}	a_{i1}	a_{i3}	$\chi 2$
PAAMePy55	3.15	315	390	10.4	62.3	226.3	0.343	0.444	0.214	1.12
			520	7	64.4		−0.255	1.00		1.05
	3.15	335	390	11	62.2	226.1	0.366	0.424	0.211	1.15
			520	10.8	62.2		−0.180	1.000		1.23
	3.15	350	390	11	60	235	0.437	0.412	0.151	1.20
			520	10.8	62.2		−0.089	1.000		1.16
	4.66	335	380	10.8	56.8	228.4	0.280	0.374	0.346	1.02
			520	10.1	52.3		−0.136	1.00		1.14
	5.2	335	380	9.7	55	228.4	0.267	0.347	0.386	1.09
			520	11.4	55		−0.156	1.00		1.15
	7.33	335	380	11.9	53.4	185.2	0.266	0.186	0.548	0.99
			520	9.89	43.8		−0.055	1.00		1.14
	8.56	335	380	13.42	45.7	172	0.248	0.163	0.584	1.03
			520	13.19	47.1		0.041	1.00		1.14
PAAMePy230	3.17	315	390	10.17	69.7	235.6	0.087	0.278	0.635	1.08
			520	9.23	73.2		−0.280	1.000		1.13
	3.17	335	390	9.66	67.9	212	0.114	0.269	0.617	1.18
			520	9.70	65.9		−0.236	1.000		1.06
	3.17	350	390	10.8	60.3	209	0.225	0.121	0.617	0.98
			520	10.4	65.8		−0.076	1.000		1.06
	5.6	315	370	8.62	56	204	0.087	0.133	0.78	0.98
			520				−0.048	0.906	0.094	1.05

larger,[6] the obtained P_A values display their minimum value (Table 1). Because it basically consists of the overlapping of two bands, the peak-to-valley difference (ΔP_A), instead of the ratio, plotted as a function of pH presents additional information (Figure 2). The diagram in Figure 2 clearly exhibits two different plateaus, which shows that a dramatic change has occurred in the absorption spectra. This is consistent with the larger contribution of associated ground-state chromophores at low pH values, up to pH $\approx$ 5, which is a result of the more coiled nature of the polymer conformation. Upon increasing pH, a higher percentage of the carboxylic groups on the PAAMePy polymers are ionized, and the repulsive interactions between them promote an expansion of the polymer chain. The total (but not relative) contribution of ground-state dimers is therefore less at pH values above 6, which is reflected in Figure 2. In fact, for basic pH values the relative percentage of ground-state aggregates gains weight when compared with that of excimers originating from a dynamic route. However, even at basic pH values excimer formation originating from a dynamic mechanism is still present.

The excitation spectra at basic pH values shown in Figure 4 are unexpected results because for those pH values it is anticipated that the expanded polymer coil would prevent any ground-state association. However, as can be seen in Figure 4, the excitation spectra collected in the monomer and excimer regions are still strongly different, and this difference is even more pronounced than for acidic pH values. For the modified PAA polymers studied here, water can be considered to be a poorer solvent than the organic solvents (dioxane, methanol) used in this investigation or even a partially selective solvent. This in turn suggests that in water internal polymer−polymer interactions (for example, between pyrene groups or other parts of the polymer chain) are more favorable than polymer−solvent interactions.[47,48] Hence, the polymer adopts a more compact conformation in water compared to its conformation in organic solvents.[6] Consequently, for these PAAMePy polymers in water at any pH value, internal polymer−polymer interactions will be present and are responsible for the observed excimer formation. At high pH, these attractive interactions compete with the additional repulsive interactions between the appearing COO^- groups, but they are still significant. Furthermore, in this case of randomly grafted polymers, an uneven distribution of unimer[48] units containing chromophores exists within the same polymer. Thus, in some parts of the polymer chain, the density of attached pyrene groups is higher than in other parts. The former will still induce a strong interaction of the aromatic moieties forming ground-state dimers, which will now be the dominant route for excimer formation. If this were not the case, then excitation at 350 nm for basic pH values (Figures 3 and 6) would not give rise to higher I_E/I_M ratios, when compared with those obtained for lower excitation wavelengths. This explains why such low (and negative) values of the preexponential factors, which are associated with the rising component in the time-resolved fluorescence decays, are obtained at high pH (Table 2 and Figure 7). In summary, because the fraction of excimer formed via ground-state dimers is larger at alkaline pH (compared with that at acidic pH values), the excitation spectra collected at the monomer and at the excimer, respectively, will present a larger shift at high pH than at low pH.

To interpret the current data correctly, the information obtained from the steady-state fluorescence measurements for PAAMePy polymers in water is complemented with that obtained from the time-resolved experiments. The monomer emission band results from the contribution of two different types of monomers, one of which is not able to produce excimers. The other is obviously a monomer able to give rise to dynamic excimer formation, here abbreviated MAGRE. The ratio I_E/I_M must therefore be evaluated by considering the presence of free monomers. In these PAAMePy systems, excimer formation may have two different origins. Both are a result of hydrophobic interactions of closely spaced pyrene chromophores. The first type of excimer formation results from dimers that are preassociated before excitation ("static excimers" or ground-state dimers),[38] and the second type evolves from a dynamic movement where one excited chromophore and one nonexcited chromophore are brought together ("dynamic excimers").[3] In terms of fluorescence spectra, the excimer emission band of static and dynamic excimers generally appears in the same region. It is consequently very difficult to extract informa-

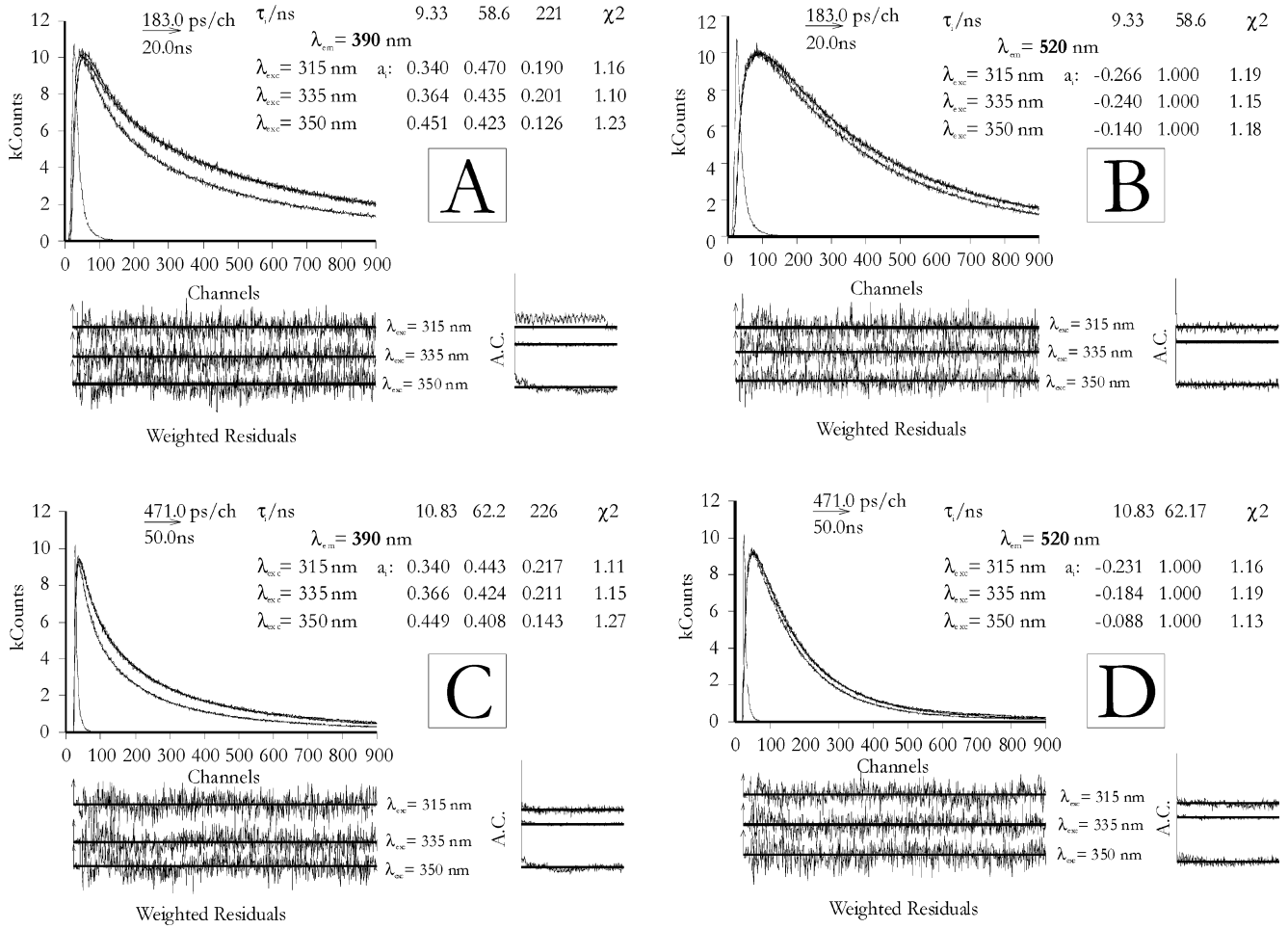


Figure 7. Fluorescence decays obtained for PAAMePy55 at pH 3.2 in the monomer (390 nm) and excimer (520 nm) regions, simultaneously analyzed with three different excitation wavelengths on two different time scales: (A and B) 0.183 ns per channel; (C and D) 0.471 ns per channel. The instrument profile curve is also shown. Decay time values and preexponential values are shown as insets of the decays. For a better judgment of the quality of the fits, shown as insets are the weighted residuals, autocorrelation (A. C.) functions, and chi-squared (χ^2) values.

tion concerning the type of excimers present solely on the basis of steady-state data.

The I_E/I_M ratio variation with pH for both PAAMePy55 and PAAMePy230 presented in Figure 6 shows that above pH $\approx$ 7−7.5 this ratio does not change significantly. For acidic pH values, the modified polymers have a low degree of dissociation ($\alpha \approx 0-0.1$ with the analogue PAAMeNp34[6] and $\alpha \approx 0$ for the unlabeled PAA; α here means the degree of dissociation and should not be mistaken for the fraction of light used to excite associated chromophores; see Scheme 2 below). For the analogue PAAMeNp34 at pH 7, 55% of the carboxylic (COOH) groups are deprotonated,[6] and we expect the same behavior here. The pyrene-labeled PAA now behaves as a strong polyelectrolyte (for the unmodified PAA, $\alpha \approx 0.9$ at pH 7). The strong pK_a dependence on α is generally attributed to an increase in the electrostatic repulsion between the COO^- groups on the unlabeled PAA polymer. Some additional important considerations can be taken from a careful observation of Figure 6. The displayed shape clearly denotes the existence of a strong dependence on the degree of deprotonation of the backbone of the labeled PAA polymers. As the pH value increases, the degree of dissociation increases, leading to a progressive predominance of repulsive electrostatic forces (between the COO^- groups) and a subsequent decrease in the I_E/I_M ratio. The shape of the curves in Figure 6 is identical for the two polymers (PAAMePy55 and PAAMePy230), following the expected trend for a titration curve with p$K_a \approx 4.5-5$, which is approximately identical to that for unlabeled PAA.[49]

One should now consider why the absolute value of the I_E/I_M ratio is significantly smaller for the PAAMePy230 polymer. In a first approximation, if this ratio was a consequence of only the equilibrium between the MAGRE monomer and the excimer, it should be identical for the two polymers. In fact, if this were the case, then all of the monomers would be able to give rise to excimer; consequently, the I_E/I_M would reflect that. The intensity of the monomer band results from the contribution of the two types of monomers (MAGRE and free). Thus, if one of them decreases, then this will automatically influence the I_E/I_M ratio. Obviously, the absolute value for the I_E/I_M ratio is lower for the less-labeled polymer (PAAMePy230) because the distance, on average, between neighboring pyrene chromophores (or monomers) has increased. As a consequence of that, it is statistically less probable for an excited monomer to encounter a nonexcited monomer to produce a dynamic excimer or even two pyrene units to be close enough together to form a preassociated dimer or a static excimer. Ultimately, this behavior would mirror the existence of more free monomers in the PAAMePy230 aqueous system than in the PAAMePy55 aqueous system. Another important aspect to be considered from the analysis of the curves in Figure 6 is their dependence on the excitation wavelength. For excitation wavelengths of 316 and 335 nm, the curves are identical both in shape and intensity. However, when excitation occurs in the red region of the

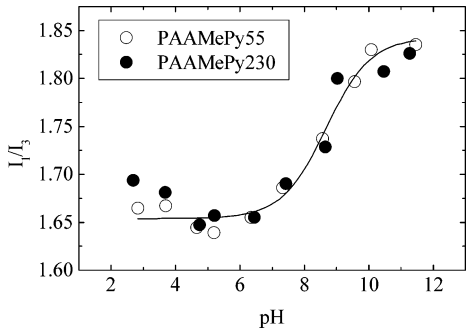


Figure 8. Variation of the fluorescence intensity ratio (I_1/I_3) with the pH for PAAMePy55 and PAAMePy230 polymers at $T = 293$ K. The solid lines are guides for the eye.

absorption spectra, the shape of the curve is kept identical, whereas the absolute value of the I_E/I_M ratio increases. This happens over the entire pH range and is a consequence of the preferred absorption of ground-state dimers in the red region of the spectra. An increase in the intensity of the excimer band is therefore observed (i.e., at this excitation wavelength, the contribution to the total excimer emission is dominated by the emission of preformed dimers (Figure 3)).

At this stage, we can conclude that the conformational change of the coiled or aggregate state to a more extended state of the hydrophobically modified PAA (by the change of pH) strongly depends on the degree of ionization of the backbone chain.

Intensity Ratio I_1/I_3. All of the previous data have yielded substantial information on the nature of the polymer structure by means of the microenvironment felt by the pyrene chromophores. In addition to the above, the fine structure of the emission from singly excited pyrene can be examined. The ratio of the fluorescence intensity of the pyrene vibronic bands $I_1[S_0(v = 0) \leftarrow S_1(v = 0)]$ and $I_3[S_0(v = 1) \leftarrow S_1(v = 0)]$ (i.e., I_1/I_3) reflects the polarity of the local medium surrounding the chromophore. As has earlier been found, the I_1/I_3 ratio increases with polarity (or dielectric constant) of the solvent.[50,51] This specific vibronic dependence that occurs in the case of pyrene is due to an efficient vibronic coupling between the two lowest lying closely spaced electronic states and has therefore been extensively used as a polarity scale.[1,5,29,50−55] The extrapolation of this I_1/I_3 dependence to other pyrene derivatives should be considered with care because substitution in pyrene leads to symmetry loss. It has been argued that methyl substitution keeps the validity of the I_1/I_3 ratio as a polarity scale, whereas if it is ethyl substitution the rule is no longer valid.[52] In our case, we have observed that the I_1/I_3 ratio can change from 1.6 to 1.85 (Figure 8) depending on the pH of the media and the excitation wavelength, thus indicating clear changes in the pyrene microenvironment. According to the study made by Dong et al.,[50] the I_1/I_3 ratio for free pyrene in water is equal to 1.87. This is very close to ours at alkaline pH values, which shows that the medium felt by the pyrene chromophores is almost as hydrophilic as water. This is further evidence that for the two PAAMePy aqueous systems the polymer coil is extended at high pH values. For pH values close to 5, the I_1/I_3 ratio approaches values around 1.6, which indicates a less polar environment because this value is close to what is found for pyrene in glycerol.[50] All of these values should, however, be taken with caution because experimental artifacts can strongly influence the determination of accurate I_1/I_3 values.[5] Thus, in general terms, our behavior clearly shows a trend that is compatible with the general observation that the pyrene probe is exposed to a more polar environment[1] at higher pH values in water for both polymers investigated.

Time-Resolved Fluorescence Data for the PAAMePy Polymers in Aqueous Solutions. A previous study on analogous systems, where time-resolved fluorescence spectroscopy was employed, revealed a lack of interpretation of the data that was in part due to the complexity of the system.[7,17] As already shown, the present systems cannot be fit to a simple Birk's kinetic scheme[3] as others have tried for similar systems; see, for example, ref 17. Different strategies, usually involving decay analysis with two exponentials, have been tried, but in all cases, additional components were found to exist and no complete elucidation of those parameters was given.[7,17] A partial analysis of the latter part of the decay curve[17] was used to extract pertinent information, but again no further elucidation was obtained. In another study, with polystyrene chains with both ends labeled with 1-pyrenyl groups, an analysis of monomer decay curves constrained with the decay values obtained from excimer decay curves was also used to obtain rate constants of monomer−excimer kinetics.[39] The important factors in the analysis of these complex decays include, among others, the careful choice of the emission and excitation wavelength and the method of analysis. It is expected that in the monomer region the chosen wavelength would avoid emission from the excimer band and vice versa, and if ground-state aggregates exist, then the excitation in different regions of the absorption spectra will give rise to different amplitudes. As earlier mentioned in the Results section, the decay analysis may either be independent or global. If components with shorter fluorescence lifetime are expected to exist, then different time scales should be tried in the measuring procedure. This was performed in the current study (Figure 7). The analysis and the interpretation of dynamic data should therefore be performed with care, as will be discussed below.

Preformed dimers coexisting with dynamic excimers are commonly observed in kinetic studies with pyrene derivatives.[38] In terms of steady-state emission spectra, it is difficult to differentiate them from each other because both normally emit in the same wavelength region. However, it is possible to prove the existence of preassociated dimers by examining the I_E/I_M ratio measured at different excitation wavelengths. The experimental evidence for dynamic excimers using time-resolved fluorescence measurements is the observation of a rising component in the excimer emission (Figure 7 and the a_{22} component in Figures 9b and 11 below). In other studies, the authors have been unable to observe a rise time for the analogous PAA polymers with different labeling degrees.[7,17] Yet it was concluded, from the dependence of the I_E/I_M ratio on the viscosity, that dynamic excimers were also present.[7] If no interaction between pyrene groups is allowed in the ground-state, then the excimer in the excited state, E*, is not produced upon direct excitation (static via), but instead it is produced from the locally excited state (dynamic via). If this is the case, then the sum of the preexponential factors (a_{21} and a_{22}) at the excimer emission should be equal to zero.[3] From Figure 9b, it can be seen that this sum clearly deviates from zero. The deviation could also be due to a contribution of monomer emission to the fluorescence decay of the excimer (monomer emission in the excimer region). However, the decays were recorded at wavelengths on the red edge of the excimer emission ($\lambda_{em} \geq 520$ nm) where no significant monomer emission is present. Another possibility for the deviation from zero is that an inadequate time resolution was used. But as shown in Figure 7, the same parameters are obtained using two different time scales. This implies that, within the experimental error, the obtained decay times are well resolved. From this discussion,

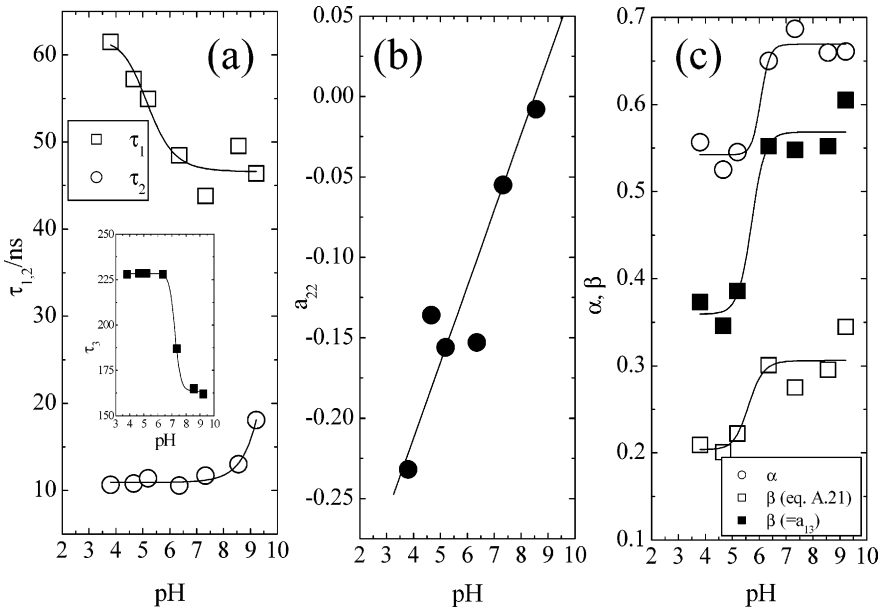


Figure 9. Variation of the fluorescence decay times (a), preexponential factor a_{22} (b), and α and β values (c) with pH for PAAMePy55 at $T = 293$ K. The inset in a shows the variation of the longer decay time as a function of pH. The lines in a and c are guides for the eye.

TABLE 3: Fluorescence Decay Times (τ_i) and Normalized Amplitudes (a_{i1}, a_{i2} and a_{i3} with $i = 1, 2$) of the Two PAAMePy Polymers in Methanol and Dioxane at Two Different Excitation Wavelengths[a]

solvent (polymer)	λ_{exc}	λ_{em}	τ_2/ns	τ_1/ns	τ_3/ns	a_{i2}	a_{i1}	a_{i3}	χ^2
dioxane (PAAMePy55)									
	315	370	25.8	124	246	0.204	0.672	0.124	1.09
		520	23.6	138		−0.721	1.000		1.13
	335	370	24.5	120	246	0.249	0.65	0.099	1.13
		520	24.5	139		−0.697	1.000		1.04
dioxane (PAAMePy230)									
	315	370	32.4	144.5	246	0.183	0.62	0.197	1.16
		520	29.1	148		−0.534	1.00		1.34
	335	370	30.1	139.4	246	0.167	0.573	0.26	1.17
		520	27.6	157		−0.467	1.000		1.24
methanol (PAAMePy55)									
	315	370	23.6	96.4	251	0.304	0.586	0.109	1.18
		520	23.3	98.1		−0.754	1.000		1.51
	335	370	24.63	90.9	254	0.343	0.515	0.142	1.21
		520	22.28	103.5		−0.763	1.000		1.17
methanol (PAAMePy230)									
	315	380	30	100	246	0.264	0.653	0.08	1.16
		520	25	124.5		−0.682	1.000		1.22
	335	380	29.6	103.6	246	0.364	0.62	0.016	1.10
		520	25.5	125.7		−0.67	1.00		1.03

[a] The presented fits were obtained with independent (i) and global (G) analyses of the decays. The chi-squared (χ^2) values are shown for a better judgment of the quality of the fit.

the obvious conclusion will be that the deviation from zero must be due to the fact that there exists a fraction of pyrene groups that interact intramolecularly in the ground state and when associated can directly absorb a portion of the exciting light. If the pyrene dimers are in the appropriate conformation (sandwichlike), then excimer formation can take place immediately by light absorption through a static mechanism. The data in Figure 9b show that this sum ($a_{21} + a_{22}$) gradually approaches unity (a_{21} is approximately constant with a value equal to 1) because the absolute value for a_{22} decreases with decreasing pH. External factors such as the temperature have also generally been shown to have an effect on this value.[56] In the present study, both the nature of the solvent and the pH of the aqueous solution are found to influence the value of the sum of the preexponential factors at the excimer emission wavelength (Tables 2 and 3).

From the time-resolved data, an additional component with a longer decay time (τ_3) has been observed, which is attributed to the existence of free excited monomers that are unable to form excimers during their lifetime. In the case of the less-labeled polymer (PAAMePy230), the expectation was that the pyrene chromophores were unable to form excimers, decaying monoexponentially with a fluorescence lifetime identical to the τ_3 decay time found for PAAMePy55. In fact, we have found this behavior with the analogue PAA polymer labeled with naphthalene, where in the case of a low degree of labeling monoexponential fluorescence decays were obtained.[42] However, in the present case even with the less-labeled polymer

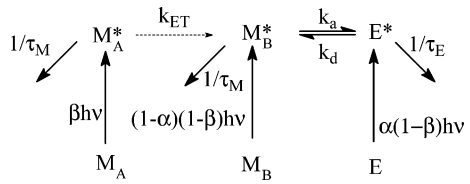
(PAAMePy230), excimer formation occurs. Consequently, the fluorescence decays are multiexponentials, as found with the PAAMePy55 polymer. This is not unexpected in view of the much longer fluorescence lifetime of pyrene when compared with that of naphthalene.[57] Also, pyrene (being more hydrophobic) is expected to associate more strongly. The 1-pyrenylmethylaminehydrochloride compound can be considered to be a primary model compound for the monomer fluorescence lifetime. For this compound, fluorescence lifetimes (τ_F) in the 156-ns (water at basic pH values) to 235-ns (in methanol) range were obtained in water and in the organic solvents methanol and dioxane.[42] Although this compound should not be regarded as an ideal model compound, the obtained τ_F values give emphasis to the fact that the slower decay time, obtained with the PAAMePy polymers, can be attributed to the fluorescence lifetimes of isolated pyrene units existing within the polymer chain. Also of relevance is the fact that for this parent analogue the fluorescence lifetime changes from 177 ns at acidic pH values to 156 ns at pH $\cong$ 10. In fact, from the inset in Figure 9a, the longer component (τ_3) shows a plateau region at acidic pH values (until pH $\cong$ 6) with a sudden decrease up to pH $\cong$ 8, thereafter remaining constant. This suggests that the removal of the nitrogen (or the carboxylic acid) proton induces a quenching effect due to electron transfer from the amine (or ionized carboxylic) lone pair to the excited pyrene, and thus an additional deactivation pathway is strongly suggested to be operative in the case of the free pyrenes in the PAAMePy polymers. This kind of quenching has also been found with other aminic aromatic-like containing molecules.[58−60]

When analyzing the decays of PAAMePy55 and PAAMePy230 in water at different pH values and in organic solvents, the longer decay time, attributed to the isolated chromophores, was found to vary from 228 to 162 ns (Figure 9a). The 228-ns decay time is kept approximately constant until pH 5.2. From there on, a decrease in this value is obtained, which is possibly a consequence of the fact that at low pH values the polymer wraps itself around pyrene, acting as a shield against the quencher (traces of oxygen or other quencher), whereas at high pH values the existing electrostatic repulsions will lead to an open structure of the polymer, which diminishes the shielding effect. The pyrene chromophore will be more accessible to the solvent (and to the quenchers therein), which will enhance the quenching effects and lead to a decrease in its lifetime. An alternative and complementary view is the previously mentioned quenching effect induced by the nitrogen lone pairs. Recent studies with another pyrene-labeled polymer in water lead to a value of 245 ns for the unquenched pyrene lifetime.[31] In this same study, different solvents and solvent mixtures gave values of this unquenched lifetime in the range of 220−256 ns.[31] This gives additional evidence to our attribution and expectation of a variation of this long decay time value with both the pH and the solvent. An additional quenching effect can be due to oxygen. It is generally considered that oxygen is a strong quencher of fluorescence probes with long lifetimes such as pyrene. Consequently, values as different as 650 ns[57] and 382 ns[51] can be found in the literature for the same solvent. Even with good degassing procedures it is likely that the local microenvironment felt by the pyrene chromophore in the PAA polymer can be responsible for some fluctuations in the decay time of the free species.

The pH dependence of the two other decay times is shown in Figure 9a. Both of them correspond to the decay times of the kinetic species involved in excimer formation. For the intermediate decay time (τ_1), the initial value around ~60 ns decays to ~50 ns above pH 6 and then remains constant. The shorter decay time (τ_2) shows a constant value until pH $\approx$ 7, from where it starts to rise.

All of the previous considerations lead to the kinetic scheme in which two types of monomers are present (Scheme 2).[24,30]

SCHEME 2



The isolated or free momomers (M_A) are unable to participate in excimer (E*) formation, whereas the other type of monomer (MAGRE), M_B, allows excimer formation. According to Scheme 2, the β value corresponds to the fraction of light that excites the isolated chromophores, M_A, and $1 - \beta$ is the remaining light that excites the chromophores, which possess one or more neighboring units (M_B and E). The fraction of light that is not absorbed by M_A can be split into two components: $\alpha(1 - \beta)$, which is the fraction of light absorbed by ground-state dimers (E), and $(1 - \alpha)(1 - \beta)$, which corresponds to the fraction of light that directly excites the MAGRE monomers. The rates of excimer formation and deactivation are given by k_a and k_d, respectively. Also displayed in Scheme 2 are the reciprocals of the unquenched lifetimes of the monomer and excimer, $1/\tau_M = k_M$ and $1/\tau_E = k_E$ (i.e., the rate constants for monomer and excimer decay, both through radiative and nonradiative pathways). In Scheme 2, we can also contemplate the rate constant for a possible energy transfer (k_{ET}) process involving the two types of monomers.[24] The reverse transfer from M_B^* to M_A^* is considered to be unimportant. The energy-transfer rate constant k_{ET} will not be considered to deduce the equations, according to the proposed kinetic model, but its possible contribution is utilized in the interpretation of the time-resolved data and is therefore included for the discussion.

According to Scheme 2, the excited-state concentration time dependence of each one of the species is given by

$$[M_A^*](t) = a_{13}e^{-\lambda_3 t} \quad (1)$$

$$[M_B^*](t) = a_{11}e^{-\lambda_1 t} + a_{12}e^{-\lambda_2 t} \quad (2)$$

$$[E^*](t) = a_{21}e^{-\lambda_1 t} + a_{22}e^{-\lambda_2 t} \quad (3)$$

where $\lambda_i = 1/\tau_i$.

Because in the monomer emission region both types of monomers contribute, the time dependence of monomer fluorescence can be described by the following equations:

$$I_M(t) = I_{370-390nm}(t) = a_{11}e^{-\lambda_1 t} + a_{12}e^{-\lambda_2 t} + a_{13}e^{-\lambda_3 t} \quad (4)$$

and that of the excimer emission by:

$$I_E(t) = I_{500-550nm}(t) = a_{21}e^{-\lambda_1 t} + a_{22}e^{-\lambda_2 t} \quad (5)$$

with λ_1 and λ_2 given by

$$2\lambda_{2,1} = \{(k_x + k_Y) \pm [((k_x - k_Y))^2 + 4k_a k_d]^{1/2}\} \quad (6)$$

where $k_X = k_a + 1/\tau_M$ and $k_Y = k_d + 1/\tau_E$ and λ_3 is the reciprocal of the decay time of the uncoupled species.

The ratio of the preexponential factors (see Appendix for individual relations) at the monomer and excimer wavelengths is respectively given by

$$A = \frac{a_{12}}{a_{11}} = \frac{(1-\alpha)(\lambda_1 - k_X) + \alpha k_d}{(1-\alpha)(k_X - \lambda_2) - \alpha k_d} \quad (7)$$

$$B = \frac{a_{22}}{a_{21}} = \frac{(k_X - \lambda_2) - (\alpha - 1)(k_X - k_M)}{(\alpha - 1)(k_X - k_M) - \alpha(k_X - \lambda_1)} \quad (8)$$

All of the unknowns in Scheme 2 (i.e., the three rate constants (k_a, k_d, and k_E) and the α and β values) can be obtained from the values of the decay constants (λ_1 and λ_2) and the ratio of the preexponential factors at the monomer and excimer wavelengths (A and B) once the monomer lifetime (τ_M) is known; see Appendix. In this particular case of pyrene-labeled polymers in aqueous solution and organic solvents, the unquenched lifetime is obtained from the decay time of the free monomer (i.e., $\tau_3 \cong \tau_M$).

It is worth noting that when setting β equal to zero we obtain the preexponential factors for the situation of the coexistence of static and dynamic dimers;[39,61,62] additionally, if α is also set equal to zero, then Scheme 2 reverts to the classic scheme where only monomers are present in the ground state.[3]

The preexponential factor of the component with the long decay time τ_3 in the monomer emission (a_{13}) is associated with the fraction of excited isolated chromophores (β). A comparison between the behavior displayed for the two polymers shows that for the same pH value the less-grafted polymer (PAAMePy230) displays a higher value at acidic pH. For example, at pH $\approx$ 3.2, a_{13} is 0.64 for PAAMePy230 compared to 0.21 for PAAMePy55 (Table 2). At higher pH values, a_{13} increases for both polymers. This is a direct consequence of electrostatic repulsions induced by the deprotonated carboxylic groups, which overcome the hydrophobic attraction between the pyrene chromophores; consequently, more free pyrenes will contribute to the increase in the β value. For the PAAMePy230 system at pH 5.6, the isolated chromophores are now clearly the most predominant emitting species (Table 2).

The dependence of α on pH, obtained from eq 8 (Appendix, eq A.20), is shown in Figure 9c. A gradual increase with pH, with two plateau regions that are more or less well defined, in the acidic and basic regions is observed. Of particular relevance is the fact that preformed aggregates are considerably more important (in terms of relative percentage) at basic pH than at acidic pH, as predicted from the excitation spectrum (Figure 4). At basic pH, the dynamic excimer formation route is much less competitive with the static process. The total amount of excimer formed is obviously less than what is obtained at acidic pH values (Figure 3). Nevertheless, the fraction of excimers produced from a static mechanism relative to the fraction formed from a dynamic mechanism is greater at high pH than at low pH. At basic pH, only locally nearby pyrene chromophores will be able to form excimers. If they are close enough, then the ground-state preformed dimer conformations will preferentially give rise to excimer formation. The encounter of an excited pyrene with another pyrene in the ground state is now less likely to occur because it will eventually involve the movement of the polymer chain. The predominant electrostatic repulsive forces will prevent that from happening.

The pH dependence of the β value is presented in Figure 9c and shows a pattern similar to that found for the α value. The gradual rise upon increasing pH with a plateau around pH 6 is an obvious consequence of the gradual vanishing of the excimer formation. This leads to an increase of free (or isolated) monomers. At pH values above 6, there is no significant further increase in β and α because the polymer has already attained its most expanded conformational state. The β value can be obtained either directly from the a_{13} preexponential factor or from a combination of the obtained α value together with the preexponential ratios in the monomer emission region;[30] see Appendix. The values obtained from both calculation methods are shown in Figure 9c as a function of pH for PAAMePy55 in water. It can be seen that although the same pattern is observed the β values estimated directly from the a_{13} preexponential factor are probably overestimated. This can be judged by analyzing the sum of α and β. In the case of the sum of α with β obtained from the a_{13} preexponential for several pH values, the resultant value is greater than 1, which is a physically impossible value, whereas if the sum is made with the β value obtained from the α and the preexponential ratio values (eq A.21 in the Appendix) the sum never exceeds 1. The reason for this apparent discrepancy may lie in the fact that when the ratio of the preexponentials in the monomer region is used an internal correction is made. Because the α value is obtained from the preexponential factors in the excimer region (eq A.20 in Appendix), the β value is therefore dependent on all five preexponential factors.

The complete kinetic scheme includes the consideration of possible energy migration between pyrene chromophores (k_{ET} in Scheme 2). When the individual analysis at each wavelength is carried out, different decay time values for MAGRE monomers are obtained. The possible reason for the difference that may be observed in the decay times of the MAGRE monomers, detected at the monomer and emission regions, is an additional deactivation pathway for the monomer. This tendency decreases as pH increases, which can be explained by an additional deactivation pathway for monomers through an energy migration mechanism. In that case, this discrepancy in decay times will not be observed in the excimer rise component because that will reflect only the decay component of the monomer that is the origin of the excimer in the excited state. This is true only if no reversibility from M_B^* to M_A^* monomers exists, contrary to what has been found for polymers containing vinyl aromatic groups[24] or polysiloxanes with methyl phenyl groups.[63] If that reversibility channel would have existed, then the decays at the excimer emission would be triexponential, presenting as rising components the lifetimes of the two types of monomers.[63] Apart from this experimental evidence, there are two reasons for the absence of reversibility: the first is the lifetime of M_B^* ($\leq$13 ns), which is reduced relative to that of M_A^* ($\geq$200 ns), and the second is the delocalized nature of the energy within the extended chromophore sequences that increases the effective separation of M_B^* and M_A^*. The M_B^*-to-M_A^* energy transfer by Förster[64] or Dexter mechanisms is therefore diminished relative to the M_A^*-to-M_B^* process.[24] For a donor−donor transfer, as is the case of pyrene−pyrene energy transfer, it was theoretically predicted, in situations where the dynamics of the polymer chain does not have a considerable influence on the obtained decays,[65] that the probability that an excited chromophore contains contributions from excitations that never leave the locally excited state and from those returning to the initially excited chromophore (after one or more energy-transfer events) depends on a critical distance and can be followed by the inhomogeneous broadening of the chromophore spectra. In our case, the emission spectra obtained for PAAMePy230 in water at different pH values do not display any differences in shape (Figure 5). This prediction for situations of low influence of the chain mobility in the decays cannot be extrapolated to

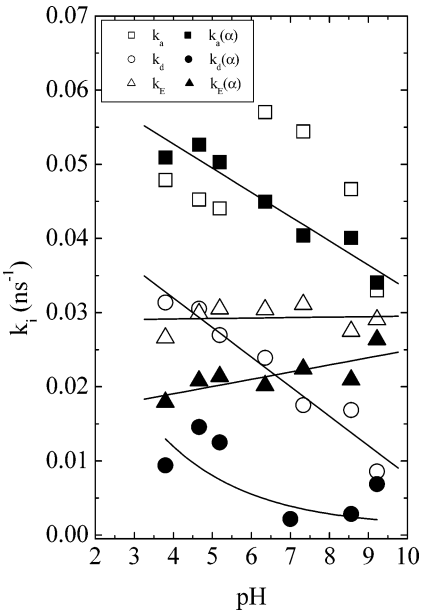


Figure 10. Variation with pH of the association [k_a and $k_a(\alpha)$], dissociation [k_d and $k_d(\alpha)$], and excimer [k_E and $k_E(\alpha)$] rate constants at $T = 293$ K.

our situation, particularly in aqueous media. However, it is likely that in a situation where the chromophores are in close proximity, as it is the case of low pH values, some parallel could be established. In fact, in the present case (Figure 9a), the decay time associated to the MAGRE monomers does not change significantly up to pH 7−8. Thus, if the energy process occurs, it should be identical and present at all pH values (until pH ≈ 8). Moreover and again on the basis of the constancy of the τ_2 values with pH, it does not seem to be a very active channel for MAGRE monomers deactivation. It is therefore logical to conclude that the experimental data, in particular for pH values higher than 5, validates the simplification made that consisted of not considering k_{ET} in Scheme 2.

Rate Constants and their Dependence on pH. In a first approach, the calculated values for k_a and k_d are obtained by setting $\alpha = 0$, as is usual in a Birks[3] scheme, and taking into account only the first two components of the decay (Appendix). Using this procedure, the obtained value for k_a is 0.05 ns^{-1} and has no general tendency or trend with pH within the experimental error (Figure 10).

However, because in the present case the system depends on the fraction of light exciting both M and E, the true rate constants are now dependent on the α value and are given by[30]

$$k_a(\alpha) = \frac{\alpha}{1-\alpha} k_d(\alpha) + k_a(\alpha = 0) \tag{9}$$

and

$$k_d(\alpha) = \frac{k_X(\alpha) k_Y(\alpha) - \lambda_1 \lambda_2}{k_a(\alpha)} \tag{10}$$

with

$$k_Y(\alpha) = \lambda_1 + \lambda_2 - k_X(\alpha) \tag{11}$$

and

$$k_X(\alpha) = \frac{\alpha}{1-\alpha} k_d(\alpha) + k_X(\alpha = 0) \tag{12}$$

Because k_E is obtained from the difference between k_Y and k_d, the new value for k_E, taking into account the α value, is now given by

$$k_E(\alpha) = k_Y(\alpha) - k_d(\alpha) \tag{13}$$

If we use eq 9−12 to solve $k_a(\alpha)$ and $k_d(\alpha)$, then a circular reference is obtained because their determination depends on the other parameter values. As a consequence of this, to deduce the $k_a(\alpha)$and $k_d(\alpha)$ values correctly, another approach has to be chosen. This approach consists of substituting $k_d(\alpha)$ in eq 12 by the value corresponding to $\alpha = 0$. The $k_X(\alpha)$ value obtained from this procedure, and consequently the $k_a(\alpha)$ value, can be improved with a new $k_d(\alpha)$ obtained from eq 10. Because the α value in eq 9 also depends on k_X (eq A.20 in the Appendix section), the true values for the rate constants could be obtained by an iterative method. This improvement of the data is now under consideration and will be explored in a forthcoming study.

The obtained data are presented in Figure 10 together with the k_a and k_d values obtained with $\alpha = 0$. Two aspects merit particular attention. One regards the fact that the corrected $k_a(\alpha)$ value obtained from eq 9 (i.e., taking into consideration both $k_X(\alpha)$ and $k_d(\alpha)$) now presents a gradual decrease with pH, which was not the case when $\alpha = 0$, where some random scattering of this value was observed and thus an averaged value had to be considered. The second observation regards the fact that the new $k_d(\alpha)$ obtained from eq 10 has now decreased ∼100% in absolute value at all of the pH values. Also interesting is the fact that the new rate constant for excimer deactivation $k_E(\alpha)$ also decreased (by ∼50%) and still remains practically constant over the entire pH range, with an average value of 2.0 × 10^7 ns^{-1} ($\tau_E \cong 50$ ns). The recovered excimer decay time is in agreement with those obtained for other pyrene-labeled polymers in water making use of the blob model.[31,34] This shows that two methods based on completely different theoretical frameworks do yield similar conclusions. In the case of the dissociation rate constant, both k_d and $k_d(\alpha)$ show a decrease with pH, although the latter is $\sim^1/_2$ of the value of the former and does not present a linear trend of decrease with pH, as was the case for k_d. From an associative point of view, pyrene being hydrophobic, it is likely that association between neighboring pyrenes will prevail. The decrease in k_d with pH can be viewed as a consequence of the competitive repulsive forces induced by the COO^- groups. In practical terms, the balance between hydrophobic attraction (association between pyrene groups) and electrostatic repulsion (between carboxylate groups) favors the latter, leading to a gradual decrease in k_d with the pH.

Time-Resolved Data in Organic Solvents. So far the steady-state measurements (in terms of I_E/I_M and I_1/I_3 ratios) of the PAAMePy polymers in aqueous media have been discussed. To further evaluate the photophysical behavior of the polymers, it is of interest to compare these results with those performed on the same polymers in organic solvents. In good solvents, such as methanol and dioxane, the dependence of the I_E/I_M ratio on λ_{exc} is strongly reduced (Figure 3). In methanol, the excitation on the red edge of the absorption band still produces more excimer emission than at the two other excitation wavelengths (316 and 335 nm), as shown in Figure 3. Nevertheless, this is much less than what is observed in water at acidic pH values. In dioxane, the dependence on the excitation wavelength is even more reduced but not totally absent.

When a polymer is dissolved in a so-called good solvent, interactions between the solvent and the polymer are energetically more favorable than polymer−polymer interactions, which may be intra- or intermolecular.[47,48] However, in our dilute

TABLE 4: Calculated Rate Constant with and without Ground-State Preformed Dimers for Excimer Formation, $k_a(\alpha)$ and k_a, Dissociation, $k_d(\alpha)$ and k_d, and Excimer Decay, $k_E(\alpha)$ and k_E, for PAAMePy Polymers in Dioxane and Methanol at Room Temperature[a]

polymer (solvent)	k_a (ns^{-1})	$k_a(\alpha)$ (ns^{-1})	k_d (ns^{-1})	$k_d(\alpha)$ (ns^{-1})	k_E (ns^{-1})	$k_E(\alpha)$ (ns^{-1})	$\bar{\alpha}$	$\bar{\beta}$	$\bar{\alpha}(1-\bar{\beta})$
PAAMePy55									
(dioxane)	0.012 ± 0.001	0.015	0.016 ± 0.003	0.014_5	0.015 ± 0.002	0.012	0.44_6	0.07	0.415
(methanol)	0.018 ± 0.005	0.025	0.012 ± 0.001	0.0163	0.018 ± 0.002	0.0123	0.51	0.05	0.485
PAAMePy230									
(dioxane)	0.009 ± 0.002	0.006_6	0.013 ± 0.004	0.012	0.015 ± 0.003	0.017	0.34_5	0.188	0.28
(methanol)	0.013 ± 0.001	0.014	0.013 ± 0.003	0.0078	0.016 ± 0.002	0.011	0.58	0.04	0.56

[a] Averaged values for the α and β values are also presented.

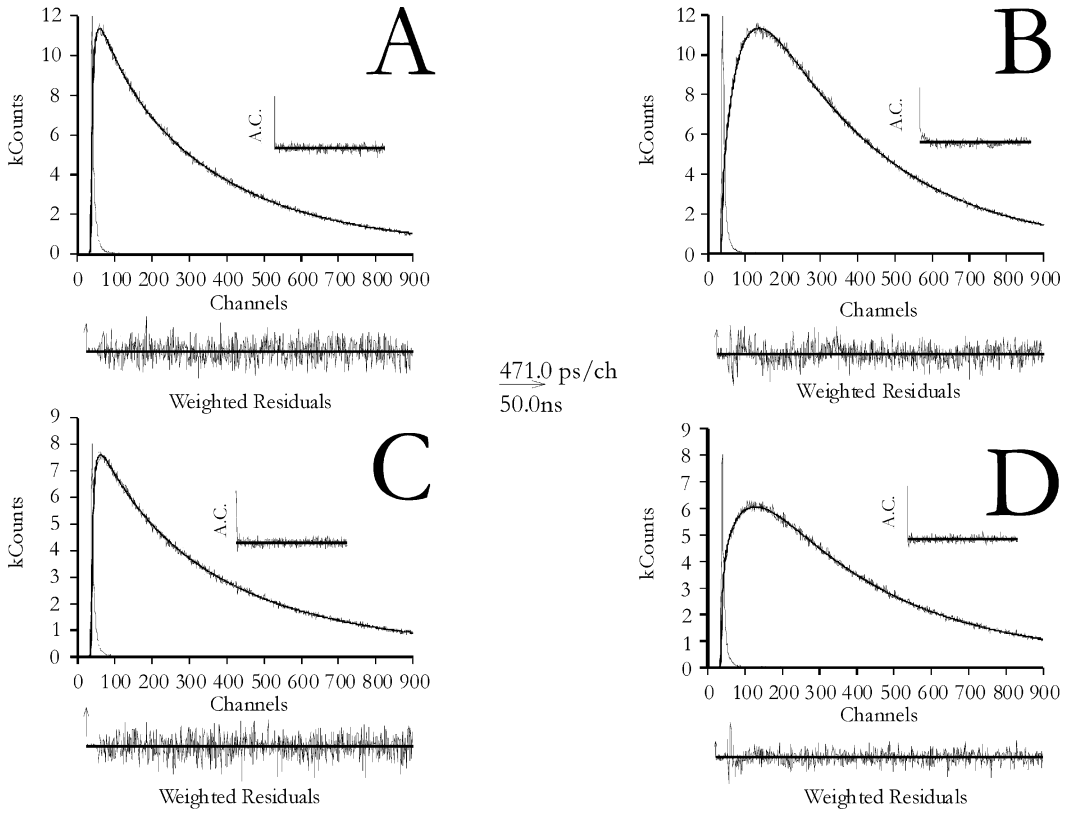


Figure 11. Fluorescence decays for PAAMePy55 (A, $\lambda_{em} = 370$ nm; B, $\lambda_{em} = 520$ nm) and PAAMePy230 (C, $\lambda_{em} = 370$ nm; D, $\lambda_{em} = 520$ nm) in dioxane at room temperature obtained with an excitation wavelength of 315 nm. The decay time values, preexponential values, and chi-squared (χ^2) values can be found in Table 3. The instrument profile curve is also shown. For a better judgment of the quality of the fits, the weighted residuals and the autocorrelation (A. C.) functions are shown as insets.

polymer systems, only internal polymer−polymer interactions are considered. The macromolecules will accordingly expand to maximize solvent−segment interactions. In such good solvents, the modified PAA polymers investigated here are more expanded, and a strong decrease is observed in the contribution of preassociated chromophores. In methanol, and to a lesser extent in dioxane, ground-state association is still present but is still less than in the aqueous solutions (Table 3 and Figure 9c for comparison). This can be attested by the fact that in dioxane different excitation wavelengths (315 and 335 nm) do not produce significant differences in the $a_{21} + a_{22}$ sum at the excimer wavelength, whereas with methanol with excitation at 350 nm an increase in this sum reveals a greater contribution from preformed aggregates (Table 3).

When comparing the decay times of PAAMePy230 and PAAMePy55 in organic solvents with those obtained in water at different pH values, it is noted that the values are generally larger in organic solution than in water. The only exception occurs with the slow decay time, which is almost identical to that found in water. This seems to obey to a general pattern found for aromatic molecules in nonpolar versus polar solvents. Also very relevant is the fact that now the decays, collected at the monomer and excimer regions, gave identical values using both analysis methods (independent or global). In fact, because the proximity of pyrene chromophores is no longer a determining factor in organic solvents, the energy migration between neighbors is now even more unlikely to occur. In general, there is close agreement between the decay times found for the two polymers.

Calculations of the α and β factors reveal that there is an important relative contribution of the former in all situations (polymer, excitation wavelength, and solvent). It should be emphasized that the α value is critically dependent on the long decay time, τ_3 ($1/\tau_M = 1/\tau_3 = k_M$ in eq A.20, see Appendix). In both dioxane and methanol, the contribution of this component is smaller than in water, which eventually leads to lower accuracy in the determination of this decay time, although longer time scales (full time scale of 965 ns) were used to get better accuracy. Also, the β value is now strongly reduced (Table 4). In the case of PAAMePy55 in methanol, the β value has an

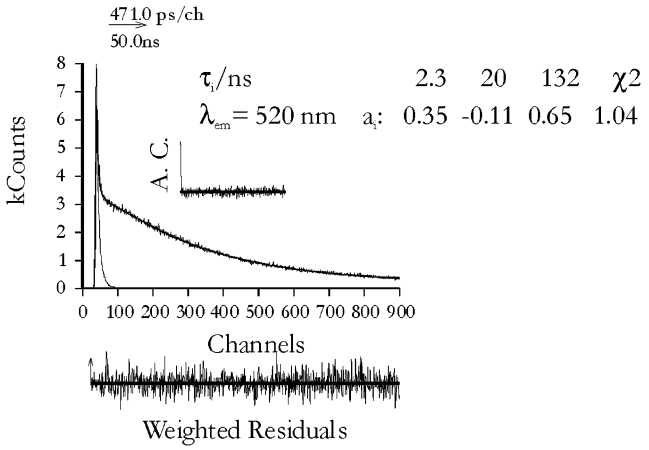


Figure 12. Fluorescence decay for PAAMePy230 in dioxane at room temperature with an excitation wavelength of 350 nm and an emission wavelength of 520 nm. Decay time values and preexponential values are shown in the inset. The instrument profile curve is also shown. For a better judgment of the quality of the fit, the weighted residuals and the autocorrelation (A. C.) function are shown as insets.

averaged value of 0.05. The significance of this decrease from water to organic solvents may lie in the fact that, on average, more spaced pyrene groups will have a lower probability of finding others in their vicinity. As a consequence, more excited free pyrene groups will decay, leading to a greater contribution of the a_{13} preexponential.

The rate constants fall within the expected values found for excimer formation in polymers labeled with pyrene.[39,62,66] The values presented in Table 4 are the result of averages of data from more than five fluorescence decays with excitation at three different wavelengths. The errors presented in Table 4 show that in the good solvents, dioxane and methanol, extremely good accuracy was obtained with the rate constant values for the PAAMePy polymers. Again, the obtained real rate constants, which are dependent on the α values, are presented in Table 4. A comparison between the absolute values of the rate constants with and without considering preformed dimers shows an increase in $k_a(\alpha)$ and a decrease in $k_d(\alpha)$, except perhaps for PAAMePy55 in methanol, when compared to the same rate constants when α is set equal to zero. This trend seems to parallel the one found in water at different pH values.

From the observation of the $\alpha(1 - \beta)$ data in Table 4, it appears that the level of aggregation is still significant. This is particularly intriguing for the low-labeled polymer. The answer to this unexpected behavior can be found in the decays in the organic solvents with excitation at the red edge of the absorption band. In fact, these decays show the presence of a small and initial decay ($\tau \cong 2-3$ ns) that should be related to a new excimer (D) formed by a static process (Figure 12).[67] This component is not found in water (Figure 7). Moreover, the emission spectra in dioxane and methanol obtained with excitation at 350 nm (Figure 3) show the absence of a full overlap of the monomer band when excitation is made at the other two more energetic wavelengths. Again, the absence of full overlap of the vibrationally resolved monomer band when obtained with different excitations is not seen in water. The static population of this new excimer most probably occurs through the formation of a nonsymmetric association of two pyrenes (partial overlap)[68] that decays very rapidly either to the E excimer, where hydrophobic interactions induce the formation of the full-overlap excimer where the two pyrene monomers are in repulsive but close contact, or to the ground state. The absence of such a component in water (Figure 7) and the presence in the good solvents, methanol and dioxane, can be explained by the particular nature of the polymer. Because the organic solvents are good solvents for the pyrene moieties, these should in principle be well separated. However, because of the particular nature of the PAA, which is less soluble in these solvents, and the random inclusion of pyrene in such backbone structure, some of those can be grafted close to one other along the polymer. This will explain the significant level of aggregation present in these two solvents (which is, for example, 28% for the low-labeled PAAMePy230 polymer in dioxane) and the presence of the new D dimer.

Conclusions

The photophysical behavior of two poly(acrylic acid) polymers randomly labeled with pyrene chromophores was studied. Two main factors were found to influence the photophysics of the polymers: the fraction of isolated pyrene monomers (β) and the degree of preassociated pyrene dimers (α). Those were found to be dependent on the degree of labeling, on the solvent, and on the hydrogen ion concentration of the medium. The balance between these factors controls the polymer conformation between a coiled and an expanded chain. Analytical expressions have been presented, allowing quantitative determinations of the α and β values, thus revealing that in water both increase with pH.

In good solvents such as dioxane and methanol, the dynamic excimer formation process increases in importance, but there is still a significant contribution of the fluorescence from the static excimer process of the preassociated chromophores. In these solvents and because of the particular nature of the polymer, it was found that excitation at the red edge of the absorption band induces the formation of a new ground-state dimer with a short decay time.

The determination of all of the rate constants with and without $\alpha = 0$ has shown that, in water and organic solvents, ground-state association always has the effect of increasing the values for the true association rate constant [$k_a(\alpha)$] and decreasing the values for the other true rate constants [$k_{d,E}(\alpha)$].

Time-resolved fluorescence spectroscopy has been shown to be a powerful tool in the interpretation of the photophysical behavior of these pyrene-labeled PAA polymers. However, it should be stressed that extremely high accuracy was demanded to obtain the set of rate constants correctly. A considerable number of independent experiments, including three different time scales and several excitation and emission wavelengths, were therefore performed for both polymers.

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Appendix

Under the transient approach (instantaneous formation of the excited species), the differential equations ruling the time-dependent concentration of the three excited species are, according to Scheme 2, given by[69]

$$\frac{d}{dt}\begin{bmatrix} M_B^* \\ E^* \\ M_A^* \end{bmatrix} = \begin{bmatrix} -k_X & k_d & 0 \\ k_a & -k_Y & 0 \\ 0 & 0 & -k_M \end{bmatrix} \cdot \begin{bmatrix} M_B^* \\ E^* \\ M_A^* \end{bmatrix} \quad \text{(A.1)}$$

where M_A^*, M_B^*, and E^* are the concentrations of M_A, M_B, and E in the excited state and

$$k_X = k_a + k_M \quad \text{(A.2)}$$

$$k_Y = k_d + k_E \quad \text{(A.3)}$$

The integration of eq A.1 leads to

$$\begin{bmatrix} M_B^* \\ E^* \\ M_A^* \end{bmatrix} = \begin{bmatrix} a_{11} & a_{12} & 0 \\ a_{21} & a_{22} & 0 \\ 0 & 0 & a_{13} \end{bmatrix} \cdot \begin{bmatrix} e^{-\lambda_1 t} \\ e^{-\lambda_2 t} \\ e^{-\lambda_3 t} \end{bmatrix} \quad \text{(A.4)}$$

where the eigenvalues λ_i are the reciprocal decay times of the shorter ($\lambda_2 = 1/\tau_2$), the longer ($\lambda_1 = 1/\tau_1$), and the isolated species ($\lambda_3 = 1/\tau_3$) and are related to the rate constants in Scheme 2 by the characteristic equation[70]

$$\begin{vmatrix} \lambda - k_X & k_d & 0 \\ k_a & \lambda - k_Y & 0 \\ 0 & 0 & \lambda - k_M \end{vmatrix} = 0 \quad \text{(A.5)}$$

the solutions of which are given by eqs A.6 (i.e., eq 6) and A.7.

$$2\lambda_{2,1} = \{(k_x + k_Y) \pm [((k_x - k_Y))^2 + 4k_a k_d]^{1/2}\} \quad \text{(A.6)}$$

$$\lambda_3 = \frac{1}{\tau_3} = k_M \quad \text{(A.7)}$$

The sum and product of the λ_1 and λ_2 values resulting from eq A.6 are respectively given by

$$k_X + k_Y = \lambda_1 + \lambda_2 \quad \text{(A.8)}$$

and

$$\lambda_1 \cdot \lambda_2 = k_X k_Y - k_a k_d \quad \text{(A.9)}$$

The preexponential factors a_{ij} are the linear combinations of the eigenvector basis set that obey the following initial conditions:

$$\sum_{j=1}^{3} a_{ij} = 1 \qquad (i = 1) \quad \text{(A.10)}$$

$$\sum_{j=1}^{2} a_{ij} = 1 - \alpha - \beta + \alpha\beta \qquad (i = 1) \quad \text{(A.11)}$$

$$\sum_{j=1}^{2} a_{ij} = \alpha(1 - \beta) \qquad (i = 2) \quad \text{(A.12)}$$

A simple manipulation of eqs A.1 and A.4, together with eq A.8 and A.9 and considering the border conditions given by eqs A.10−A.12, leads to the following relations for the preexponential amplitudes a_{ij}:

$$a_{11} = \frac{[(k_X - \lambda_2)(1 - \alpha) - \alpha k_d]}{(\lambda_1 - \lambda_2)}(1 - \beta) \quad \text{(A.13)}$$

$$a_{12} = \frac{[(\lambda_1 - k_X)(1 - \alpha) + \alpha k_d]}{(\lambda_1 - \lambda_2)}(1 - \beta) \quad \text{(A.14)}$$

$$a_{13} = \beta \quad \text{(A.15)}$$

$$a_{21} = \frac{[(k_X - k_M)(\alpha - 1) - \alpha(k_X - \lambda_1)]}{(\lambda_1 - \lambda_2)}(1 - \beta) \quad \text{(A.16)}$$

$$a_{22} = \frac{\alpha(k_X - \lambda_2) - (\alpha - 1)(k_X - k_M)}{(\lambda_1 - \lambda_2)}(1 - \beta) \quad \text{(A.17)}$$

The use of the preexponential ratio factors at the monomer emission, *A*, with simple algebraic manipulations leads to

$$k_a = \frac{\lambda_1 + A\lambda_2}{A + 1} - k_M \quad \text{(A.18)}$$

The value for k_d comes from a rearrangement of eq A.9,

$$k_d = \frac{k_X k_Y - \lambda_1 \lambda_2}{k_a} \quad \text{(A.19)}$$

and can now be obtained from the now known values for k_X and k_Y (previously obtained from k_a eqs A.18 and A.8).

The intrinsic excimer fluorescence lifetime, τ_E ($= 1/k_E$) can now be obtained through the relation $k_Y = k_d + 1/\tau_E$.

Finally, the α value can be obtained by a reorganization of the ratio of the preexponential (*B*) factors at the excimer wavelength, given by eq 8, leading to

$$\alpha = \frac{(a_{21} + a_{22})(k_X - k_M)}{a_{22}(\lambda_1 - k_M) + (a_{21} + a_{22})(\lambda_2 - k_M)} \quad \text{(A.20)}$$

After knowing the α value, from a simple manipulation of eq A.10 we obtain a new relation for β:

$$\beta = \frac{1 - \alpha}{1 - \alpha + \frac{a_{11}}{a_{13}} + \frac{a_{12}}{a_{13}}} \quad \text{(A.21)}$$

When the effect of α is considered in the determination of the rate constants, the use of the preexponential ratio factors at the monomer emission (eqs A.13 and A.14) leads to the new relations translated by eqs 9−14.

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