

Self-Assembly of a Hydrophobically Modified Naphthalene-Labeled Poly(acrylic acid) Polyelectrolyte in Water:Organic Solvent Mixtures Followed by Steady-State and Time-Resolved Fluorescence

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The solution properties of two water-soluble polymers, poly(acrylic acid) (PAA), covalently labeled with the fluorescent hydrophobic dye naphthalene (Np), have been investigated in water:organic solvent mixtures. The naphthalene chromophores have been randomly attached, onto the polymer, with two different degrees of labeling. Fluorescence measurements (steady-state and time-resolved) have been used to follow the photophysical behavior of the polymers and consequently report on the self-association of the polymers in the mixed organic (methanol or dioxane):aqueous solutions. The emission spectra of the high-labeled Np PAA reveal the presence of monomer and excimer bands whereas with the low-labeled polymer only monomer emission is observed. The excitation spectra collected at the monomer and excimer emission bands show significant differences, depending on the water content of the mixture, which indicate the simultaneous presence of preformed and dynamic dimers as routes to excimer formation. The time-resolved data decay profiles of the high-labeled polymer in the mixtures were always triple exponential whereas in pure methanol and dioxane they follow biexponential laws. The data in the mixtures are consistent with two types of monomers and one excimer. Both monomers are able to give rise to excimer in the excited state, one type involving the movement of long distant Np chromophores and the other involving a local reorientation of adjacent Np chromophores. These correspond to different decay times: (1) a long which corresponds to the long distant approach of nonneighboring Np chromophores forming an excimer and (2) a short corresponding to the fast adjustment of two neighboring Np chromophores in order to have the adequate parallel geometry. An additional decay time corresponding to the excimer decay was found to be present at all wavelengths. All the decay times were dependent on the water content of the mixture. An estimation of the two excimer forming rate constants values is made for the mixed media considered in this work. On the whole, using both steady-state and time-resolved fluorescence parameters, and by comparing data for a polymer with a small number of hydrophobes with a more highly modified one, it is possible in great detail to demonstrate how association is controlled by solvent quality for the hydrophobes and by the distance between hydrophobes.

Introduction

Water-soluble polymers grafted with aromatic fluorescent probes have recently gained increased interest because they can provide information at a molecular level on the kind of existent interactions. From photophysical (essentially fluorescence) studies it is possible to follow, at a molecular level, events occurring in a very short time scale. Among a large variety of molecular probes, particular relevance has been devoted to pyrene, carbazole, and naphthalene because of their additional property of displaying excimer emission in addition to the fluorescence of the fluorophore itself (monomer emission). This additional emission results whenever the excited fluorophore can collide with another nonexcited fluorophore, leading to quenching of the monomer fluorescence by excimer formation. It was first reported by Förster and Kasper for the intermolecular

association of pyrene in liquid solution.¹ This type of association was later on reported to also occur intramolecularly with diphenyl- and triphenylalkanes by Hirayama.² In this last case, Hirayama postulated that the number of carbon atoms connecting the two chromophores (involved in excimer formation) that would allow maximum overlap, and therefore a more efficient excimer formation, should be 3. This is known as the $n = 3$ or Hirayama rule.^{2,3} The light emitted by the monomer and excimer occurs in different regions of the emission spectra with the latter red-shifted with regards to the former.

The more or less pronounced fluorescence intensity of the excimer band relative to the monomer band, or even its absence, can be directly related with movements of the polymer where the probe is found attached. Since excimer formation occurs only when one electronically excited chromophore and a ground-state chromophore achieve a specific conformation (preferentially full overlap between them) at close contact (distance of ca. 3 Å) with each other, the excimer fluorescence is often used as a probe for molecular associations. For example, pyrene end-

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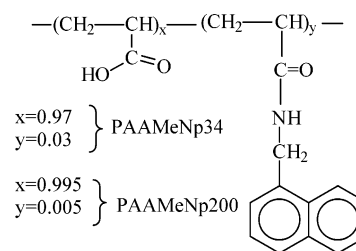
capped polymers are used to study the conformational structure and dynamics of the chain connecting the two chromophores.^{4,5} In a randomly labeled polymer with aromatic moieties, such as the presently studied polymers, there are regions within the polymer richer in the fluorophore, where preformed pairs are more probable to occur, coexisting with other regions where the fluorophore is isolated.⁶ If the macromolecule is completely immobilized, a situation not very probable to occur in solution, then the relative intensity of the excimer-to-monomer bands (I_E/I_M) will only depend on the quantity of sites rich in the fluorophore or excimer-forming sites (EFS).⁷ This is close to the situation found for the highly labeled naphthalene PAA in alkaline solution, where electrostatic repulsions between the ionized carboxylic acid groups inhibit the necessary movements of segments of the polymer in order to get two chromophores in close proximity, a condition needed to induce excimer formation.⁸ If movements, within the polymer, are allowed then the I_E/I_M ratio will vary, thus providing information concerning the conformational state of the polymer.

Hydrophobically modified polymers (HMP) are water-soluble polymers modified with hydrophobic moieties, which can be randomly distributed or located in the two extremities of the polymer chain. In aqueous solution the HMPs can orient themselves in a way that they expose their hydrophilic groups toward water and simultaneously protect their hydrophobic groups from the contact with the water, and consequently they are able to form hydrophobic microdomains by inter- and intramolecular interactions. Because of this characteristic, they are very important in several industrial applications, for example, surface modification, rheology control, and dispersion stabilization. The hydrophobic modification of a polyelectrolyte, with alkyl^{9,10} or aromatic groups,^{6,8} allows a balance between electrostatic forces and hydrophobic interactions. The grafting of the fluorescent probe into the polymer backbone provides also the possibility to employ luminescent techniques at very low concentrations, i.e., so that conformational properties of the isolated polymer can be studied with no additional interpolymeric interactions. The presence of the aromatic hydrophobic groups, randomly introduced into the chain, also provides hydrophobic associations, which compete with the electrostatic repulsions resulting from the ionized carboxyl groups. In view of these competing processes, the polymer chain can undergo conformational changes, which again can be probed by the hydrophobic aromatic units.

The transition from a coiled to an extended conformation can be induced by several external factors.^{6,8} The pH and temperature effects were previously studied in aqueous solutions of poly(acrylic acid) labeled with naphthalene.^{8,11} Conformational changes can also be induced by changes in the nature of the solvent, this being the aim of the present work. Water is a bad solvent for the aromatic probe. The gradual introduction of a good solvent for the aromatic probe (but not so good for the backbone chain), such as dioxane or methanol, into the water solution induces an additional controlling factor. In fact, the gradual introduction of a second solvent allows the balance between hydrophobic and hydrophilic influence to be controlled. In the present work, two naphthalene-labeled PAA polymers were studied using steady-state and time-resolved fluorescence techniques, one high-labeled (denoted PAAMeNp34) and one low-labeled (denoted PAAMeNp200).

The area of polymer photophysics has gained a renewed interest during the past years. This is so because new approaches have given a new light into the complex excited-state kinetics occurring with these polymers. There are several reasons for

SCHEME 1



polymers, grafted with fluorescent probes, to present complex decay laws, particularly for polymers carrying probes where excimer formation is allowed. In the case of randomly labeled polymers it has been argued that different distances between the interacting chromophores will give rise to a distribution of excimer-forming rate constants, and this has been considered within the so-called *blob model*.^{12,13} However, this does not seem to be the general rule for this kind of polymers, and alternative modified classical models seem to adequately fit the decays with a rate constant that can be considered as representing an average value of that rate constant distribution.⁶ Other possible complications arise from the possibility of existence of more than one excimer, the presence of dimers, and kinetically different monomers. The presence of more than one monomer has been discussed with the present polymer¹¹ and also with poly(acrylic acid) labeled with pyrene,⁶ where a model for excimer formation, and which includes two types of monomers (isolated and able to give rise to excimer formation), has been developed and used in the analysis of the time-resolved fluorescence measurements. The presence and identification of the isolated monomer, which is unable to reach the contact distance and therefore to induce excimer formation, is discussed. This pattern was also found for different kinds of polymers¹⁴ and can also be due to the existence of high-energy barriers separating the two types of monomers.¹⁵

Experimental Section

The synthesis and characterization of the polymers was reported in previous works.^{8,16} Briefly, the designation of the polymers is made according to the number of naphthalene (Np) units per acrylic acid units in the polymer. The PAA polymer has a nominal molecular weight of 150 000 g/mol and was purchased from Wako Chemicals, Japan. The amounts of naphthalene were determined both by ultraviolet (UV) absorption measurements using 1-naphthylmethylamine hydrochloride as a model compound and by ¹H nuclear magnetic resonance (¹H NMR) measurements in deuterium oxide. The naphthalene content of the high-labeled PAA polymer was 1 Np unit per 34 acrylic acid unimer units, and the sample is denoted PAA-MeNp34 (see Scheme 1). The naphthalene content obtained for the second labeled PAA polymer corresponds to 1 Np unit per 200 acrylic acid unimer units, and the sample is denoted PAA-MeNp200 (see Scheme 1).

The solvents used for the polymer solutions were of spectroscopic or equivalent grade. 1,4-Dioxane was purified by the procedure described in ref 17. Methanol (Merck Uvsol) was previously dried over CaO and then distilled. Water was twice distilled and passed through a Millipore apparatus. The measured pH values were obtained with a Crison micro pH 2000, and adjustments of the hydrogen ion concentration of the solutions were made with diluted HCl and NaOH solutions. The chromophore concentration of the PAA-MeNp34 and PAA-MeNp200 solutions used in all fluorescence experiments ranged from

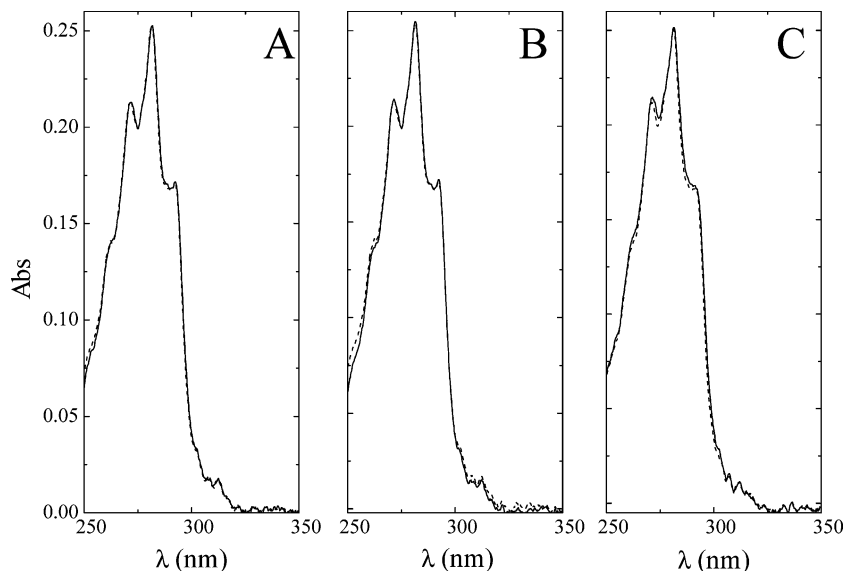


Figure 1. Absorption spectra of PAAMeNp34 (full line) and PAAMeNp200 (dashed line) in (A) dioxane, (B) dioxane:water 7:3 (v:v), and (C) dependence of the dioxane:water 1:9 (v:v) at $T = 293$ K. The pH of the water before mixing with dioxane is 5.5.

1×10^{-5} to 10^{-6} M. Prior to experiments, solutions were deoxygenated by bubbling with N_2 or Ar and sealed. This procedure was utilized for both time-resolved and steady-state experiments. The polymer concentration in the different solutions was 0.05 g/L, which for this molecular weight is well below the critical value for coil overlap, c^* , and intermolecular chain contacts are therefore improbable in homogeneous solutions. In fact, as stated elsewhere, a concentration of 0.05 g/L is still far below the overlap concentration ($c^* \cong 1/[\eta]$, where η stands for the viscosity of the medium¹⁸) of the unlabeled PAA, which was found to be approximately 10 g/L in water at $pH \cong 3$.⁸

Absorption and fluorescence spectra were recorded on Shimadzu UV-2100, Olis-Cary 14, and Jobin-Ivon SPEX Fluorog 3-22 spectrometers, respectively. All the fluorescence spectra were corrected for the wavelength response of the system. The low optical density of the samples used prevents self-absorption or inner filter effects.

Fluorescence decays were measured using a home-built time-correlated single photon timing apparatus previously described.^{19,20} In short, it consists of a D_2 or N_2 gas filled IBH 5000 coaxial flashlamp as excitation source, excitation and emission Jobin-Ivon monochromators, Philips XP2020Q photomultiplier, and Canberra instruments time-to-amplitude converter and multichannel analyzer. Alternate measurements (1000 counts per cycle at the maximum) of the pulse profile at 285 nm (or 295 nm) and the sample emission were performed until $(5-10) \times 10^3$ counts at the maximum were reached. The fluorescence decays were analyzed in a Pentium 500 PC, running LINUX Red Hat as the operating system, using the method of modulating functions of Striker with automatic correction for the photomultiplier "wavelength shift".²¹

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 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). The I_E/I_M ratio results from the decomposed area under the monomer and excimer bands. The general procedure to

obtain the I_E/I_M ratio values consisted in matching the emission spectra of the less labeled polymer (PAAMeNp200), where only the monomer band is displayed, with the monomer band of the PAAMeNp34 polymer. The resulting differential spectrum is the excimer emission band.

Results and Discussion

Figure 1 shows the absorption spectra of the PAAMeNp200 and PAAMeNp34 polymers in pure dioxane and in two dioxane:water mixtures with a low and a high content of the organic solvent. The existence of a perfect match between the absorption of the Np chromophore of the two polymers for all mixtures can be observed; only at 90% water (Figure 1C) there is a slight departure. An identical behavior is observed for other dioxane:water and methanol:water mixtures (not shown). This perfect overlap of the absorption spectra for the PAAMeNp34 and PAAMeNp200 polymers in all solvents suggests that the level of ground-state dimers (GSD) is approximately identical in dioxane, methanol, and mixtures of these two solvents with water. In water, as was previously shown, the level of ground-state dimers strongly depends on the pH of the media.^{6,11} In the case of pyrene-labeled PAA polymers and in good solvents (for the chromophore), such as dioxane and methanol, the level of GSD strongly decreases.⁶

Figure 2 shows the normalized (at the emission maximum) fluorescence spectra for the PAAMeNp34 polymer in dioxane:water and methanol:water mixtures. Note that the increment in the percentage of the organic solvent leads to an increase followed by a decrease of the excimer to monomer fluorescence intensity (I_E/I_M) ratio (see also Figure 3). It can be seen in Figure 3 that in both organic solvent:water mixtures, and in consonance with previous findings,⁸ the dependence of I_E/I_M ratio with the molar fraction of the organic solvent shows a similar shape to the situation observed in water at different pH values.¹¹ For methanol:water mixtures the data display an increase from $x_{MeOH} = 0$ to $\cong 0.2$, followed by a gradual decrease (Figure 3A). In dioxane:water mixtures the same pattern is observed (Figure 3B), but with slightly different values for the molar fraction of dioxane, x_{Dx} . In this case, the maximum for the I_E/I_M ratio occurs at $x_{Dx} \cong 0.1$. This behavior reflects that the degree of excimer formation has a maximum and decreases thereon with the increase in methanol (or dioxane) in the mixture. In addition, note that the inflection points in the I_E/I_M curves are found for

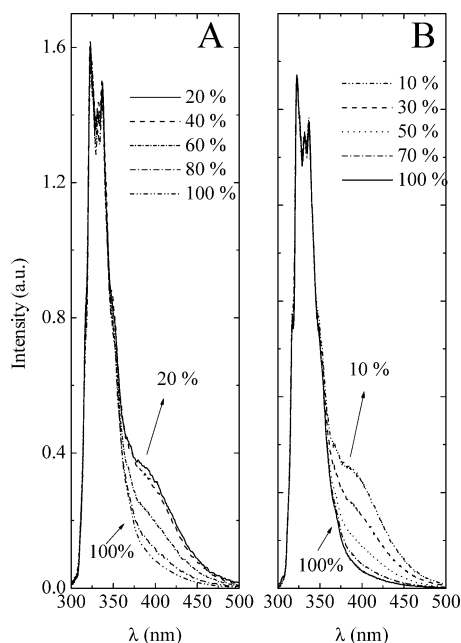


Figure 2. Fluorescence emission spectra for PAAMeNp34 in (A) methanol:water mixtures and (B) dioxane:water mixtures. The spectra are normalized at the emission maximum. The percentage (in volume) is relative to the organic solvent.

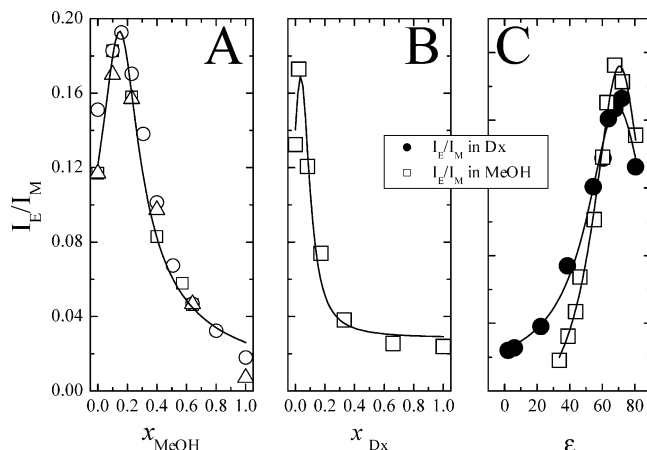


Figure 3. I_E/I_M ratio as a function of the molar fraction of (A) methanol, (B) dioxane in water for the PAAMeNp34 system [water at pH = 1.37 (○), pH = 5.22 (△), and pH = 5.52 (□)] and (C) dependence of the I_E/I_M ratio with the dielectric constant (ϵ) of the dioxane and methanol solvents. The lines are guides for the eye.

different values (Figure 3A,B), whereas the plot against the dielectric constant of the mixture shows the inflection at approximately the same value (Figure 3C). This indicates that this variation and in particular the inflection are dependent on the solvation ability of the mixture.

In a previous work we have shown that in the pure organic solvents dioxane and methanol the analogous PAA polymer labeled with pyrene (PAAMePy) showed increased dynamic contribution to excimer formation when compared to water solutions.⁶ In the case of the PAAMePy polymers it was possible to extract such information based on steady-state and time-resolved data.⁶ In fact, excitation at different wavelengths in the case of pyrene⁶ (and naphthalene¹¹) results in different I_E/I_M ratio values, namely at the red edge of the absorption band. This constitutes an additional evidence for the presence of GSD in such systems. Moreover, the emission excitation spectra, with collection at the monomer and excimer regions, gives rise to

TABLE 1: Spectroscopic Parameters Obtained from the Absorption and Fluorescence Excitation Spectra for PAAMeNp200 and PAAMeNp34, at $T = 293$ K, in Methanol:Water Mixtures^a

% methanol (v:v)	0	20	30	40	50	100
$\Delta\lambda_1/\text{nm}$ (± 0.3)	1.6	1.3	1.2 ₅	0.5 ₇	0.4 ₅	0.1
(± 0.3)	(1.1)	(0.9)	(0.6)	(0.5)	(0.5)	
$\Delta\lambda_2/\text{nm}$ (± 0.19)	1.5	0.9	0.9	0.5	0.3 ₅	0.0
(± 0.20)	(0.8)	(0.5)	(0.5)	(0.5)	(0.5)	
P_A (± 0.06)	1.18	1.18	1.17	1.20	1.23	1.28
(± 0.08)	(1.15)	(1.18)	(1.21)	(1.21)	(1.21)	
P_M (± 0.03)	1.13	1.14	1.13	1.11	1.14	1.11
(± 0.03)	(1.11)	(1.10)	(1.11)	(1.14)	(1.13)	
P_E (± 0.02)	1.11	1.12	1.09	1.10	1.12	1.07
(± 0.02)	(1.10)	(1.10)	(1.11)	(1.05)	(1.10)	

^a The values were obtained for water at two different pH values: 2.55 and 5.22 (in parentheses). The P_A value is for PAAMeNp34. Also presented is the associate error for all the parameters (resulting from 4 to 5 independent measurements).

TABLE 2: Spectroscopic Parameters Obtained from the Absorption and Fluorescence Excitation Spectra for PAAMeNp200 and PAAMeNp34, at $T = 293$ K, in Dioxane:Water Mixtures^a

% dioxane (v:v)	0	10	30	50	70	90	100
$\Delta\lambda_1/\text{nm}$ (± 0.2)	1.1	0.4	0.3	0.2	0.2	0.2	0
$\Delta\lambda_2/\text{nm}$ (± 0.3)	0.8	0.4	0.7	0.6	0.4	0.2	0.1
P_A (± 0.04)	1.18	1.19	1.19	1.22	1.25	1.24	1.42
P_M ($\pm 0.01_6$)	1.11	1.13	1.13	1.14	1.15	1.14	1.14
P_E ($\pm 0.01_8$)	1.10	1.11	1.12	1.12	1.10	1.10	1.07

^a The water pH value is 5.47. The P_A value is for PAAMeNp34. Also presented is the associate error for all the parameters (resulting from 4 to 5 independent measurements).

parameters (P_M and P_E) which can also be related, in a qualitative manner, to the degree of GSD present in solution.^{6,22}

Several parameters can be extracted from the absorption and excitation spectra in the different studied mixtures. In Table 1, the values for $\Delta\lambda_1$ represent the difference between the wavelength maxima of the $S_0 \rightarrow S_2$ transition obtained in the absorption spectra for the two polymers; $\Delta\lambda_2$ is the difference between the wavelength maxima obtained from the fluorescence excitation spectra obtained at 320 and 440 nm for PAAMeNp34; P_A is the ratio, in the absorption spectra, between the maximum absorption value and the absorption value in the valley for the $S_0 \rightarrow S_2$ transition (see Figure 1) made for the PAAMeNp34 polymer; P_M is the ratio between the maximum and the valley in the fluorescence excitation spectra of PAAMeNp34 when collected at 320 nm (monomer region); and finally, P_E is the ratio between the maximum and the valley in the fluorescence excitation spectra of PAAMeNp34 when collected at 440 nm (excimer region).

In systems where preformed pyrene aggregates occur, the red shift observed is in the range of $\Delta\lambda_2 = 1-4$ nm.^{6,21-24} In the case of the present PAAMeNp34 naphthalene polymer $\Delta\lambda_2$ values of ca. 1 nm have validated direct absorption of light by ground-state preassociated Np dimers.⁸ In Tables 1 and 2 the margin of error associated with each parameter (or the associated uncertainty) is given for the data that results from, at least, four independent measurements.

From the gradual decrease of the $\Delta\lambda_1$ value (Table 1), a decrease in the percentage of GSD with the increase in methanol content in the mixture is inferred. Note that in the case of water at higher pH (Table 1, values in parentheses), the $\Delta\lambda$ values (in particular the $\Delta\lambda_1$ value) present, in general, lower absolute values than those found at the acidic pH value. This seems to suggest that the level of GSD is in all the mixtures more

pronounced at the acidic pH value. In the case of the mixture with water at pH = 2.55, the $\Delta\lambda_1$ parameter displays, for low methanol contents, values above one, which is a clear sign that significant differences exist between the two polymers. At this pH value the PAA polymer is totally protonated, and thus a coiled structure would result in an increased degree of the hydrophobic association between the naphthalene groups. For the PAAMeNp34 polymer, the result is that ground-state aggregation between Np units will be highly pronounced. In the case of the PAAMeNp200, the low degree of labeling leads to an absence of intrachain interactions between the Np groups irrespective of the PAA backbone conformation.

The $\Delta\lambda_2$ parameter (obtained only for PAAMeNp34) also shows larger changes for the methanol:water mixture at acidic pH values. Note that we are now comparing data resulting from "observation" of the excimer and the monomer emissions. Moreover, at both pH values (2.55 and 5.22), there is a gradual decrease of the $\Delta\lambda_2$ parameter with the increase in methanol in the mixture. In methanol $\Delta\lambda_2$ is virtually zero. This is again in consonance with the gradual decrease of the contribution of GSD.

In the case of the P_A parameter, it has been argued that with pyrene-labeled polymers values close to 3.0 will indicate the absence of ground-state association and that the lowering of that value will indicate the presence of those aggregates.²⁵ As previously mentioned, the situation with naphthalene derivatives, with regard to parameters related with absorption and excitation spectra, is not as clear as with pyrene derivatives when information regarding the quantitative level of GSD is to be obtained. The relatively low sensitivity of this parameter in methanol:water and dioxane:water mixtures is revealed by the constancy of the obtained P_A values, which vary between 1.18 at 0% and 1.28 at 100% methanol (two pH values were investigated: 2.55 and 5.22) and 1.18 at 0% and 1.42 at 100% dioxane (at pH = 5.5). Albeit the sensitivity of this parameter with naphthalene derivatives is low, the message that can be taken from the data in Tables 1 and 2 is that in fact the P_A parameter increases with the methanol (and dioxane) content in the mixture, which seems to indicate a decrease in the contribution of GSD. With respect to the P_M and P_E parameters, there is basically no change in the difference between these two values (they lay around 1.07–1.14) for both organic:water mixtures at the different conditions studied (organic solvent content and pH). From this behavior of the P_M and the P_E parameters, we may infer that GSD aggregates either contribute equally for the emission at both wavelengths or contribute very little for the emission at either wavelength. Other recent studies with naphthalene macromolecular structures have used excitation spectra together with time-resolved analysis to prove the existence of GSD.²⁶ From the analysis of these steady-state fluorescence data, we can conclude that time-resolved fluorescence measurements are needed to fully explore the GSD and excimer formation in these polymer systems (see below). Note that despite the substantial information concerning the $\Delta\lambda$ and P parameters that validates these parameters in Tables 1 and 2, the data should be regarded with caution, and the information here obtained only helps to understand the gradual pattern found. In this case, water induces GSD formation whereas dioxane (and methanol) reduces it strongly.

Time-Resolved Fluorescence Data. Methanol:Water Mixtures. Time-resolved fluorescence measurements were performed on both the Np-labeled PAA polymers in methanol:water and dioxane:water mixtures. The fluorescence decays for PAAMeNp34 in methanol:water mixtures were fitted with sums

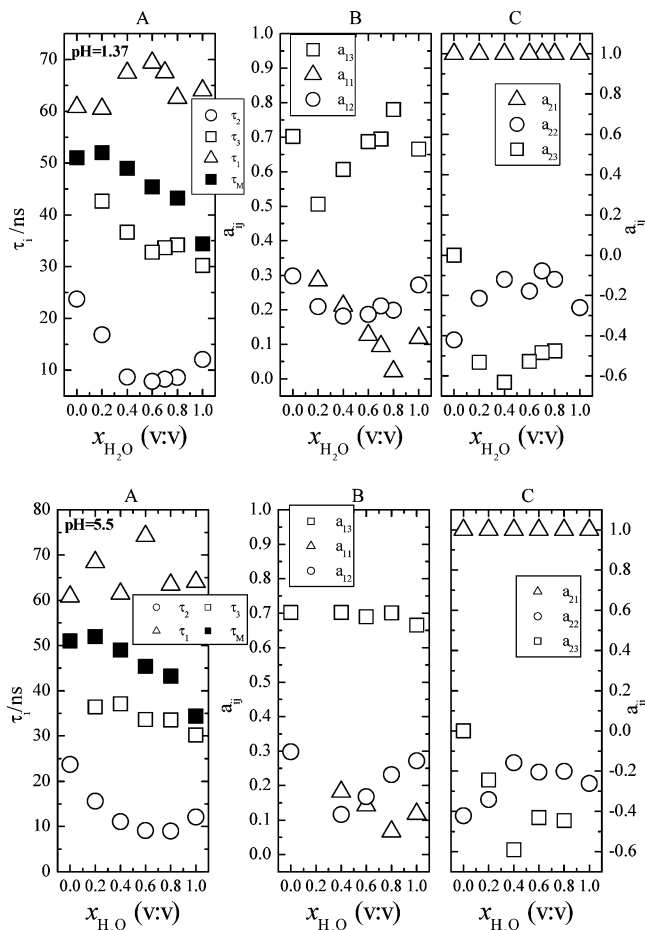


Figure 4. Fluorescence decay times (A) and amplitudes at the (B) monomer (a_{1j}) and (C) excimer (a_{2j}) emission of PAAMeNp34 as a function of water content in methanol:water mixtures (water at pH = 1.37, top panels; water at pH = 5.5, bottom panels).

of three exponentials at the monomer and excimer emission regions. At the two emission regions (monomer and excimer) the obtained decay times, τ_1 , τ_2 , and τ_3 are identical. This does not apply to the preexponential factors which are related with the excited-state concentrations at time zero (see Figures 4 and 5A).

In water and in pure dioxane and methanol we have observed for the PAAMePy and PAAMeNp polymers that the coexistence of isolated monomers (unable to form excimers) with monomers able to give rise to excimer was responsible for two of the decay times in the monomer emission region.^{6,11} The third decay time in the monomer emission was, for the PAAMePy polymers, attributed to the excimer-to-monomer reversibility.⁶ In the case of the PAAMeNp derivatives again in water at room temperature the decays were biexponential with decays associated with the "free" monomer and to the monomer giving rise to excimers.¹¹ At the excimer emission wavelength the decays were basically found to be biexponential for the two PAA derivatives (Py and Np) with the presence of a negative preexponential, associated with the faster time (rise time).^{6,11} In the present situation, for the organic solvent:water mixtures, the decays are more complex. In fact, the global analysis (simultaneous analysis) of the monomer (320 nm) and excimer (390 nm) decays gives triexponential laws for all mixtures. The individual analysis at each wavelength also gives decays that can be fitted to sums of three exponentials. The three decay times associated with the exponential laws are identical at the monomer and excimer

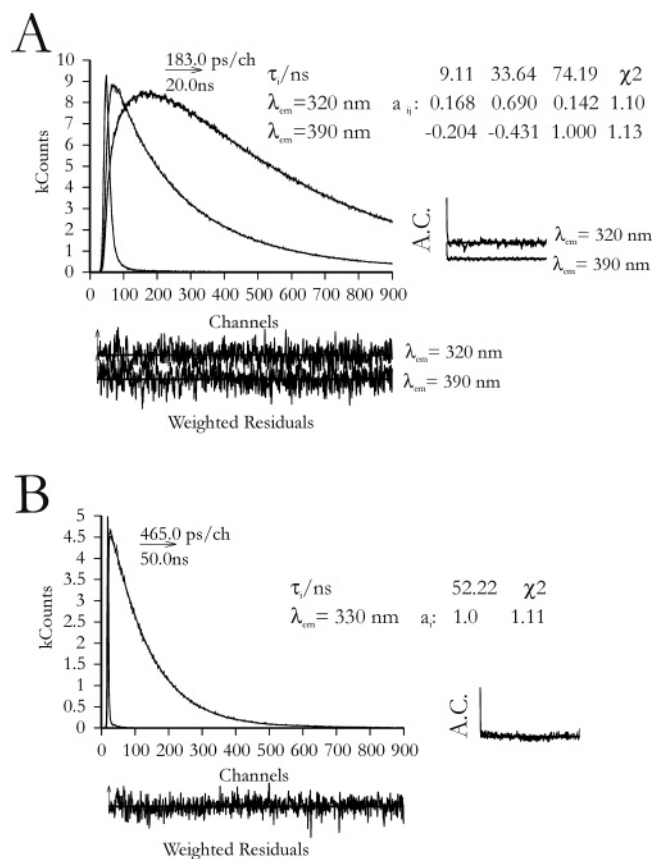


Figure 5. Fluorescence decays for (A) PAAMeNp34 in a methanol:water (pH = 5.5) mixtures 4:6 and (B) PAAMeNp200 in a methanol:water mixture 5:5.

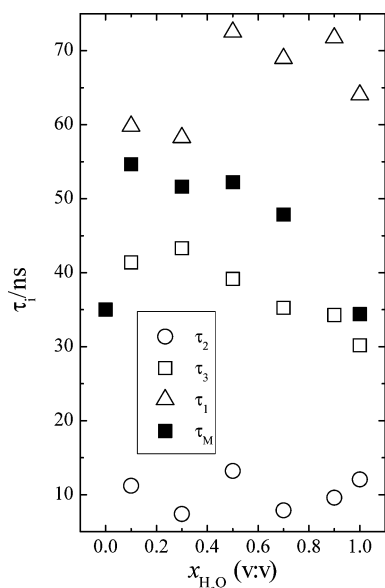


Figure 6. Fluorescence decay times as a function of the water content in dioxane:water mixtures (water at pH = 5.5) for the PAAMeNp34 polymer (Δ , \square , \circ) and PAAMeNp200 (\blacksquare).

emissions, meaning that at least three different species are kinetically coupled in the excited state. Data for the three decay times (τ_i) of PAAMeNp34 in methanol and the amplitudes in the monomer (a_{1j}) and excimer (a_{2j}) decays are shown in Figures 4 and 5. Emphasis should be placed on the comparison between the present decays and those obtained in pure water.¹¹ In the present case, two rise times are obtained at the excimer emission

whereas in water only one was observed. The question arising now is the correct attribution to these three species.

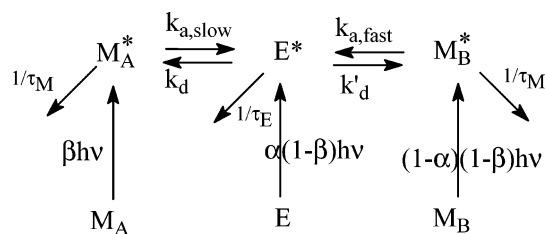
The presence of more than one excimer can result from the coexistence of ground-state dimers (GSD) that can be either directly excited and decay with a short lifetime^{6,27} or rapidly rearrange in the excited state to give a dynamic excimer.²⁸ Also in the case of 1,3-dipyrenylpropanes, full- and partial-overlap excimers were found to coexist, giving rise to different emission bands.^{29,30} With polymers labeled with pyrene, reports exist on systems where only one monomer and two different excimers are found to coexist.^{29,31,32}

In the case of randomly labeled polymers giving rise to multiexponential decays, other models imply a distribution of sites for excimer formation that originate a distribution of rate constants for the intramolecular excimer process.^{13,33} Yet in the present case, clearly three exponentials can fit the fluorescence decays, and consequently the physical model here proposed constitutes an additional and alternative method to others where distributions of rate constants are obtained depending on the degree and localization of the labeling.

In the case of the poly(methylphenylsiloxanes), Dias et al. have found, by time-resolved fluorescence, at several temperatures, that two different types of monomer species were operative.^{15,34,35} These were attributed to long- and short-range phenyl interactions. To establish the two types of interactions, one has to be able to differentiate them. From the stationary fluorescence data it is unlikely that contributions from two monomers could be distinguished. However, time-resolved fluorescence data can give important insight, since if the contributions of these two monomers are different enough, they will give different decay times. This is what happens for the PAAMeNp34 polymer in methanol:water and dioxane:water mixtures (Figure 6). In fact, good fits for the fluorescence decays of PAAMeNp34 in methanol:water mixtures were only obtained with sums of three exponential functions (see Figure 5). The simultaneous analysis of the fluorescence decays gives rise to identical decay times (τ_i) and different amplitudes (a_{ij}) when collected in the monomer ($\lambda_{em} = 320$ nm) and excimer regions ($\lambda_{em} = 390$ nm) for all mixtures. Pure dioxane and water present a different behavior. In the case of water the decays are virtually biexponentials at the two emission wavelengths but with one single common decay time.¹¹ The decay time and amplitude values were found to be strongly dependent on the pH value of the media.¹¹ In Figure 5 we can observe that the two shorter decay times have associated negative preexponentials at the excimer emission wavelength. Moreover, the longer component, associated with the decay time of the excimer, has a strong contribution at the monomer emission wavelength. This shows that the backward excimer-to-monomer reaction is operative in these mixtures. This was not observed in pure water.¹¹ The observation of the PAAMeNp200 polymer lifetime (τ_M) in the same mixtures reveals that this polymer decays single exponentially, meaning that no excimer formation is present (Figure 5B). This happens because of the nature of the grafting process, which results in the nonexistence of neighboring Np groups in this polymer. Moreover, these observations also provide information on the nature, and value, of the unquenched decay time of the PAAMeNp34. In fact, in the case of the PAAMeNp200 polymer, the chromophore is found alone with no possible interaction with other neighbor naphthalene. Its decay time can only be influenced by the nature of the solvent medium.

Figures 4–6 show that the intermediate decay time (τ_3) in PAAMeNp34 has a value close, but not identical, to the PAAMeNp200 value (τ_M) in the same mixtures. The difference

SCHEME 2



between these two values (τ_3 and τ_M , see Figure 4) can be understood from the ability of the long distance monomers (nonneighboring), in the two polymers, to reach the excimer conformation during its lifetime; i.e., it can be considered as a chromophore intramolecular concentration dependence of the average pseudo-unimolecular rate constant, $k_{a,slow}$. In fact, since the ratio of monomers in the two polymers is $200/34 = 5.88$, $k_{a,slow}$ in the PAAMeNp200 polymer is estimated to be $<0.002 \text{ ns}^{-1}$. As a consequence, with the low-labeled polymer the excimer formation cannot compete with the monomer decay $k_M = 0.02 \text{ ns}^{-1}$. In PAAMeNp34 the excimer formation rate constant between nonneighboring monomers ($k_{a,slow} = 0.01 \text{ ns}^{-1}$) is of the order of magnitude of k_M , and excimer formation is observed.

The aforementioned two negative preexponential factors occurring in the excimer region should be linked to two monomer states, since their lifetimes are identical to the two faster components in the monomer emission. Moreover, since they possess different decay times, this means that when the excited chromophore (monomer) is formed, it can be in one of the following two situations: (a) near excimer conformation (e.g., neighboring monomers) where the excimer can be reached with a localized movement (single rotation of a bond or bending to reach parallel position); (b) "isolated", where the excimer conformation is achieved by movements of large polymer segments (several rotations and diffusion). If the times that it takes to deactivate these two different monomer states are sufficiently different, then the excimer formation would reflect this, by showing that its formation will occur during the decay of two different monomers. This is what we believe is happening in the present case. The random nature of the naphthalene labeling will provide intramolecular short- and long-range encounters between one excited and one nonexcited monomer. The present excited-state behavior is consequently in agreement with the proposed model schematically illustrated by Scheme 2.

The excited-state kinetic mechanism for PAAMeNp34 in methanol:water and dioxane:water mixtures, here considered, includes therefore three kinetically coupled species which are the two monomers (M_A and M_B) and one excimer (E). In the case of the monomers, these differentiate into long-range (M_A) and short-range (M_B) interacting chromophores leading to excimer formation. Consequently, the excimer formation due to the long-range interaction will give rise to the slow rate constant ($k_{a,slow}$) and the short-range naphthalene interaction to the fast ($k_{a,fast}$) rate constant. Since the two types of monomers can exist in the ground state, the fraction of light exciting these two types of monomers must be divided into β and $(1 - \alpha)$ ($1 - \beta$). The kinetic scheme also contemplates the possibility that excimers can be preformed in the ground state by the direct absorption of a fraction of light [$\alpha(1 - \beta)$].

Others^{34–36} have also considered the involvement of two processes, one of which is slow and the other rapid, in excimer formation reactions occurring with labeled polymers. In their case,^{34–36} it involves a sequential process where two monomer-

like species are directly connected, and the last gives rise to the excimer. We believe that our system behaves differently, although we cannot discard one mechanism relative to the other since phenomenologically the two are indistinguishable. In the present situation the results appear to account for the presence of two kinetically independent monomers giving rise to one excimer.

To evaluate the possible influence of the hydrogen ion concentration on the dynamic behavior of the PAAMeNp34, fluorescence decays were obtained in methanol:water mixtures at two different pH values (1.37 and 5.5) (see Figure 4). Comparison between the data obtained at the two pH values shows that no significant difference exists albeit the fact that the plots cannot be considered strictly identical since there are some fluctuations due to the analysis procedure. The electrostatic repulsions between the carboxylate groups in the main polymer backbone were of fundamental importance in pure water, decreasing the level of association between naphthalene groups.^{6,8,11} In fact, the analysis of the $\Delta\lambda_1$ values at two pH values (Table 1) shows that for low methanol contents ($<40\%$) protonation of the carboxylates leads to chromophoric aggregation, while for higher methanol contents both protonated and unprotonated forms show similar extended conformations. This difference for mixtures below 40% methanol is not seen in the time-resolved data at pH = 1.37 and pH = 5.5 because it reflects the presence of preformed ground-state dimers with no dynamic contribution. The lowering of the dielectric constant induced by the addition of organic solvent makes (for methanol contents above 40%) the mixture a better solvent for the chromophores and eliminates the differences in aggregation between the protonated and unprotonated forms of the polymer. The organic solvent in the mixture also induces changes in the relative contribution of the short- and long-range interacting chromophores. The increase of organic solvent most likely results in a more homogeneous medium, leading to identical contributions of the two types of monomers. In fact, in pure methanol the decays display double-exponential behavior (see Figure 4).

Under the transient approach (instantaneous formation of the excited species), the differential equations ruling the time dependence of concentrations of the three excited species are, according to Scheme 2, given by³⁷

$$\frac{d}{dt} \begin{bmatrix} M_A^* \\ E^* \\ M_B^* \end{bmatrix} = \begin{bmatrix} -k_X & k_d & 0 \\ k_{a,slow} & -k_Y & k'_d \\ 0 & k_{a,fast} & -k_Z \end{bmatrix} \cdot \begin{bmatrix} M_A^* \\ E^* \\ M_B^* \end{bmatrix} \quad (1)$$

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

$$k_X = k_{a,slow} + k_M \quad (2)$$

$$k_Y = k_{a,fast} + k_d + k_E \quad (3)$$

$$k_Z = k'_d + k_M \quad (4)$$

The integration of eq 1 leads to

$$\begin{bmatrix} M_A^* \\ E^* \\ M_B^* \end{bmatrix} = \begin{bmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ a_{31} & a_{32} & a_{33} \end{bmatrix} \cdot \begin{bmatrix} e^{-\lambda_1 t} \\ e^{-\lambda_2 t} \\ e^{-\lambda_3 t} \end{bmatrix} \quad (5)$$

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 of the long-range excimer-forming monomer species ($\lambda_3 = 1/\tau_3$) and are

related to the rate constants in Scheme 2 by the characteristic equation:³⁹

$$\begin{vmatrix} \lambda - k_X & k_d & 0 \\ k_{a,\text{slow}} & \lambda - k_Y & k'_d \\ 0 & k_{a,\text{fast}} & \lambda - k_Z \end{vmatrix} = 0 \quad (6)$$

The λ values are obtained by solving the characteristic equation leading to

$$\lambda^3 - \lambda^2(k_X + k_Y + k_Z) + \lambda(k_X k_Y + k_X k_Z + k_Y k_Z - k_{a,\text{fast}} k'_d - k_d k_{a,\text{slow}}) + k_X k_{a,\text{fast}} k'_d + k_d k_{a,\text{slow}} k_Z - k_X k_Y k_Z = 0 \quad (7)$$

The complete solution of analogous systems has been performed elsewhere by obtaining additional relations between the pre-exponential factors (a_{ij}), rate constants (k_i), and experimental decay times (λ_i).^{15,35} However, as others have also noted, it is possible to simplify the solution in order to have estimated values for the excimer formation rate constant.³⁴ In our case, rate constants for the fast excimer formation, $k_{a,\text{fast}}$, and slow excimer formation, $k_{a,\text{slow}}$, can be estimated (approximate values) from the difference between the reciprocal of the shortest decay time (τ_2) and k_M (eq 8) for the case of the fast excimer formation rate constant and from the difference between the reciprocal of the intermediate decay time (τ_3) and k_M for the slow excimer formation rate constant (eq 9). The value for k_M ($=1/\tau_M$) is obtained with PAAMeNp200 under the same experimental conditions.

$$k_{a,\text{fast}} = \frac{1}{\tau_2} - k_M \quad (8)$$

$$k_{a,\text{slow}} = \frac{1}{\tau_3} - k_M \quad (9)$$

The obtained rate constants for excimer formation due to fast ($k_{a,\text{fast}}$) and slow ($k_{a,\text{slow}}$) processes display for the fast rate constant an increase with increasing water content of the mixture, whereas the water content of the mixture seems to have no significant influence on the rate of the slow process. The increase in the $k_{a,\text{fast}}$ value with $x_{\text{H}_2\text{O}}$ can be explained by the gradual departure of the solvent medium from a good solvent for the Np probe. Because this rate constant essentially results from the interaction of nearby chromophores, the gradual addition of a bad solvent (water) will promote the approach of the hydrophobic probes. As a consequence of this, the Np groups will be closer, and therefore the rate constant for excimer formation will be faster. Also, the preexponential associated with the fast decay, at the excimer emission wavelength (a_{22}), becomes less negative with increasing water content, which indicates a formation of ground-state dimers. Moreover, the reversibility (measured by the weight of the component associated with the excimer decay at the monomer emission wavelength) decreases with increasing water content of the mixture. This could be evidence of a more rigid structure. As mentioned above, long-range interacting chromophores, corresponding to the slow process, suffer no significant change in the rate with the addition of water to the mixture. This is expected if these Np chromophores are in chromophoric poor regions, since in that case the chain solvation (hydrophilic) will dominate chromophore solvation (hydrophobic) and no aggregation occurs. The present model does not allow the determination of the fraction of each type of monomer present in the ground state (α and β values in Scheme 2) as well as the values for the

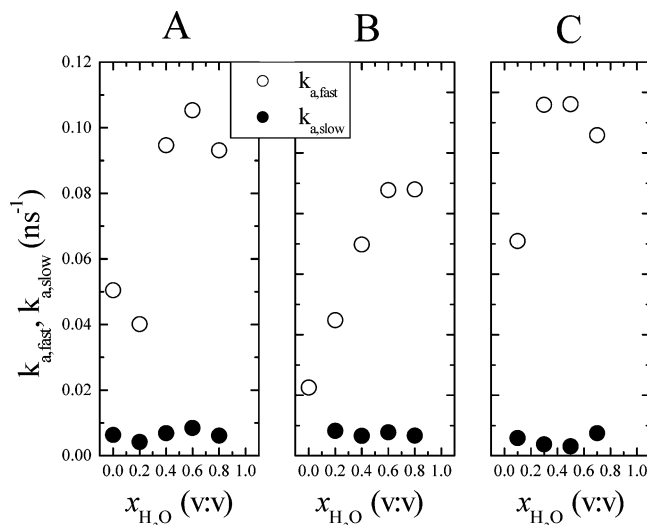


Figure 7. Rate constants for the fast ($k_{a,\text{fast}}$) and slow ($k_{a,\text{slow}}$) excimer formation processes occurring in the PAAMeNp34 polymer in methanol:water mixtures (water at (A) pH = 1.37 and (B) pH = 5.5) and (C) dioxane:water mixtures.

reversible reactions (k_d and k'_d in Scheme 2), as were obtained in previous works.^{6,34,35} These and other aspects are currently under study,³⁵ and their solution will be presented in a forthcoming work.

Conclusions

The study of poly(acrylic) acids hydrophobically modified with naphthalene has been performed in organic solvent:water mixtures. We have shown that for both polymers the existence of two different types of monomers lead to excimer formation in organic solvent:water mixtures. The two different channels for excimer formation involves in one situation the approach of two chromophores at long distance, whereas in the other situation a single local rearrangement (probably resulting from the correct rotation around the bond connecting the chromophore to the chain backbone) is enough to have the Np rings parallel to each other and thus to induce excimer formation. The short- and long-range interacting naphthalene groups give origin to excimer formation with different rate constants. The simultaneous presence of these two excimer-forming channels occurs for PAAMeNp34 in the aforementioned organic solvent:water mixtures. The low Np level polymer, PAAMeNp200, was also studied in the same mixtures, leading, in all situations, to single-exponential decays with lifetime values close to the decay time of the PAAMeNp34 polymer associated with the approach (long fold approach). The fast process was found to be dependent on the water content of the mixtures whereas the slow process is not. The gradual addition of water to the organic solvent was found to induce an increase in the excimer formation, resulting from an increase in the GSD contribution. Globally, it was shown that the comparison between two different labeled polymers (high and low level of hydrophobes) allows the visualization of how association is ruled by solvent ability toward the naphthalene chromophore as well as by the distance between them.

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