

Picosecond Dynamics of Dimer Formation in a Pyrene Labeled Polymer

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A commercial poly(acrylic acid) (PAA, $M_n = 450\,000\text{ g mol}^{-1}$) has been labeled with high levels (9.1 mol %) of pyrene by reaction with 1-pyrenylmethylamine in the presence of boric acid. The modified polymer was found to display an unusual photophysical behavior closely resembling that found for pyrene in constrained environments. The appearance of a band at longer wavelengths in the excitation spectra when collected at the long wavelength emission band was attributed to the spectra of a dimer. The emission of this dimer was particularly enhanced in dioxane:water solvent mixtures with low dioxane content. From time-resolved fluorescence measurements in the picosecond time domain, two decay components were obtained: a fast decay (4–10 ps) at short wavelengths, which becomes a rising component at longer wavelengths, and a second exponential (2–4 ns) related to the emission of the relaxed dimer. Time-resolved emission spectra were seen to change with time, revealing the emission contribution of two species. This is one of the first reports where the dynamics of dimer formation in a pyrene derivative have been observed.

Introduction

Fluorescence spectroscopy has been extensively used to gain information on the long-range dynamics, aggregation and conformational behavior in solution of polymers labeled with photoactive groups.^{1–3} Of particular interest is the detection of intermolecular and/or intramolecular association phenomena in aqueous media of hydrophobically modified water-soluble polymers. Pyrene is the most popular fluorophore because of its high quantum efficiency in both monomer and excimer fluorescence. Excimer fluorescence appears if an excited pyrene encounters a ground-state pyrene via diffusion. An excimer can also be generated instantaneously when aggregated pyrenes absorb a photon. The low spectral superposition between the monomer and excimer emission bands means that excimer formation is easily detected in fluorescence, and time-resolved experiments can distinguish whether the excimer is formed instantaneously or by diffusion.

The water-soluble polymers that have been labeled with pyrene units include polyelectrolytes like poly(methacrylic acid)s,⁴ poly(acrylic acid)s,^{5–8} poly(maleic acid)s,⁹ polysulfonates,¹⁰ and their modified polymers and copolymers, and nonionic polymers like poly(acrylamide)s,^{3,11–15} poly(ethylene oxide)s,^{16–20} hydroxyalkylcelluloses,^{21,22} polypeptides, DNA, and nucleic acid macromolecules.^{1,23–26} The pyrene molecules can be attached at either one or both ends of a monodisperse polymer, in which case end-to-end cyclization dynamics and/or intermolecular association are monitored,^{16,19,20} or incorporated into the chain at either random or regular intervals, which allows the investigation of intrachain dynamics and hydrophobic interactions.¹⁷ Previous works showed that the association processes are very dependent on external influences such as pH,^{4–9} solvent,^{7,13} temperature, concentration,^{11,12,19} and the presence of amphiphilic molecules, such as surfactants,^{6,14,18,27}

and cyclodextrins.²⁸ The balance between intramolecular static and dynamic dimers in gels vs water has been recently investigated with naphthalene derivatives, and it was shown that the relative contribution of the two is strongly dependent on the media.²⁹ Other factors of critical importance are the size of the polymer, the pyrene content and placement, the method of pyrene incorporation, the nature of the linker connecting pyrene to the backbone, and (where appropriate) the pyrene distribution along the polymer backbone.^{5,8,15} Ideally, the labeling strategy should be straightforward and the linker connecting pyrene to the polymer should be stable in the solvents under study. Taking poly(acrylic acid)s (PAAs) labeled with pyrenyl units as an example, the two main synthetic methods are postsynthesis amidation of PAA with pyrenyl-substituted *N*-alkylamines,⁵ and copolymerization of acrylic acid and a pyrenyl-substituted comonomer with azobisisobutyronitrile (AIBN) as initiator.^{4,30}

Recently, we have studied the intramolecular association of PAAs randomly grafted with pyrene (Py) units to different degrees in solution (aqueous and organic solvents) using steady-state and time-resolved fluorescence.^{7,8} Specifically, polymers with low [2000 g mol^{−1} and abbreviated as PAA(2)], high [150 000 g mol^{−1} and abbreviated as PAA(150)], and very high [450 000 g mol^{−1} and abbreviated as PAA(450)] molecular weight were labeled with low levels of pyrene (0.43–2.0 mol %, or 50–230 monomer units per Py chromophore) by reaction with 1-pyrenylmethylamine in an aprotic solvent, 1-methylpyrrolidone, and in the presence of 1,3-dicyclohexylcarbodiimide. The photophysical behavior of these polymers in aqueous solution is dependent on the pH, solvent, degree of labeling, and size of the PAA chain. With the long chain polymers [PAA(150) and PAA(450)] in aqueous solution, deprotonation of the carboxylic acids of the PAA chain induces a change from a coiled to an extended conformation (due to electrostatic repulsions), leading to less excimer formation (essentially due to the existence of fewer ground-state dimers). In a continuation of this work, we now wish to report on the

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photophysical behavior in solution of a pyrene-labeled PAA sample prepared by reaction with 1-pyrenylmethylamine in the presence of boric acid. Unlike the previous method, this method is very efficient and allowed a high pyrene loading of ca. 10 mol % to be reproducibly obtained, which corresponds to 10 monomer units per Py chromophore. As a consequence of the high loading, excimer formation mainly occurs through the excitation of preassociated dimers, and exceptionally for these types of systems the dynamics of dimer formation have been probed through picosecond time-resolved measurements.

Experimental Section

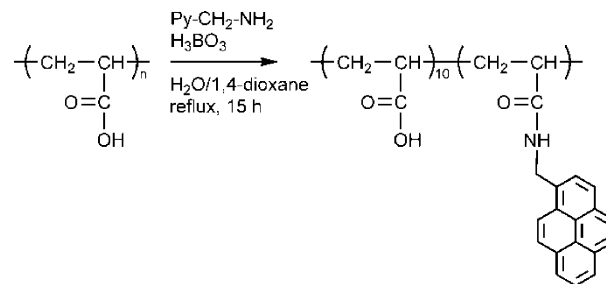
Materials. Poly(acrylic acid) with nominal $M_n = 450\,000$ g mol⁻¹ (Aldrich), 1-pyrenylmethylamine hydrochloride (Aldrich), boric acid (Panreac), 1,4-dioxane (Panreac), dichloromethane (Aldrich), and dimethylformamide (Aldrich) were obtained from commercial sources and used as received. The pyrene-labeled PAA samples referred to as PAAMePy(450)53 and PAAMePy(450)87 were prepared as described previously.⁸

1-Pyrenylmethylamine hydrochloride was neutralized by treatment with 1 M K₂CO₃, followed by addition of CH₂Cl₂. After 2 h, the organic layer was extracted and dried over anhydrous Na₂SO₄. The solvent was removed in a rotary evaporator, giving a yellow solid.

Instrumentation. Solution ¹H spectra were acquired using a Bruker CXP 300 spectrometer. ¹³C{¹H} CP/MAS NMR spectra were recorded at 125.72 MHz on a (11.7 T) Bruker Avance 500 spectrometer, with an optimized $\pi/2$ pulse for ¹H of 3.5 μ s, 2 ms contact time, spinning rates of 7–9 kHz, and 4 s recycle delays. Chemical shifts are quoted in parts per million from tetramethylsilane. IR spectra were obtained as KBr pellets using a FTIR Mattson-7000 infrared spectrophotometer. Absorption spectra were recorded on a Shimadzu UV-2100 spectrophotometer with a minimum resolution of 0.2 nm. For the steady-state measurements, fluorescence spectra were recorded with a Horiba-Jobin-Ivon SPEX Fluorog 3-22 spectrometer and were corrected for the instrumental response. Fluorescence decays on the nanosecond time scale were measured using a home-built time-correlated single photon counting (TCSPC) apparatus as described elsewhere,^{31,32} except that a Horiba-JI-IBH NanoLED, $\lambda_{exc} = 339$ nm, was used as the excitation source. Picosecond time-resolved fluorescence measurements were performed using a home-built picosecond TCSPC apparatus, in which the excitation source consists of a picosecond Spectra Physics mode-lock Tsunami laser (Ti:sapphire) Model 3950 (repetition rate of about 82 MHz, tuning range 700–1000 nm), pumped by a Millennia Pro-10s, frequency-doubled continuous wave (CW), diode-pumped, solid-state laser ($\lambda_{em} = 532$ nm). Fluorescence decays and the instrumental response function (IRF) were collected using 1024 channels in a 3.26 ns/channel scale, or 4096 channels in a 0.814 ps/channel, until 5×10^3 counts at maximum were reached. The full width at half-maximum (FWHM) of the IRF was about 22 ps and was highly reproducible with identical system parameters. A more detailed description of this equipment can be found in ref 33.

PAAMePy(450)11. PAA (1.0 g) was dissolved in distilled water (10 mL) in a round-bottomed flask and 1,4-dioxane (75 mL) added with stirring. H₃BO₃ (0.109 g, 1.76 mmol) and 1-pyrenylmethylamine (0.350 g, 1.52 mmol) were added and the reaction was carried out under reflux for 15 h. The resultant yellow solution was concentrated under reduced pressure and the product precipitated with CH₂Cl₂. After the solvent was removed by decantation, the product was precipitated twice from dimethylformamide/CH₂Cl₂, giving a dark yellow solid which

SCHEME 1: Preparation of the Labeled Poly(acrylic acid) Referred to as PAAMePy(450)11



was vacuum-dried at 90 °C for 2 h. Finally, the solid was ground to a powder and washed several times with CH₂Cl₂ (yield: 1.1 g). IR (cm⁻¹): 3484 br, 3429 br, 3233 sh, 2951 sh, 2858 sh, 2281 sh, 2036 w, 1950 w, 1706 m, 1637 m, 1619 sh, 1540 m, 1452 m, 1419 w, 1385 sh, 1255 sh, 1232 sh, 1192 m, 1115 m, 1079 w, 1045 sh, 949 sh, 889 w, 867 w, 847 m, 799 m, 767 sh, 615 m, 546 w, 485 w. ¹H NMR (300 MHz, rt, DMSO-*d*₆, TMS): $\delta = 8.49$ – 8.11 (series of overlapping multiplet signals, Py), 4.83 (s, CH₂-Py), 3.5 (vbr, NH), 2.24 (br, CH-chain), 1.75 (br, CH₂ chain), 1.44 (br, CH₂ chain). ¹³C CP/MAS NMR: $\delta = 177$ (COOH and NHCO), 123 (Py-C), 40 (CH and CH₂).

The above procedure was repeated using PAA (1.0 g), distilled water (10 mL), 1,4-dioxane (75 mL), H₃BO₃ (0.010 g, 0.16 mmol), 1-pyrenylmethylamine (0.010 g, 0.043 mmol), and a reflux time of 19 h, which resulted in a second modified polymer with a much lower degree of pyrene labeling (PAAMePy(450)517). The pyrene labeling contents were determined by UV spectroscopy by comparison with 1-pyrenylmethylamine ($\epsilon = 37\,070$ M⁻¹ cm⁻¹ at $\lambda = 340$ nm in methanol). In the designation PAAMePy(450)*X*, *X* stands for the average number of PAA monomer units per Py chromophore, which means a Py content of 9.1 mol % in the case of PAAMePy(450)11. A similar value of 9.4 mol % was obtained from the ¹H NMR spectrum by using the expression $3y/(x + y) = I_{8.0-8.5}/I_{0.9-2.4}$, where *x* and *y* are the mol % values of underivatized and derivatized carboxylic acid groups, respectively ($x + y = 100$), $I_{8.0-8.5}$ is the sum of the integrals for the Py signals in the range 8.0–8.5 ppm, and $I_{0.9-2.4}$ is the sum of the integrals for the CH and CH₂ signals in the range 0.9–2.4 ppm.

Results and Discussion

Synthesis and Characterization of Labeled PAA. The method used to functionalize the commercial PAA ($M_n = 450\,000$ g mol⁻¹) with pyrenyl groups was adapted from the boric acid catalyzed procedure described by Tang for amide formation from carboxylic acids and amines (Scheme 1).³⁴ Both UV–vis and ¹H NMR spectroscopy indicated that the modified polymer had on average one pyrene group per 11 PAA monomer units, or approximately 600 pyrenes per macromolecule. The Py content of 9.1 mol % is only slightly lower than the maximum theoretical value of about 11 mol % (based on the quantities of reagents used), which indicates that the grafting procedure is very efficient. Furthermore, the reproducibility of the method was shown by carrying out a duplicate reaction with the same quantities of reagents, which gave a modified polymer with a Py content of 10 mol %. The anchoring of the pyrenyl groups to the PAA main chain via a secondary amide linkage was indicated by the bands at 3240 cm⁻¹ (NH stretching), 1637 cm⁻¹ (amide I band, mainly C=O stretching), and 1540 cm⁻¹ (amide II band, mainly N–H bending) in the IR spectrum (Supporting Information). Unreacted carboxylic acid groups

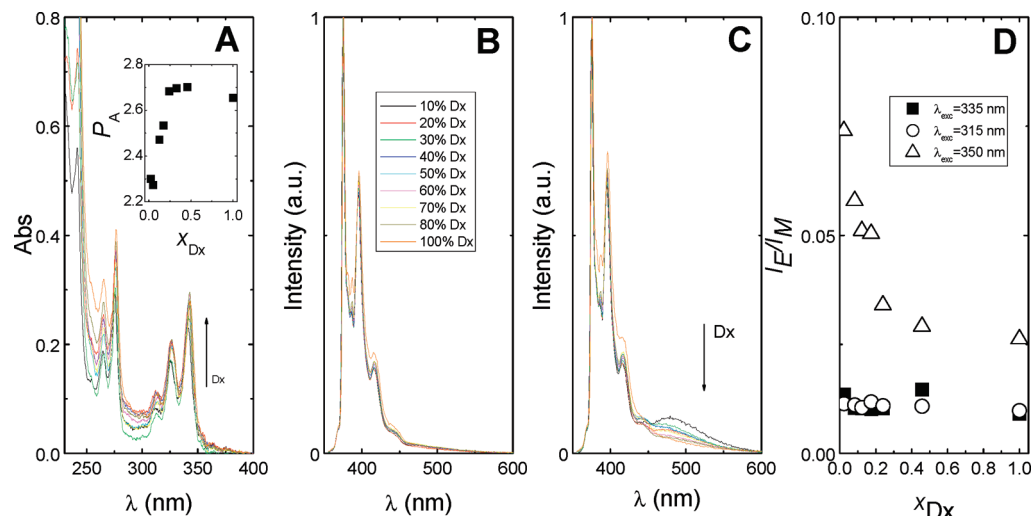


Figure 1. Absorption (A) and emission spectra obtained at $\lambda_{\text{exc}} = 335$ (B) and 350 nm (C) for PAAMePy(450)11 in dioxane/water mixtures. Dependence of the I_E/I_M ratio on the molar fraction of dioxane obtained at three different excitation wavelengths is presented in (D).

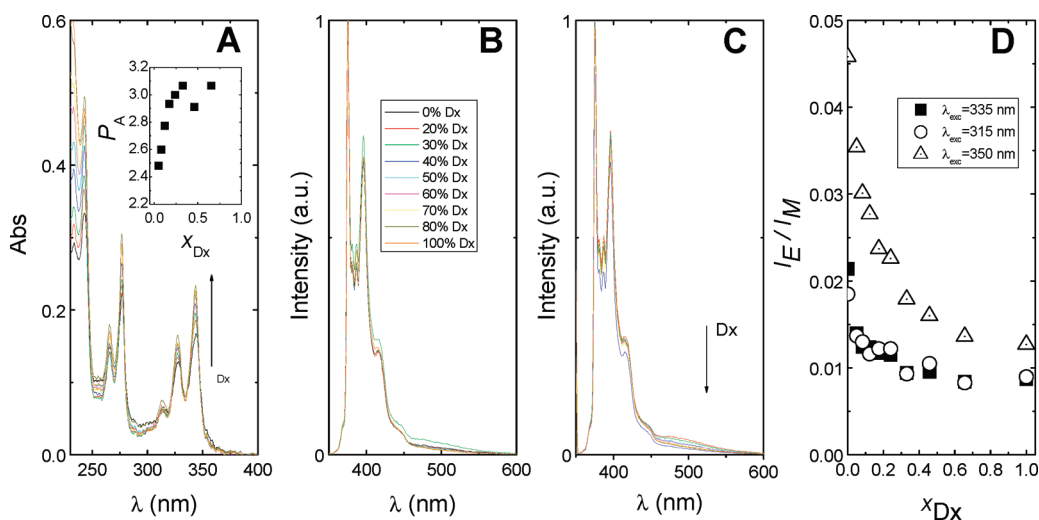


Figure 2. Absorption (A) and emission spectra obtained at $\lambda_{\text{exc}} = 335$ (B) and 350 nm (C) for PAAMePy(450)517 in dioxane/water mixtures. Dependence of the I_E/I_M ratio on the molar fraction of dioxane obtained at three different excitation wavelengths is presented in (D).

gave rise to a C=O stretching vibration at 1706 cm^{-1} . These assignments are supported by reference to the spectrum of the unlabeled PAA starting material, which exhibits the C=O stretching vibration at 1709 cm^{-1} and does not present a band in the range $1500\text{--}1600\text{ cm}^{-1}$. The anchoring of a measurable fraction of the pyrenyl groups via a cyclic imide linkage can be ruled out because the ^1H NMR spectrum of the modified polymer showed only one sharp singlet for CH_2 (pyrenyl) groups (at 4.83 ppm), which was assigned to PyCH_2NHCO . The ^{13}C CP/MAS NMR spectrum of PAAMePy(450)11 contained three intense, broad, and/or structured peaks centered around $\delta = 40$ (overlapping resonances due to the methylene and methine carbon atoms of the polymer backbone), 123 (aromatic carbons), and 177 ppm (overlapping resonances due to the carbonyl functions of the carboxylic acid and amide groups).

Absorption and Steady-State Fluorescence. The absorption spectra of the PAAMePy(450)11 and PAAMePy(450)517 polymers in dioxane/water solutions are shown in Figures 1A and 2A. Due to the high amount of pyrene grafted to the polymer backbone in PAAMePy(450)11, the material is barely soluble in water, and therefore mixed solutions were chosen for the investigation of the photophysical properties. The peak-to-valley ratio (P_A) of the first vibronic $S_2 \leftarrow S_0$ transition of the absorption spectrum gives important information regarding the

presence or absence of GSD: P_A values equal or higher than 3 correspond to the situation where GSD are absent. In the present systems the trend of variation of the P_A parameter, as a function of the molar fraction of dioxane, shows that the absorption of the two polymers mainly results from the contribution of pyrene monomer and ground-state dimers (GSD) with this contribution decreasing with the molar fraction of dioxane. As will be seen below, the presence of ground-state association in PAAMePy(450)11 is much more evident from the excitation spectra in comparison with the absorption spectra. This may be explained by the lower extinction coefficient of the 0–0 absorption peak of pyrene aggregates when compared with those of the pyrene monomers, i.e., $21\,000$ vs $34\,700\text{ M}^{-1}\text{ cm}^{-1}$.¹⁵

The fluorescence emission spectra of PAAMePy(450)11 and PAAMePy(450)517 in dioxane/water mixtures show the characteristic monomer (with maxima at $\sim 375\text{ nm}$) and excimer (with maxima centered at $\sim 470\text{ nm}$) emission bands (Figures 1 and 2). Two clear observations can be made concerning the dependence of these spectra on the excitation wavelength regarding the highly labeled PAAMePy(450)11. First, excitation at $\lambda_{\text{exc}} \geq 350\text{ nm}$ enhances the excimer emission band when compared with excitation at 335 nm (and 315 nm), indicating that the long wavelength emission band is essentially promoted by the direct excitation of the *dimer* (or GSD), and that the

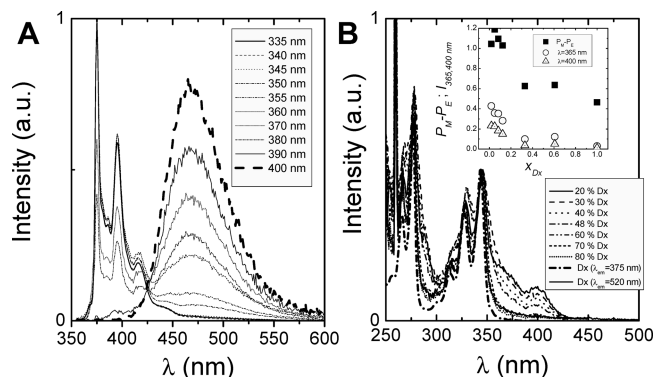


Figure 3. Emission spectra collected at different excitation wavelengths for PAAMePy(450)11 in a 2:8 (v:v) dioxane/water mixture (A). Excitation spectra collected at 520 nm (B) and dependence of the $P_M - P_E$ difference and of the intensities at 365 and 400 nm obtained from the excitation spectra (inset in B), at the excimer emission wavelength ($\lambda_{em} = 520$ nm), for this same polymer in different dioxane: water mixtures.

contribution from excimers formed from a dynamic process is strongly reduced (or absent). This is also supported by the dependence of the I_E/I_M ratio on the dioxane molar fraction, x_{Dx} (Figure 1D); i.e., the I_E/I_M ratio is higher at $\lambda_{exc} = 350$ nm than at the other two (lower) excitation wavelengths. The I_E/I_M ratio sharply decreases with the addition of dioxane, leveling off for $x_{Dx} \geq 0.33$. This evidences the increase of the dynamic route of excimer formation and a concomitant decrease of the ground-state association. The same behavior is observed with a low labeled polymer, PAAMePy(450)517 (see Figure 2D).

Taking into consideration that PAAMePy(450)517 has a very low degree of labeling, i.e., approximately 12 pyrene groups per chain, this could be indicative of an improbable excimer (and dimer) formation; nevertheless, and due to the random nature of the labeling process in these polymers, pyrene groups may be in neighboring positions within the same polymer chain, giving rise to excimer and/or dimer emission. Indeed, this was also found to occur in other situations^{7,35} together with the relevant fact that polymers synthesized by copolymerization show a higher degree of ground-state association than those where pyrene groups are grafted onto the polymer chain.³⁵

In addition to the above, a detailed analysis of the emission spectra in a mixture with a low dioxane content [Dx:H₂O 2:8 (v:v), molar fraction of dioxane $x_{Dx} = 0.05$] shows that an increase in the excitation wavelength leads to a gradual loss of the vibrational resolution at the monomer emission band, together with an increase of the excimer emission band intensity (Figure 3A). This is due to the spectral overlap between the dimer and monomer absorption bands, such that excitation between 390 and 400 nm affects the shape of the monomer emission band.

The most relevant observation comes from the comparison of the excitation spectra collected at the monomer ($\lambda_{em} = 375$ nm) and excimer ($\lambda_{em} = 520$ nm) emission wavelengths (Figure 3B). There is a significant difference between the two sets of spectra, particularly in the mixtures with a high content of water. For the different mixtures, the excitation spectra collected at $\lambda_{em} = 375$ nm are independent of the solvent content and match the absorption spectra of pyrene monomer. However, when the excitation spectra are collected at the excimer emission region, the shape strongly differs from that of the monomer absorption. Moreover, in mixtures with a dioxane content lower than 80% (in volume), a very intense band between 350 and 400 nm is observed in the excitation spectra. Its intensity increases with

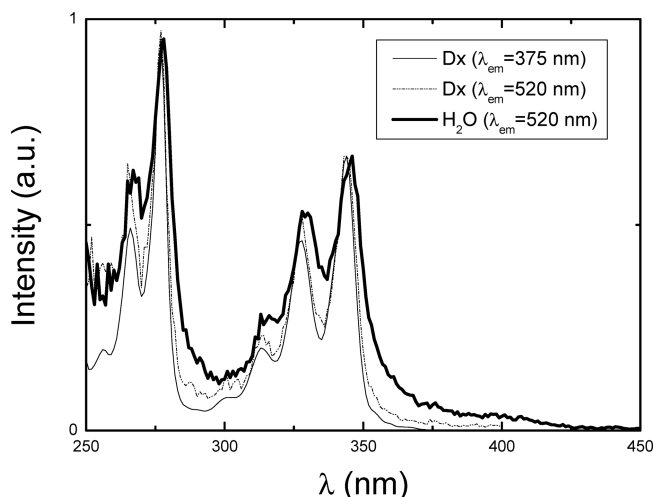


Figure 4. Excitation spectra of PAAMePy(450)517 in dioxane and water, collected at the monomer (375 nm) and excimer (520 nm) wavelength.

the water content in the mixture (Figure 3B). Additionally, an increase (but not so pronounced) of the band intensity in the wavelength region between 250 and 300 nm is also observed.

Usually, the changes in the excitation spectra are presented by the 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 ($P_M - P_E$), and also by the shift in the maximum wavelength of the two spectra ($\Delta\lambda_2$).^{1,7} Both $P_M - P_E$ (see inset of Figure 3B) and $\Delta\lambda_2$ parameters decrease with increasing molar fraction of dioxane in the mixture. The $\Delta\lambda_2$ values decrease from 4 to 5 nm at low molar fraction of dioxane and reach zero at pure dioxane. Although this result would ideally mean that ground-state association is completely absent, the broadening of the excitation spectrum (when collected at excimer emission wavelength), when compared with that collected at the monomer emission wavelength, shows that this is not the present situation. Indeed, in the present case these qualitative parameters do not seem to mirror the true effect the solvent promotes in the system, since the major changes occur in the 350–400 nm range where the P_M and P_E parameters have no expression. Consequently, the effect of the solvent on the ground-state association is better expressed by the dependence of the intensities (taken from the excitation spectra) at $\lambda = 365$ and 400 nm on the dioxane content (see the inset of Figure 3B). In pure dioxane the intensities at $\lambda = 365$ and 400 nm are practically zero, showing a negligible contribution of the dimer in this solvent.

The photophysical properties of PAAMePy(450)11 are very uncommon for these type of systems, where the dimer is usually detected by a slight increase in the red-edge of the absorption spectra, as can be seen in Figure 4 for PAAMePy(450)517 in aqueous solution. Such a pronounced difference between the excitation spectra collected at different wavelengths is normally seen when dimers are formed in constrained environments, examples of which include anionic pyrene derivatives intercalated into layered double hydroxides,³⁶ pyrene adsorbed or bound to silicas, aluminas, clays, and zeolites,^{1,33} pyrene encapsulated in cyclodextrins (1:2^{28,37} and 2:2³⁸ CD:Py inclusion complexes), and metal complexes of pyrene derivatives [for instance the 1:2 (metal/ligand) complex formed between Zn^{2+} and the deprotonated form of 1-pyrenebutyric acid³⁹].

Furthermore, it is surprising to note that the I_E/I_M ratio obtained for PAAMePy(450)11 ($I_E/I_M \sim 0.01$ in dioxane, λ_{exc}

= 335 nm) is lower than the ratios obtained for the modified polymers PAAMePy(450)87 and PAAMePy(450)53 ($I_E/I_M \sim 0.12$ and ~ 0.27 in dioxane, respectively), both of which have a lower degree of pyrene labeling (1 pyrene per 87 and 53 carboxylic groups, respectively).⁸ Indeed, this behavior is very peculiar and the explanation can only lie in the relative position of the pyrene groups within the polymer chain. In the case of a regular distribution along the PAA chain, the pyrene groups would be separated by 11 acrylic acid groups, which would be equivalent to approximately 22 methylene groups in an alkyl chain. Previous studies on the dependence of the chain length on the intramolecular excimer formation in 1,*n*-bis(1-pyrenyl-carboxy)alkanes showed that for $n > 12$ the efficiency of excimer formation is strongly decreased.⁴⁰ Although in the PAA chain the pyrene groups are separated by acrylic acid groups, where other additional contributions are likely to be found, this would explain the low I_E/I_M ratios obtained for this polymer in solution. Indeed, the strong evidence for ground-state association is likely to be essentially due to the existence of a low fraction of pyrene groups densely located in a particular region of the polymer. In the presence of water, polymer–solvent interactions become less favorable and the highly labeled regions “collapse”, leading to a hydrophobic association and a strong interaction of the aromatic moieties, associated in the form of ground-state dimers, which will now be the dominant route for excimer formation.

Nanosecond Time-Resolved Fluorescence. The photophysical behavior of PAAMePy(450)11 is, as stated above, strongly dependent on the excitation wavelength. Accordingly, the decay profiles are simultaneously strongly dependent on the excitation and emission wavelengths (of the dimer/excimer band). Indeed, the global analysis of the fluorescence decays (obtained with a nanosecond time resolution) of PAAMePy(450)11 in dioxane leads to fits with sums of three exponentials (Figure 5), similar to those found for the modified PAAMePy(2), PAAMePy(150), and PAAMePy(450) polymers in organic solvents.^{6,7} The decay times found are attributed to monomers that in the excited state give rise to excimer (shorter decay time, τ_2 , associated with a negative pre-exponential at 520 nm), free monomers (longer decay time, τ_0) and excimer (intermediate decay time, τ_1).

In Figure 5 the global and independent analysis of decays is presented. It can be observed that with the global (simultaneous) analysis of the decays, three decay times are present at the three selected emission wavelengths. This is a consequence of the global analysis procedure. However, in the present case, the decay time associated with isolated monomers, longer lifetime τ_0 , cannot be present in the excimer emission region, which indeed comes as the output of an independent analysis at the excimer emission wavelength, where the decay is now fitted with a double exponential decay law with decay time associated with the monomers able to give rise to excimer (*MAGRE*) and excimer decays, Figure 5C. It is also noteworthy that the independent analysis at the monomer emission wavelength (375 nm in Figure 5B) generates basically the same analysis output as that obtained with the global analysis procedure (Figure 5A), with a three exponential analysis with decay times associated to the *MAGRE*, isolated, and excimer species. Moreover, at the excimer emission wavelength the sum of the pre-exponential factors differs from zero, thus showing once more the existence of a fraction of pyrene groups that intramolecularly interact in the ground state and when associated can directly absorb a fraction of the exciting light. Increasing the water content of the mixture leads to a general decrease of the decay time values and, most importantly, to the absence of the negative pre-exponential at the excimer emission wavelengths, which again

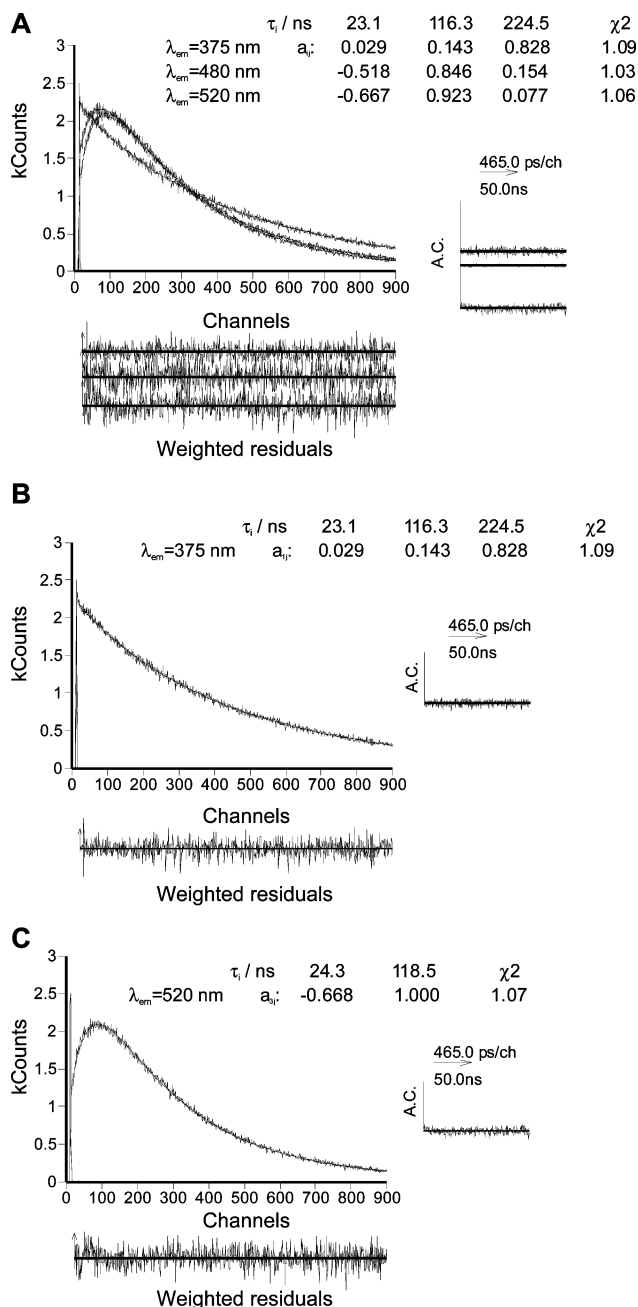


Figure 5. Fluorescence decays collected at the monomer ($\lambda_{em} = 375$ nm) and excimer ($\lambda_{em} = 480$ and 520 nm) emission wavelengths for PAAMePy(450)11 in dioxane at room temperature and $\lambda_{exc} = 339$ nm): global (simultaneous) analysis (A) and independent analysis at 375 nm (B) and 520 nm (C) of the decays. For a better judgment of the quality of the fits, autocorrelation functions (A.C.), weighted residuals (W.R.), and χ^2 values are presented as insets.

is in full agreement with the steady-state fluorescence data in Figures 1 and 3, showing that excimer formation now mainly occurs through the direct excitation of preassociated dimers.

In the case of the dioxane/water 2:8 (v:v) mixture, the fluorescence decays collected along the emission spectra mainly consist in two contributions: the isolated monomers (~ 200 ns) and the dimer (3–4 ns). Figure 6 shows the decays collected at 375 nm (single exponential) and at 495 nm. In this last situation, although the decay is not single exponential, the decay time associated with the dimer represents the major component; the two other components (34 and 112 ns) should be associated with the excimers with twisted and parallel conformations,

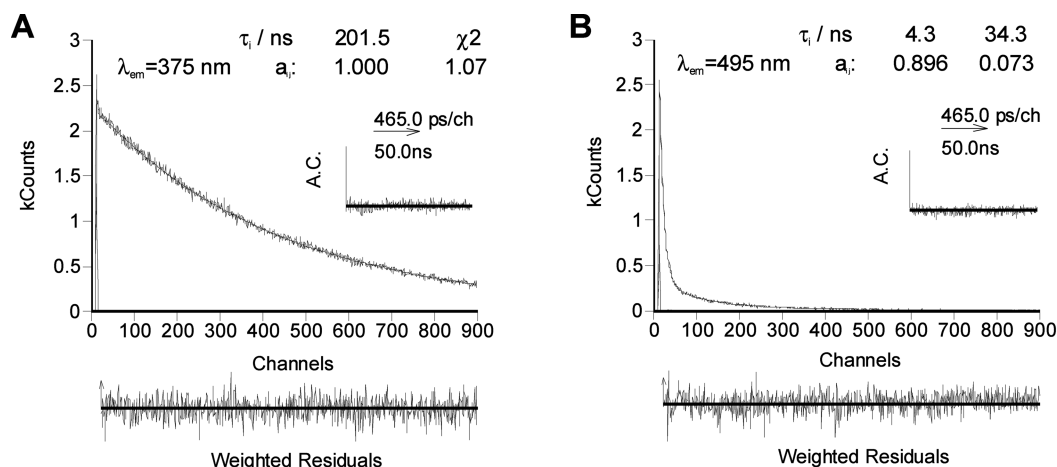


Figure 6. Fluorescence decays collected at the (A) monomer ($\lambda_{em} = 375$ nm) and (B) excimer ($\lambda_{em} = 495$ nm) emission wavelengths for PAAMePy(450)11 in $x_{Dx} = 0.05$ at room temperature and $\lambda_{exc} = 339$ nm. For a better judgment of the quality of the fits, autocorrelation functions (A.C.), weighted residuals (W.R.), and χ^2 values are presented as insets.

respectively. However, even with a negligible contribution (confirmed from the almost negligible associated pre-exponential factors) these are needed to properly fit the decay at longer wavelengths. These two excimers are obtained from the *MAGRE* monomers.⁸ Nevertheless, at this emission wavelength (495 nm) this component (*MAGRE*) is no longer needed to fit the decay, but in the range between 385 and 465 nm it has a non-negligible contribution with a decay time of ~ 9 ns.

The fluorescence decays were collected along the wavelength range from 375 to 545 nm and the nanosecond time-resolved fluorescence spectra (ns-TRES) were constructed by plotting the fluorescence intensity dependence with time vs the wavelength [$I(\lambda, t)$] and using the amplitude pre-exponential factors, $a_i(\lambda)$, the decay times, $\tau_i(\lambda)$, and the steady-state intensity (I_{SS}) according to the following equation:⁴¹

$$I(\lambda, t) = I_{SS} \frac{\sum_i a_i(\lambda) e^{-t/\tau_i(\lambda)}}{\sum_i a_i(\lambda) \tau_i(\lambda)} \quad (1)$$

The obtained ns-TRES spectra are shown in Figure 7 where it is clear that, essentially, two components are present: the free monomers and the dimers. These have respectively maxima at 375 and 465 nm, with the former existing at longer times and the latter only at shorter times.

Picosecond Time-Resolved Fluorescence. The most interesting finding comes when the PAAMePy(450)11 system is observed on a picosecond time-scale. Indeed, by adopting a coiled conformation, due to the strong intrapolymeric pyrene–pyrene interactions, the number of preassociated pyrene dimers increases. Since this is now the dominant process, it is possible to follow it in more detail. Upon excitation, the nearby pyrene groups reorient themselves to adopt a preferred dimer conformation and this fast process can be followed on a picosecond time scale.

In dioxane/water mixtures with a high water content (from 1:9 to 4:6, v:v) and with excitation at 392 nm (a region where the pyrene monomer absorption is basically absent), the investigation of the observed emission is essentially from the dimers (see Figure 3A). The fluorescence decays in a mixture with $x_{Dx} = 0.018$ (Dx:H₂O 0.08:9.2, v:v) and $x_{Dx} = 0.05$ (Dx:H₂O 2:8, v:v) were collected at different emission wavelengths,

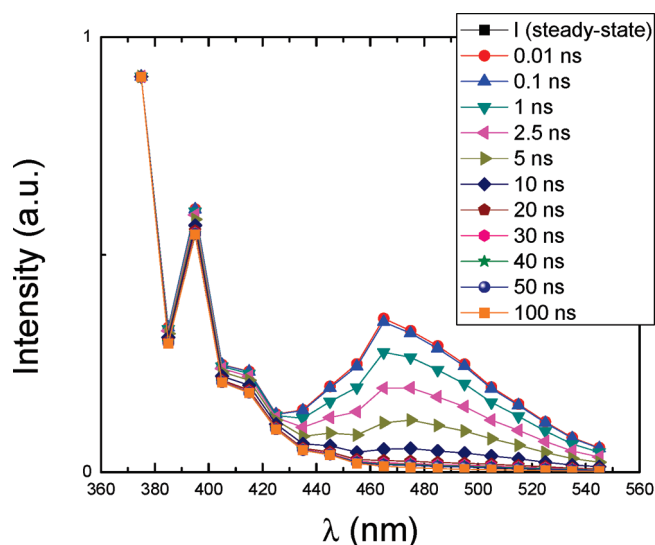


Figure 7. Nanosecond time-resolved emission spectra (ns-TRES) of PAAMePy(450)11 in $x_{Dx} = 0.05$. The fluorescence decays were collected in the range from 375 to 545 nm, using $\lambda_{exc} = 339$ nm.

from 420 to 520 nm, i.e., along the excimer emission band (Figure 8). Although the best fits are obtained with triexponential decay laws, a biexponential decay law was found to correctly fit the decays whose values, $\tau_1 = 2.5$ –3 ns and $\tau_2 = 4$ –10 ps, depending on the mixture, were found to remain constant with the emission wavelength. The third exponential displays decay time values of ~ 140 –200 ps but with a pre-exponential factor value lower than 0.06. Values of 2–3.7 ns have previously been observed for pyrene in silica sol–gel systems⁴² and for 1,3-bis(1-pyrenyl)propane in *n*-heptane.⁴³ Similar values were obtained in Zn–Al layered double hydroxides intercalated by anionic pyrene derivatives (2.9–3.4 ns, with no rise-time at the excimer emission wavelength) and were attributed to a dimer emission.³⁶

By comparing the literature lifetime values with those obtained for PAAMePy(450)11, we can reasonably conclude that the longer decay time should be associated with the emission decay of the excited dimer. Moreover, by collecting the decay profiles along the emission band, we can see that the shorter decay time (τ_2) appears as a decay time at $\lambda_{em} < 440$ nm and as a rise time at $\lambda_{em} \geq 480$ nm. This constitutes strong evidence for the presence of a two-state mechanism, with the emission

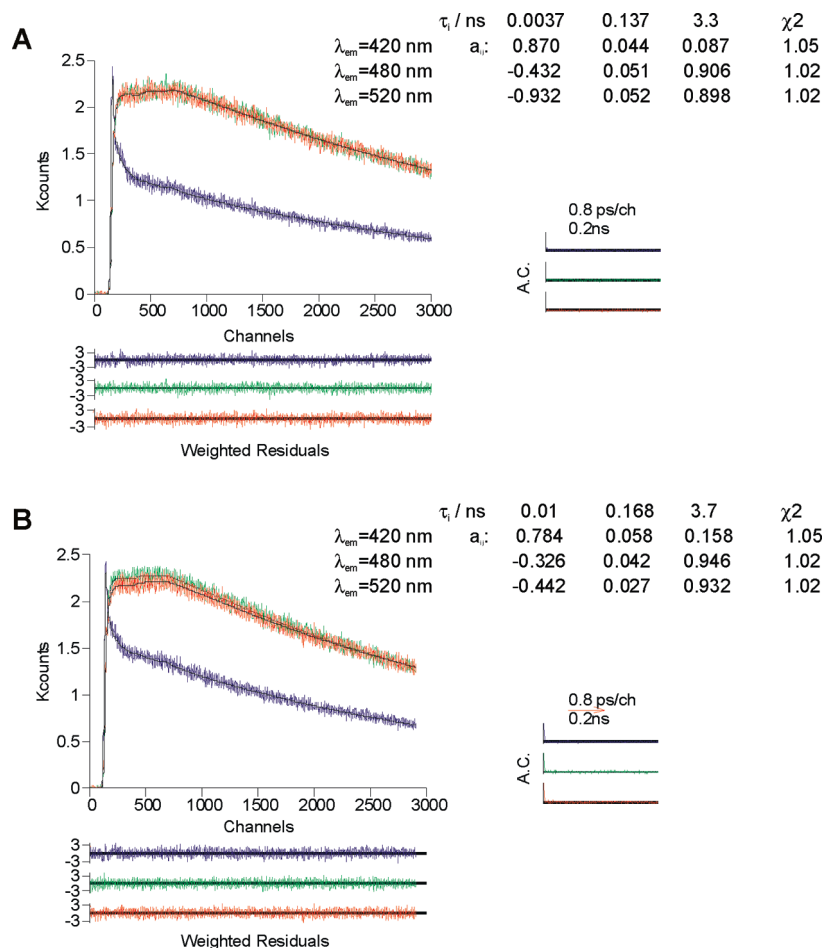


Figure 8. Fluorescence decays, with picosecond time resolution, for PAAMePy(450)11 in dioxane/water mixtures of 8% (A) and 20% (B) dioxane, at three emission wavelengths (λ_{em} = 420, 480, and 520 nm) at room temperature and λ_{exc} = 392 nm. For a better judgment of the quality of the fits, autocorrelation functions (A.C.), weighted residuals (W.R.), and χ^2 values are presented as insets.

spectra resulting from the superposition of two distinct emissions. The presence of a rise-time seems to exclude the possibility of two independent processes. Furthermore, the time-resolved emission spectrum in the 420–545 nm range of the dimer can provide additional evidence for the presence of the instantaneously formed dimer (with a decay time of ~ 10 ps) and of the more stable conformer (~ 3 –4 ns). Indeed, we have obtained the picosecond time-resolved emission spectra (ps-TRES) with biexponential (decay times of 10 ps and 3.1 ns) and triexponential decays (decay times of 10 ps, 200 ps and 3.1 ns), using eq 1.

Several important conclusions can be drawn from the spectra obtained with this procedure (Figure 9). First, the fact that there is no difference between the analyses with two or three exponentials adds weight to the hypothesis that apparently only two species are fundamental to interpret the results. Second, there is clear evidence that the ps-TRES changes for different times, which supports the conclusion that different species emit for different times. Figure 9 shows that for long lifetimes the band is broad and seems to be the result of the spectral envelope consisting of two bands with maxima at ~ 445 and 470 nm. For short times (≤ 15 ps), the ps-TRES spectra clearly show the predominance of the band with maxima at ~ 445 nm. With the increase in time this band decreases its intensity whereas the band with maxima at 470 nm grows in intensity. For times > 20 ps the TRES is virtually identical to the steady-state spectra. In addition it is worth noting that evidence for different pyrene emission bands in pyrene labeled polymers was reported in the

pioneering work of Yamazaki et al.⁴⁴ In this work, hydroxypropyl cellulose polymers labeled with pyrene were investigated, and two emission bands with maxima at 420 and 470 nm could also be clearly split by TRES.

There are some examples in the literature where in the same emission band the decay collected in the red or blue edge of the band gives rise to different decay profiles.^{45,46} For conjugated organic polymers,⁴⁵ the different decay profiles were assigned to the instantaneously formed species (shorter decay time) and to the conformationally relaxed species (more planar) of the polymer. A similar pattern was observed with indigo and some of its derivatives.⁴⁶ In the present work, a parallel can be made such that the observed behavior can be attributed to the relaxation of the instantaneously formed dimer into the stable conformer (decay time of 2–3.7 ns) whose emission is predominant at the long wavelength region of the band. It is worth stressing that a decay time of 140 fs, assigned to excimer formation in pyrene crystals, has been previously reported,⁴⁷ and from molecular dynamics simulations Schuetz et al. predicted that dimers can survive for times longer than 10 ps.⁴⁸

Although the prompt rearrangement of the pyrene groups in the dimer to a more stable (dimer) conformation seems a logical and reasonable explanation for the observed behavior, a complementary explanation, based on the same experimental evidence, is possible and is related to the reorientation of the solvent around the dimer. In this case the short-lived component refers, in the blue edge of the spectra, to the instantaneous emission, i.e., from a Franck–Condon dimer excited state. At

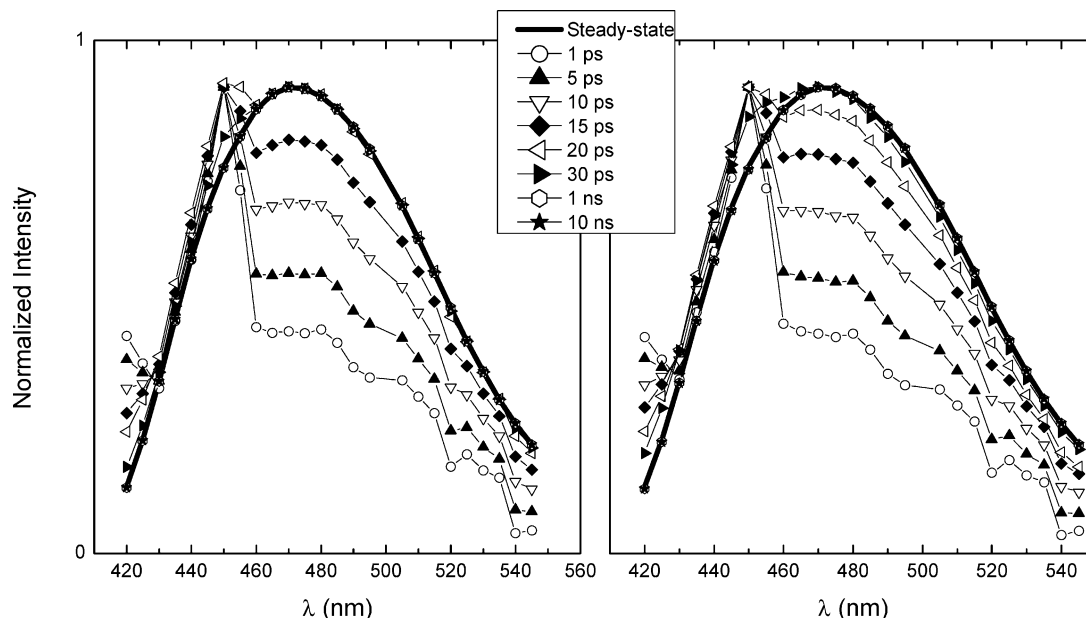


Figure 9. Picosecond time-resolved emission spectra (ps-TRES) of PAAMePy(450)11 in $x_{Dx} = 0.05$. The fluorescence decays were collected from 420 to 550 nm, using $\lambda_{exc} = 392$ nm. The left and the right-hand panels regard the TRES obtained using bi- and triexponential decays, respectively.

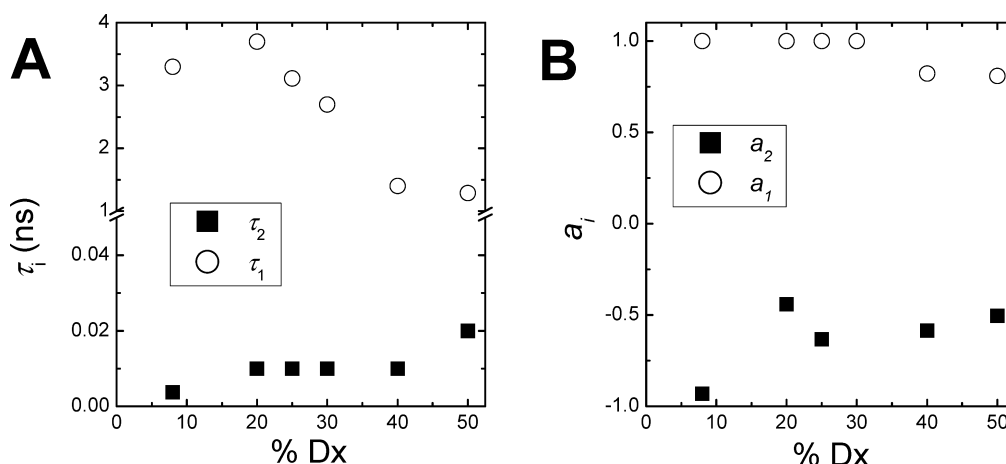


Figure 10. Fluorescence decays times (A) and pre-exponential factors (B) collected at the excimer emission wavelength ($\lambda_{em} = 520$ nm) for PAAMePy(450)11 in different dioxane/water mixtures at room temperature ($\lambda_{exc} = 392$ nm). The pre-exponential factors a_1 and a_2 are associated to respectively the decay times τ_1 and τ_2 .

the long wavelength region of the emission band, the rising component is now related to a relaxed excited state (solvent shell as reoriented into a preferred state).⁴⁹

The decay times and preexponential factors for PAAMePy(450)11 in several different dioxane/water mixtures, obtained at the excimer emission wavelength ($\lambda_{em} = 520$ nm), are shown in Figure 10. The fluorescence decays are biexponential. The longest decay time (τ_1) is observed in all mixtures and decreases, varying from ~ 3.7 to ~ 1.3 ns, with x_{Dx} . With the increase in the dioxane content, the decrease of the decay time values indicates the disappearance of the short-lived dimer. The shorter decay time (τ_2) assumes values between ~ 4 and 10 ps, with the sole exception being the mixture with $x_{Dx} = 0.12$ (20 ps).

In summary, the labeled polymer PAAMePy(450)11 with high pyrene content exhibits properties that are markedly dependent on the solvent, a strong associative behavior being found when the quality of solvent (introduction of water) is decreased. A very intense band was observed in the wavelength range 350–400 nm in the excitation spectra collected at the

excimer emission wavelength, indicating a strong absorption from a pyrene dimer structure. Picosecond time-resolved fluorescence allowed us to follow the fast reorientation of the pyrene groups within this structure, which occurs in a lifetime of 4–10 ps.

Conclusions

A pyrene labeled PAA polymer, PAAMePy(450)11, found to be practically insoluble in water due to the high amount of grafted pyrene groups, was investigated in dioxane/water mixtures using absorption, steady-state, and time-resolved (with nanosecond and picosecond time resolution) fluorescence techniques. Further details on the mechanism of dimer formation and decay could be investigated and a rise-time in the range ~ 4 –10 ps for the instantaneously formed dimer followed by a decay of 2–4 ns could be obtained in different dioxane/water mixtures. Time resolved emission spectra in the picosecond time domain have shown that the emission of these two species has a signature of two bands emitting with maxima at 445 and 470

nm. The 470 nm band was also observed in the time-resolved emission spectra obtained in the nanosecond time domain.

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Supporting Information Available: Figures showing the FT-IR, ^1H NMR, and ^{13}C CP/MAS NMR spectra of PAAMe-Py(450)11. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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