Synthesis and Characterization of Pyrene-Labeled Poly(ethylenimine)

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ABSTRACT: Pyrene-labeled poly(ethylenimine) (PEI) containing different amounts of pyrene per polymer were prepared by reductive amination of PEI with 1-pyrenecarboxaldehyde. Aqueous solutions of the pyrene-labeled PEI (PEI−Py) exhibit typical pyrene monomer and excimer emission, and the excimer-to-monomer ratio I_E/I_M decreases with decreasing Py content of the macromolecule. The excimer emission arises from preassociated pyrene aggregates. Both the monomer and excimer emission intensities increase with decreasing pH of the solution. A decrease in pH of the solution from pH = 10 to pH ≈ 1.5 led to approximately a 100-fold increase in I_M, whereas I_E increased just 2- to 3-fold. A major feature of this system is the quenching of the Py fluorescence by amino groups of the PEI. This quenching depends on the degree of protonation of these groups. Model experiments with pyrenemethanol show that triethylamine is 20 times more effective as a quencher than diethylamine in water, implying that most of the quenching in PEI is due to the tertiary amine groups. More interesting is the relative insensitivity of the excimer emission to this quenching process. This selective quenching may be due to the lower energy of the excimer state or the environment of the preassociated pyrenes in the polymer.

Introduction

Water-soluble polymers have been extensively studied by the fluorescence technique. Some experiments involve fluorescence probes added to the solution, whereas others involve chemical labeling of the dye to the polymer backbone. One of the special features of water-soluble polymers to which fluorescent dyes are attached is that the dyes change the hydrophilic−lipophilic balance of the polymer and often serve as association sites for the polymer.[1−3] Of the various dyes that have been employed for these types of studies, pyrene (Py) is often the chromophore of choice. The features of pyrene that make it an attractive chromophore include its well-characterized long-lived excited state, the sensitivity of its fluorescence to quenching, the sensitivity of its excitation spectra to microenvironment changes, and its propensity for forming excimers. These various fluorescence properties of pyrene have been used to study a variety of different kinds of polymers: nonionic water-soluble polymers including the class of hydrophobically modified polymers used as associative thickeners, polyelectrolytes, and block copolymers.

For example, the intramolecular cyclization dynamics of poly(ethylene oxide) (PEO) chains was studied using end-labeled PEO samples carrying a pyrene at both chain ends.[4−7] Since excimer emission is diffusion controlled, the ratio of excimer-to-monomer emission, I_E/I_M, of these samples in organic solvents decreases with increasing solvent viscosity. In contrast, for solutions of the polymer in water, unexpectedly high I_E/I_M values were obtained, due to hydrophobic association of the Py groups. Duhamel et al.[8] estimated the fraction of pyrene excimer originating from preformed dimers to be ca. 7% for a Py−PEO−Py of M_w = 8000.

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Hydroxypropyl cellulose (HPC) and other cellulose ethers, such as methyl cellulose and hydroxypropyl methyl cellulose, labeled randomly with pyrene derivatives exhibit both monomer emission and excimer emission.[9,10,37] In aqueous solution, excimer emission often originates from preassociated pyrene aggregates. In samples of pyrene-labeled HPC,[10,11] the I_E/I_M ratio is sensitive to temperature. For example, in the case of HPC−Py/26 (one Py per 26 glucose units), I_E/I_M increases from 0 to 29 °C. Upon further heating, I_E/I_M abruptly decreases and reaches approximately a constant value above 40 °C, close to the cloud point for the polymer.[11] The abrupt change in I_E/I_M reflects the phase transition of the polymer solution. Above the phase transition temperature, pyrene monomer emission increases at the expense of the excimer fluorescence intensity, indicating the disruption of the associated pyrene in the polymer.

The fluorescence of pyrene-labeled poly(*N*-isopropylacrylamide) (PNIPAM−Py) is also characterized by both monomer and excimer emission. In the case where Py is the only hydrophobic substituent on the polymer, excimer emission originates from "static" pyrene pairs or aggregates.[12−14] In a different set of samples in which the PNIPAM contained both C_{18} chains and pyrene groups, excimer emission was also intense, but here the excimers formed after light absorption by the Py moieties.

The structural, dynamic, and conformational properties of pyrene-labeled polyelectrolytes have been described in a number of publications.[15−23] For example, the pyrene association for two pyrene-labeled polyelectrolytes, a polycation and a polyanion, in aqueous solution was reported by Herkstroeter et al.[15] Since the I_E/I_M ratio was insensitive to dilution with water, the authors[15] concluded that association occurred between Py moieties attached to the same polyelectrolyte chain. It is well-known that the conformation of poly(carboxylic

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acid)s depends on the pH of solution: at low pH, the polymers have a coiled structure, whereas at high pH the chains expand as a result of intrapolymeric electrostatic repulsion.[16] The influence of this kind of conformational transition on pyrene-labeled poly(acrylic acid) (PAA-Py) was reported by Turro and Arora.[17] They observed that upon neutralization of the polymer, the excimer emission decreased, indicating the dissociation of the pyrene aggregates. Chu and Thomas[18] and Guillemet and Piculell[19] used pyrene and its derivatives to study various properties of hydrophobically modified polyelectrolytes. The properties of pyrene-labeled polysulfonates were reported in refs 20−22. For some systems, particularly, a pyrene-labeled poly(styrene sulfonate), no excimer emission was detected.[20] Wielema and Engberts studied a pyrene-labeled poly(vinylsulfobetaine), poly[dimethyl(2-(methacryloxy)ethyl)(1-(2-sulfoethyl))ammonium betaine] (PVSB−Py).[23] Unlike the corresponding unlabeled poly(vinylsulfobetaine), PVSB−Py is soluble in water. Upon addition of increasing amounts of salt, PVSB−Py first precipitated; then at a sufficiently high salt concentration, it went into solution again. Surprisingly, the I_E/I_M ratio measured for the pyrene-labeled polymer was found to be only slightly affected by the solubility behavior of this polymer.

The studies referred to above all involved linear polymers. In contrast, studies of branched polymers labeled with fluorescence dyes are rare.[50] It is in this context that we have prepared samples of pyrene-labeled poly(ethylenimine). Poly(ethylenimine) (PEI) is often thought of as a hyperbranched polymer. It is a polybase with a weak polyelectrolyte character in unbuffered water[24] and behaves as a strong polyelectrolyte upon protonation with acid. This polymer was recently characterized by laser light scattering and viscometry measurements by Park and Choi.[25] They fractionated the polymer to obtain samples of different molecular weights. All of the solution properties they determined at 35 °C were characteristic of a branched polymer. Viscometry measurements gave $[\eta] = 0.513M_w^{0.31\pm0.01}$, where $[\eta]$ is the intrinsic viscosity. The normal exponent of the Mark−Houwink relation for flexible linear polymer system is usually 0.7 in a good solvent and 0.5 under Θ conditions. The exponents of scaling relations between M_w and the *z*-average radius of gyration, R_G, the hydrodynamic radius R_H, the viscometric radius, R_V, and the thermodynamic radius, R_T, were lower than those for linear polymer in a good solvent. These low exponents were attributed to the compact structure of a highly branched polymer.

We report here the synthesis and fluorescent properties of PEI labeled with pyrene (PEI−Py). The PEI sample we examine is of high molecular weight (M_w = 750 000) but very broad molecular weight distribution ($M_w/M_n \approx 10$). Here we describe the steady-state fluorescence properties of this PEI−Py sample in water, in unbuffered water, and at pH 6.6, where approximately 20−40% of the amino groups are protonated. The fluorescence measurements are particularly interesting because of the quenching of Py fluorescence by secondary and tertiary amino groups in the polymer backbone, and because of the very different sensitivity to quenching of the monomer and excimer emission.

Experimental Section

Materials. Two poly(ethylenimine) (PEI) samples (50 wt % solution in water, commercial name Polymin P) were obtained from BASF and from Aldrich and were used as received. The two polymer samples both have a nominal molecular weight of 70 000 (M_w = 750 000). BASF is the world's major producer of PEI, and virtually all samples available in North America and Europe, as for example, from Aldrich, originate from BASF. NaCl and NaOH were purchased from BDH, Inc. The solution concentrations of PEI are given as weight percents (grams per 100 mL). HCl standard solutions, 1-pyrenecarboxaldehyde, pyrenemethanol, and pyrenemethylamine hydrochloride were obtained from Aldrich and were used without purification. Aqueous solutions were made up by using distilled water that was deionized in a Millipore Milli-Q water system.

Potentiometric Titrations. All potentiometric titrations were performed with a pH-meter purchased from EXTECH Instruments (Waltham, MA). To the aqueous solutions of PEI, small aliquots of a standard solution of HCl were added. All experiments were carried out under constant stirring at room temperature. To avoid disturbance of pH measurements, the titrations never lasted longer than 15 min, and after each run, the electrodes were rinsed with water and calibrated using standard calibration solutions.

Labeling PEI with 1-Pyrenecarboxaldehyde. PEI, 10 g of 50% aqueous solution, was first diluted with 15 mL of water, and then, 80 mL of methanol was added. This solution was neutralized with concentrated HCl to pH 6.0 and then was mixed with a methanol solution of 1-pyrenecarboxaldehyde (51.3 mg in 10 mL). After the mixed solution was incubated for 10 min, excess $NaBH_3CN$ (68 mg) was added. The mixture was stirred for 4 h to yield an almost colorless solution, and then the solvent was evaporated at 40 °C using a Buchi (Switzerland) vacuum evaporator. The oil residue was redissolved in 50 mL of deionized water and washed four times with chloroform. The aqueous phase was bubbled with nitrogen for 1 h to remove any traces of chloroform. Different labeling contents on PEI were adjusted by changing the amount of 1-pyrenecarboxaldehyde added, while the reducing agent $NaBH_3CN$ was always kept at a 5-fold molar excess.

The degree of pyrene substitution was determined by UV absorption measurements using ϵ_{344}= 39 800 L^{-1} cm^{-1} taken from the value of pyrenemethylamine in THF.[26] We describe the polymer with a notation that indicates the approximate number of EI units per pyrene (*n*) in the polymer (PEI−Py/*n*); Thus PEI−Py/900 and PEI−Py/6000 have one pyrene per 906 ethyleneimine (EI) units and one pyrene per 6170 EI units, respectively.

Fluorescence Measurements. Fluorescence spectra were recorded at room temperature on a SPEX Fluorolog 2 spectrometer equipped with a DM3000F data system. The emission spectra were not corrected. The excimer-to-monomer intensity ratio (I_E/I_M) was calculated by dividing the integrated fluorescence intensity between 450 and 600 nm (I_E) by the integrated fluorescence intensity between 368 and 395 nm (I_M). The excitation wavelength was 343 nm in all experiments. Polymer solutions examined here were prepared by dilution from an aqueous stock polymer solution (3 wt %) in water. Polymer solutions at different pH were obtained by adding concentrated aqueous solutions of HCl or NaOH to the aqueous solutions of the polymers. Decay curves were obtained by the time-correlated single-photon-counting technique.[27] The excitation source was a coaxial flash lamp (Edinburgh Instruments, Model 199F) filled with deuterium. The analysis of the decay curves was performed with the δ-pulse deconvolution method[28] using iterative weighted linear least-squares analysis to find the parameters of the fluorescence decay curves having the best fit. The excitation wavelength was 343 nm; the fluorescence from the pyrene "monomer" was monitored at 377 nm.

Results and Discussion

Protonation of PEI. Before characterizing pyrene-labeled PEI, we will consider the structural and physicochemical properties of unlabeled PEI. The branched PEI studied in this work is known to contain three

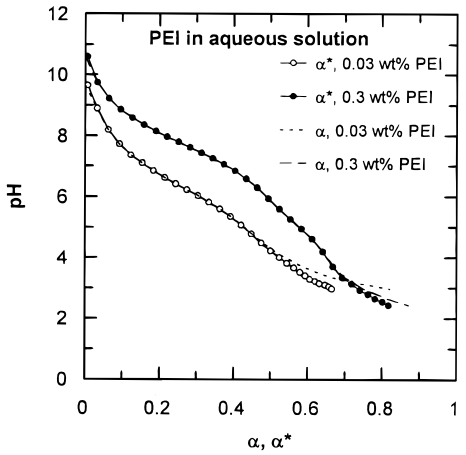


Figure 1. Potentiometric titration of PEI solutions.

different types of amino groups: secondary and tertiary amino groups in the main chain and secondary and primary amino groups in the side chain.[29] The ratio of primary-to-secondary-to-tertiary amino groups is 1:2:1.[30] Thus, PEI is a weak polybase. The degree of protonation α* of the PEI amino groups can be easily changed by adding acid to the solutions, and can be calculated from the electroneutrality condition:

$$\alpha^* = [(C_H)_{added} - (C_H)_{free} + (C_{OH})_{free}]/[EI] \quad (1)$$

Here, $(C_H)_{added}$ is the concentration of added acid (HCl); $(C_H)_{free}$ and $(C_{OH})_{free}$ are the concentrations of free protons and hydroxyl ions, which can be determined from pH measurements by equating concentrations and activities, along with the relationship between them $(C_H)_{free}(C_{OH})_{free} = 1.0 \times 10^{-14}$; [EI] is the polymer concentration expressed as monomeric equivalents of PEI. As follows from (1), α* is related to degree of neutralization $\alpha = (C_H)_{added}/[EI]$ by the following equation:

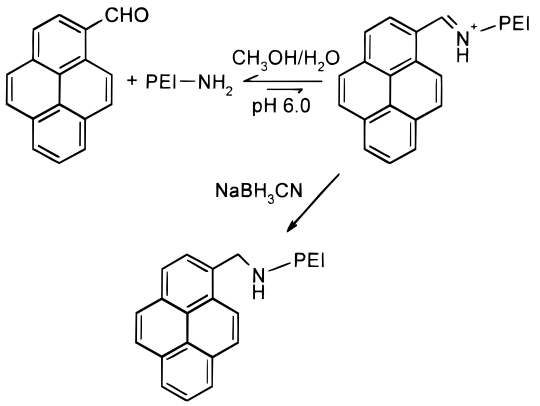
$$\alpha^* = \alpha + [(C_{OH})_{free} - (C_H)_{free}]/[EI] \quad (2)$$

In Figure 1 we plot pH versus α* and α for 0.03 and 0.3 wt % PEI. As can be seen in this figure, the α and α* values differ at very acidic and very basic pH. As expected, at neutral pH α and α* values are practically the same.

Since PEI is a polybase, its dilution in unbuffered water solution leads to an increase of pH of these solutions up to around pH 10, and the degree of ionization α* of these PEI samples of different concentrations equals approximately 0.01. Thus, under these conditions the PEI is essentially an uncharged polymer, and for the sake of simplicity, we refer to them as high pH solutions or α* = 0 solutions. Under more acidic conditions, PEI amine groups are protonated (α* increases), and PEI must be considered as a polyelectrolyte.

The characteristic feature of the PEI solutions is that at the same pH, the degree of protonation α* of amino groups is, in general, different for different concentrations of PEI (see Figure 1). This feature of PEI in water has been investigated by van Treslong and Staverman.[31] We find, for example, that at pH 6.6, α* equals 0.22 for 0.03 wt % PEI and 0.43 for 0.3 wt % PEI. This aspect of the degree of protonation of PEI solutions should be taken into account when considering the fluorescent properties of pyrene-labeled PEI described below.

Scheme 1



Labeling PEI with 1-Pyrenecarboxaldehyde. Samples of pyrene-labeled poly(ethylenimine) were prepared by reductive amination[32] with 1-pyrenecarboxaldehyde with $NaCNBH_3$ in aqueous methanol at pH 6.0 (Scheme 1). The reaction was complete within 3 h. Unreacted pyrene derivatives were removed by simple extraction of the polymer solution in water with chloroform. UV measurements showed that only trace quantities of the pyrene derivatives were in the organic phase compared to that in the aqueous solution, implying that the labeling reaction is nearly quantitative. While reductive amination can in principle occur at either primary or secondary amino groups, we assume that the primary amine is the preferred reaction site, as this reaction is often used to prepare secondary amines from primary amines in high yield.[32]

Quenching of Pyrene by Amine Groups. Aromatic and aliphatic amines are known to be efficient quenchers of aromatic hydrocarbons, and they are believed to effect quenching through formation of an excited charge-transfer complex.[1,38,39] Before describing the fluorescence spectra of the pyrene-labeled polymer, we will anticipate the quenching of pyrene fluorescence by amino groups on the polymer. To build a basis for understanding these spectra, we first describe the quenching of pyrene monomer fluorescence in water by simple secondary and tertiary amines. We choose 1-pyrenemethanol as our water-soluble pyrene derivative and ethylamine, diethylamine, and triethylamine as model quenchers. The quenching constants, K_{SV}, and the rate constant, k_q, were obtained using a Stern−Volmer analysis of steady-state fluorescence quenching experiments.

$$I_0/I - 1 = k_q\tau_0[Q] = K_{SV}[Q] \quad (3)$$

Here I_0 and I are the intensities of the fluorescence without and with quencher, respectively, τ_0 is the lifetime of the fluorescence in the absence of quencher, and [Q] is the molar concentration of quencher. Plots of $(I_0/I - 1)$ against concentration of diethylamine and triethylamine gave straight lines (Figure 2). A time-resolved fluorescence measurement of pyrenemethanol in water showed that the decay curve is well fitted by a single exponential with τ_0 equal to 121 ns. This value was used for the calculation of k_q. The K_{SV} and k_q values for diethylamine and triethylamine are gathered in Table 1.

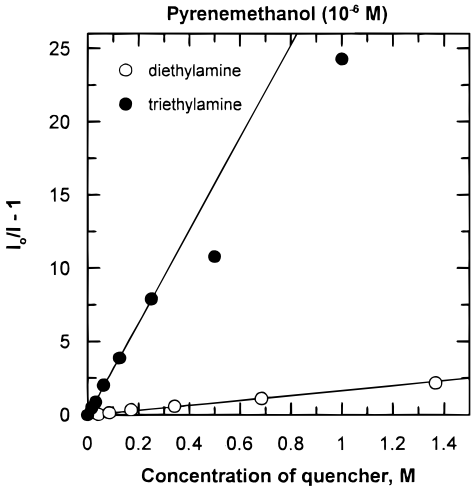


Figure 2. Stern−Volmer plots for the pyrenemethanol in water quenched by triethylamine and diethylamine.

Table 1. Stern−Volmer Constants and Rate Constants of Quenching of Pyrenemethanol for Diethylamine and Triethylamine

quencher	K_{SV} (M^{-1})	k_q (M^{-1} s^{-1})
diethylamine	1.60	1.33×10^7
triethylamine	31.6	2.64×10^8

As can be seen from the data in Table 1, the Stern−Volmer constant for triethylamine is approximately 20 times larger than that for diethylamine. The K_{SV} and k_q for ethylamine are so much smaller than those for diethylamine that meaningful values could not be obtained: one needs to add so much ethylamine that the final solutions are actually a mixed solvent system (no detectable quenching for up to 15 vol % ethylamine in water). One sees that tertiary amino groups are much more effective quenchers than secondary amino groups for pyrene fluorescence in aqueous solution. Of course, triethylamine is known to be a quencher of excited pyrene.[44,45] Even for triethylamine, however, the rate constant for quenching here is more than 1 order of magnitude smaller than the diffusion-controlled limit in water ($k_{diff} = 6.5 \times 10^9$ M^{-1} s^{-1}).[46]

We examined the influence of protonation on the quenching efficiencies of triethylamine and diethylamine. A series of aqueous solutions were prepared, at different pH values, each solution containing pyrenemethanol and either 1.0 M triethylamine or 1.37 M diethylamine. In Figure 3 we show the influence of pH on the relative fluorescence intensities. These model experiments show that upon protonation of tertiary and secondary amino groups, the fluorescence of pyrenemethanol increases. These observations serve as a model for similar processes that occur in pyrene-labeled PEI upon decrease of pH (see Figures 7 and 8). That is, upon the protonation of the PEI secondary and tertiary amino groups, the Py-label fluorescence increases due to a diminished concentration of quenching groups.

Fluorescence Spectra of Pyrene-Labeled PEI. In aqueous solutions, pyrene-labeled PEIs with very low levels of pyrene incorporation exhibit only monomer emission due to the locally excited pyrene chromophores (intensity I_M) with the (0,0) band at 377 nm. Samples containing a higher level of pyrene substitution also exhibit a broad featureless excimer emission centered at 489 nm (of intensity I_E). Fluorescence spectra,

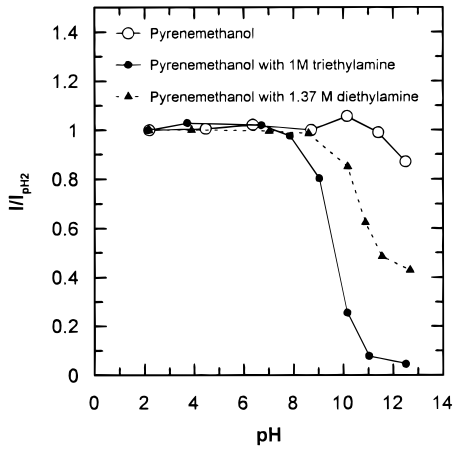


Figure 3. Changes in the I/I_{pH2} ratio of pyrenemethanol in the absence and in the presence of 1 M triethylamine or 1.37 M diethylamine at various pH in aqueous solution.

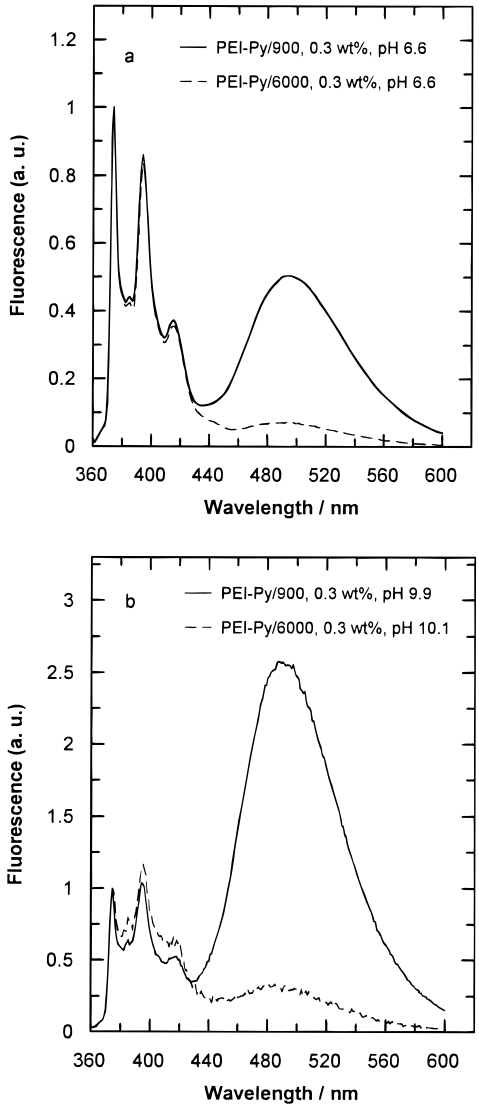


Figure 4. Fluorescence spectra of PEI−Py/900 and PEI−Py/6000, normalized to the (0,0) peak at 377 nm, at pH 6.6 (a) and pH 10 (b).

normalized to the 0−0 peak, of samples PEI−Py/900 and PEI−Py/6000 in water at pH 6.6 are shown in Figure 4a, and samples in unbuffered water (pH ≈ 10) are shown in Figure 4b. As can be seen in these figures, the excimer-to-monomer ratio, I_E/I_M, for PEI−Py/900 is

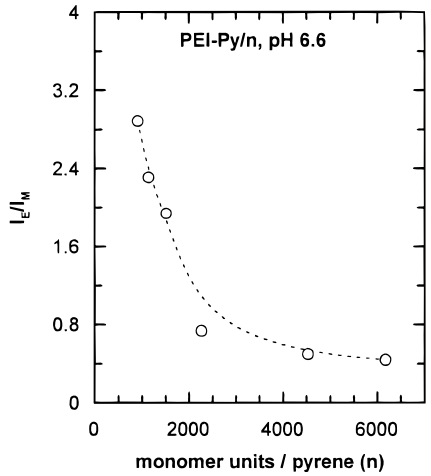


Figure 5. Dependence of the excimer-to-monomer ratio I_E/I_M for the PEI−Py derivatives containing different numbers of pyrene substituents. The *x*-axis represents the number (*n*) of EI monomer units per pyrene, and we refer to each polymer as PEI−Py/*n*.

much larger than that of PEI−Py/6000. The dependence of I_E/I_M on the number of PEI monomer units per Py group is shown in Figure 5. As expected, the excimer-to-monomer ratio decreases with the decreasing average content of Py moieties on the PEI macromolecule.

Static Excimers. Excimer emission of pyrene-labeled polymers can arise via a dynamic process or via preassociated pyrenes.[33] In the former case, the incident light excites isolated pyrene groups, which then diffuse and encounter a ground-state pyrene to form an excimer. "Static excimers" are formed by direct excitation of pyrene dimers or aggregates. These processes of excimer formation exhibit different excitation spectra, and thus can be distinguished: Dynamic excimers have excitation spectra identical to that of monomer emission. When associated pyrene groups are present, the spectrum monitored at the excimer emission is red-shifted compared to the spectrum obtained for the monomer, and the peak-to-valley ratio, $P_M = I_M{}^{Peak}/I_M{}^{Valley}$, for the (0,0) transition in the excitation spectrum monitored at the monomer emission is larger than that $P_E = I_E{}^{Peak}/I_E{}^{Valley}$, of the (0,0) transition in the excitation spectrum viewed at the excimer emission.[34]

The excitation spectra for solutions of 0.3 wt % PEI−Py/900 at $\alpha^* = 0.43$ (pH 6.6) and $\alpha^* = 0$ (pH 10), monitored at the excimer and monomer emission, are presented in parts a and b of Figure 6, respectively. At $\alpha^* = 0.43$, the excimer excitation shows a pronounced red-shift (8 nm) compared to that of the monomer emission. The peak-to-valley ratios are 2.0 for P_M and 1.35 for P_E. Similar results were obtained for the PEI−Py/900 at both high and low pH. For example, at 0.3 wt % PEI−Py/900 in unbuffered water at pH 10, we found a red-shift of 7 nm for the excimer (0,0) band, and P_M and P_E equal 2.06 and 1.46, respectively. These results indicate that for the pyrene-labeled PEI the excimer emission occurs from direct excitation of ground-state preassociated pyrene aggregates, both at $\alpha^* = 0$ and $\alpha^* = 0.43$.

The formation of "dynamic" or "static" Py excimers on polymer macromolecules depends on many factors, of which hydrophobic association of the Py groups can be a major contributor. For example, Winnik et al.[35] found that the excimer emission of pyrene-labeled hydroxypropyl cellulose (HPC) in water originated from

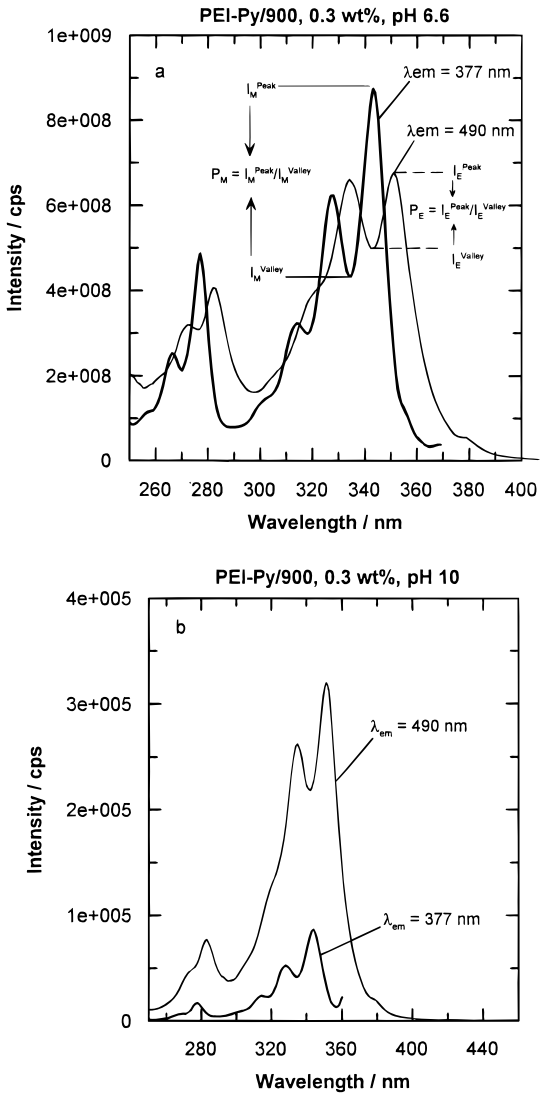


Figure 6. Excitation spectra of 0.3 wt % PEI−Py/900, monitored at 377 nm (monomer, thick line) and at 490 nm (excimer, thin line) at pH 6.6 (a) and at pH 10 (b).

pairs or aggregates of pyrene groups which exist prior to excitation. A similar situation was found for aqueous solutions of pyrene-labeled PNIPAM, PNIPAM−Py/200.[14] If, however, the PNIPAM−Py also contains flexible hydrocarbon chains, as in PNIPAM−C_{18}Py/200 and PNIPAM−C_{18}Py/400, the excimer emission originates from "dynamic excimers".[14] It is likely that the C_{18} chains provide a fluid micelle environment that allows the Py groups to be in proximity without forming face-to-face aggregates.

In systems where preassociation of pyrene molecules occurs, the red-shift usually takes values ranging from 1 to 4 nm.[34] However, there are examples in the literature of more pronounced differences between maxima of the excitation spectra monitored at monomer and excimer emissions. One such example is the 1-pyrenebutyric acid (PBA)−poly((vinylbenzyl)trimethylammonium chloride) (PVBTA) system.[36] Here it was shown that in the presence of 8.3×10^{-5} M of PVBTA, the excitation spectrum of PBA, monitored at the wavelength corresponding to the maximum of the excimer spectrum, is red-shifted by around 8 nm relative to that monitored at the maximum of the monomer emission. The red-shift we observe here is similar to that of PBA−PVBTA system.

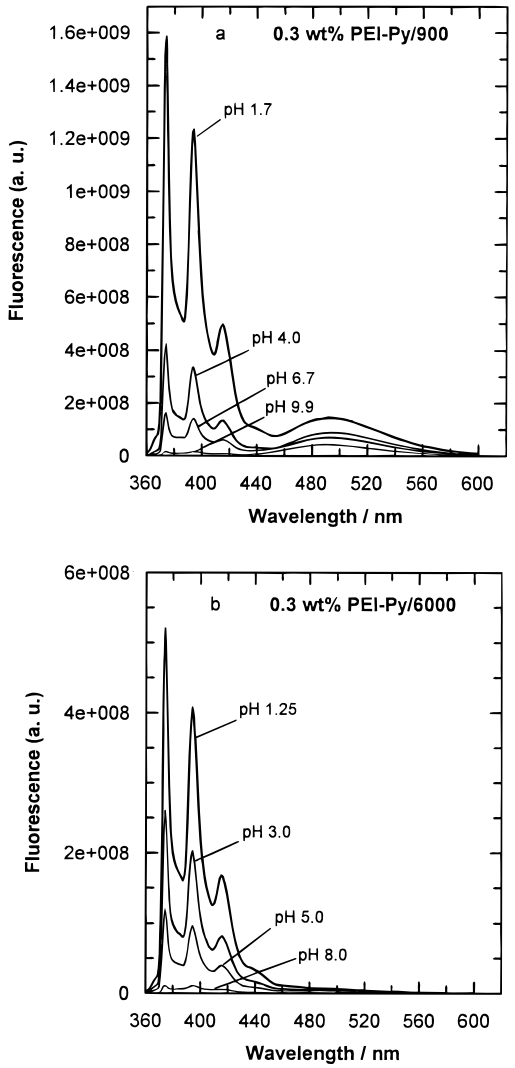


Figure 7. Fluorescence spectra of 0.3 wt % PEI−Py/900 (a) and 0.3 wt % PEI−Py/6000 (b), in water at various pH.

pH Effects. The data presented in Figure 4a,b suggest that the excimer-to-monomer ratio for pyrene-labeled PEI depends not only on the content of Py moieties but also on the pH of the solution. To investigate this effect, we performed a series of additional experiments. Emission spectra of PEI−Py/900 (0.3 wt %) obtained at different pH are shown in Figure 7a, and corresponding emission spectra of PEI−Py/6000 (0.3 wt %) at various pH are shown in Figure 7b. It can be seen in these figures that the intensity of the monomer emission I_M increases significantly with decreasing pH of the solution, whereas intensity of the excimer emission I_E increases to a much smaller degree.

Relative changes in I_M, expressed as I_M/I_{Mo}, are presented in Figure 8 as a function of pH, where I_{Mo} refers to the value of I_M at pH 1.75 for the PEI−Py/6000 and I_M at pH 1.75 for PEI−Py/900. This figure also shows the corresponding changes in I_E/I_M. As can be seen in Figure 8, I_E/I_M increases with decreasing α^* (increasing pH) for both pyrene-labeled PEI samples. I_M increases by nearly 100-fold for the both PEI samples over this range of acidities, whereas the excimer emission of PEI−Py/900 increases 2-fold, and that of PEI−Py/6000 increases by a factor of 3. Note that the fluorescence intensity of aqueous solutions of the model compound pyrenemethylamine does not depend significantly on pH over this range (Figure 9). Thus the

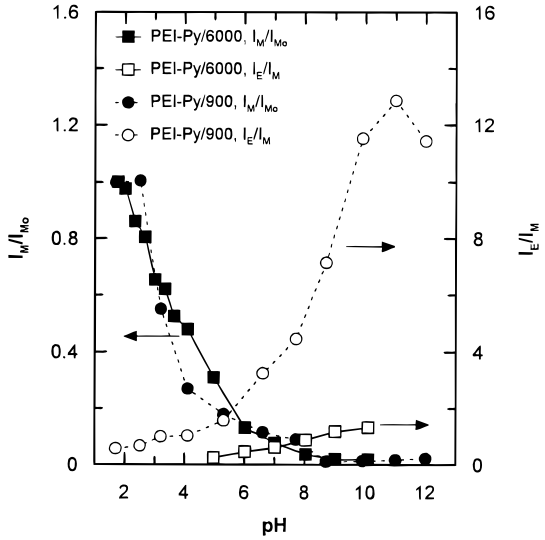


Figure 8. Ratio of I_M to I_{Mo} measured at pH 2 and the ratio of I_E/I_M for the PEI−Py/900 and PEI−Py/6000, 0.3 wt % in water at different pH.

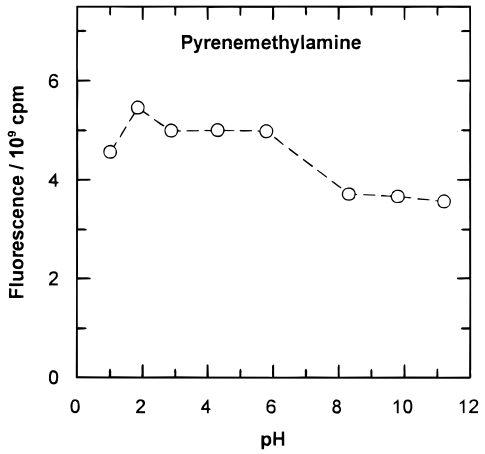


Figure 9. Relative fluorescence intensity of pyrenemethylamine (1×10^{-5} M) in water at different pH.

monomer emission from individual Py groups are not affected by the pH of the solution, but those Py groups on PEI experience a much lower local concentration of unprotonated amino groups as acid is added to the solution.

We have seen that tertiary and secondary amines are effective quenchers of pyrene fluorescence in water. Thus we assume that the fluorescence of the Py groups on PEI are quenched by uncharged amino groups on the PEI polymer, while protonated amino groups are much weaker quenchers. Since the decrease in pH of the solution results in the protonation of the PEI amino groups, the decrease in quenching of Py fluorescence parallels the decrease in the number of unprotonated amino groups.

Arora and Turro[40] examined the fluorescent properties of a pyrene-labeled poly(acrylic acid) (PAA) and its dependence on the pH of its solutions. They also examined its interaction in water with poly(vinylamine hydrochloride) (PVAm), poly(l-aminoacrylic acid) (PDA) and poly(l-(acetylamino)acrylic acid) (PADA). They showed that the I_M/I_E ratio for the pyrene-substituted PAA is constant at pH values less than 4.5 but increases above this point. They proposed that, due to low charge density and hydrophobic interactions, Py-labeled PAA

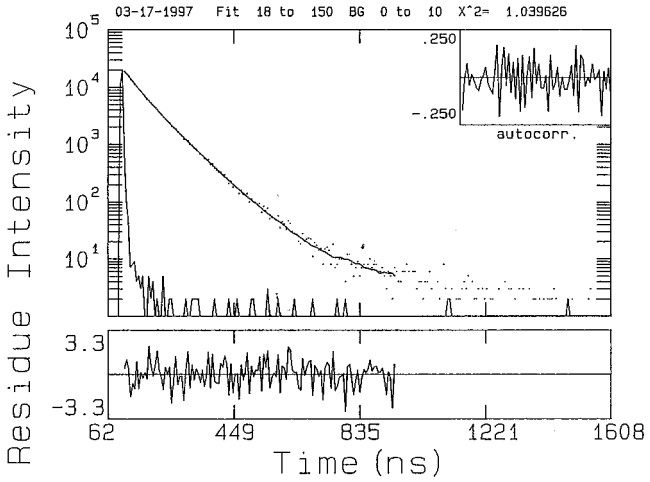


Figure 10. Fluorescence decay curve and computer fit (double exponential decay) for the PEI−Py/900, 0.3 wt % at pH 1.25.

is in a more compact conformation at low pH than at high pH. The expansion of the Py-labeled PAA at pH values greater than 4.5, caused by electrostatic repulsion between negative charges on the polymer chains, results in disruption of Py-group association and, hence, an increase in the intensity ratio I_M/I_E.

The mechanism of the change in I_E/I_M is likely to be different for PEI−Py from that of PAA−Py. In our view, the main reason for the change in I_E/I_M for the PEI−Py is a change in the efficiency of Py quenching with the change in pH, not a conformational perturbation of the PEI macromolecule. Arora and Turro[40] also showed that there were significant differences in the interpolymer interaction of the PAA−Py with PDA compared to the interaction of PAA−Py with PVAm. They observed exciplex emission in solutions of PAA−Py plus PVAm, due to excited state interactions between pyrene and amino groups on PVAm. In mixtures of PAA−Py and PDA, only fluorescence quenching was observed.

Even at very low pH, we observe some quenching of the polymer-bound pyrene. For example, at pH 1.25 the decay of the monomer emission of the 0.3 wt % PEI−Py/900 solution is nonexponential but can be fitted by a sum of two exponential terms with lifetimes of 86.7 and 44.4 ns (Figure 10). Both the short and the long decay times are much smaller than the lifetime of the fluorescence of the pyrenemethylamine in aqueous solution. It is well-known for PEI that at this pH not all amino groups are protonated, and that, in general, it is impossible to protonate all amino groups of the PEI because of the strong Coulombic repulsion between protonated amino groups and additional protons in the solution.[31,41−43] The time-resolved experiments with PEI−Py/900 are consistent with this picture and clearly indicate that quenching of pyrene label by uncharged amino groups occurs even at pH 1.25.

Excimer vs Monomer Quenching. One of the most interesting and unusual features of the fluorescence behavior described above is the much weaker susceptibility of pyrene excimer emission from the polymer-bound Py to quenching by amine groups from the polymer. If the locally excited pyrene were a precursor to a significant fraction of the excimer emission, any process that quenched the monomer emission would also quench excimer emission. All of our evidence is consistent with excimer emission from pyrenes associated prior to excitation. Nevertheless, the medium must play an important role in determining the efficiency of quenching. It may be, for example, that charge transfer from secondary and tertiary amines is less favorable to the excimer than to the locally excited pyrene. In the paragraphs below, we describe other systems in which the medium has a strong effect on the efficiency of various quenchers in quenching pyrene monomer and excimer emission.

Neal and Villegas[44] used triethylamine as a quencher to examine lipid-associated pyrene in order to measure the capacity of the medium to protect the fluorophore from quenching through pyrene partitioning into the vesicle from the aqueous phase. The Stern−Volmer constants and corresponding rate quenching constants, for the pyrene embedded in different lipid vesicles, were 2−4 times larger than those we found for the pyrenemethanol−triethylamine system presented in Table 1. In this example, it is likely that triethylamine partitions into the lipid bilayer, so that its local concentration in lipid bilayer may be higher in the lipid phase than in the bulk solution.

Nickel and Roden[45] studied pyrene-delayed fluorescence (DF) in methylcyclohexane solution at 193 K. They found that triethylamine was a more effective quencher of $S_1 - S_0$ DF than of either $S_2 - S_0$ DF or the pyrene T_1 triplet state. Here the important point is that the lower energy of the triplet state makes it less susceptible to charge-transfer quenching than the pyrene S_1 state.

Hrdlovic et al.[47] reported examples of pyrene derivatives for which the monomer emission and the excimer emission are quenched with different efficiencies. For example, acrylamide is an efficient quencher for the monomer emission of the 2,2,6,6-tetramethyl-4-hydroxypiperidinium ester of 4-(1-pyrene)butyric acid (TMP1PyBu HCl) when this ester is associated with sodium dodecyl sulfate micelles, but it is ineffective for quenching for the corresponding excimer emission of this chromophore. Another quencher, the free radical 1-oxo-2,2,6,6-tetramethyl-4-hydroxypiperidine, is 6 times more effective at quenching the monomer emission of this pyrene derivative than its excimer emission. The authors[47] explained the reduced efficiency of quenching by assuming that the quenchers must overcome a hydrophobic barrier formed by the alkyl chains of the anionic surfactant. These authors found, in contrast, that excimer emission of pyrenemethylamine in SDS micelles is quenched by quenchers such as I^- and Cu^{2+} and also by the above-mentioned nitroxide free radical. Here the efficiency of quenching of the excimer was similar to that of quenching the monomer emission. From these various observations, Hrdlovic et al.[47] proposed that the pyrenemethylamine is less tightly associated in SDS micelles than the TMP1PyBu HCl derivative.

Duhamel et al.[8] showed that for dilabeled poly(ethylene oxide), Py−PEO−Py in aqueous solution the monomer emission intensity is quenched by ions of I^- much stronger than the excimer emission. These examples show that quenching of the monomer and excimer fluorescence, and different excited states of pyrene depend on the type of quencher, the particular excited-state quenched, and the nature of the packing of pyrene aggregates in micelles and polymers.

Intra- and Interpolymer Interaction. Pyrene excimers can in principle be formed from pyrenes within one polymer (intrapolymeric) or from pyrenes on different polymers (interpolymeric association). If these

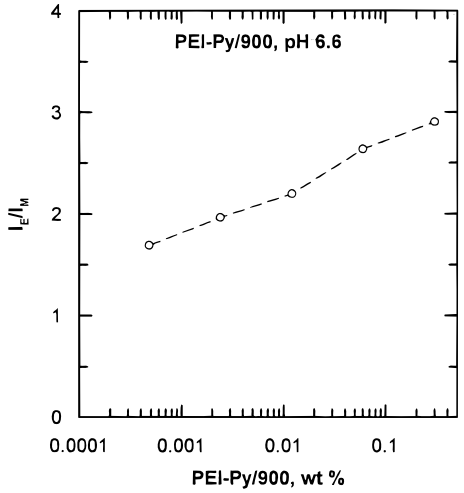


Figure 11. Change in I_E/I_M ratio as a function of the concentration for PEI−Py/900 at pH 6.6.

species are in dynamic equilibrium, dilution of the polymer solution will minimize interpolymeric association and lead to a decrease of the I_E/I_M ratio. The dependence of the I_E/I_M ratio on the concentration of PEI−Py/900 at pH 6.6 is shown in Figure 11. I_E/I_M does in fact decrease with decreasing PEI−Py concentration, but the concentration dependence is not that expected for a simple dynamic equilibrium. For example, I_E/I_M is very similar at 0.3 wt % polymer as at 0.03 wt % polymer.

A complicating feature of these experiments is the preferential quenching of I_M by secondary and tertiary amine groups of the polymer. We imagine that monomer Py moieties on one PEI macromolecule may be quenched not only by tertiary and secondary amino groups of the same macromolecule but also by amino groups of other PEI macromolecules. Upon dilution of the pyrene-labeled PEI, the relative concentration of polymer aggregates decreases, and intermolecular quenching of the Py monomer emission by the amino groups should also decrease. This will result in a decrease of the I_E/I_M ratio since the excimer emission is less quenched by these amino groups than the monomer emission.

Another complication is due to concentration effects on the degree of protonation of the polymer. We showed above that I_E/I_M increases with decreasing α^* (Figure 8). In the solutions of the same bulk pH, the degree of protonation is smaller for lower PEI concentrations (Figure 1). Consequently, the local concentration of unprotonated amino groups within the polymer is higher at lower PEI−Py concentrations. This should result in an increase in the I_E/I_M ratio upon dilution of the PEI−Py. Thus the change in the I_E/I_M ratio upon dilution of PEI−Py/900 at pH 6.6 may have several origins. First, the I_E/I_M ratio may decrease due to dissociation of interpolymeric Py aggregates. Second, the I_E/I_M ratio may decrease due to a preferential reduction in interpolymeric quenching of I_M by PEI amino groups in the solution. Third, the I_E/I_M ratio may increase because of changes of the degree of protonation of PEI. We also found that 20-fold dilution of 0.3 wt % unbuffered PEI−Py/900 at high pH does not lead to a significant decrease of the I_E/I_M ratio. The relative independence of I_E/I_M vs PEI−Py polymer concentration is likely due to a superposition of these different effects.

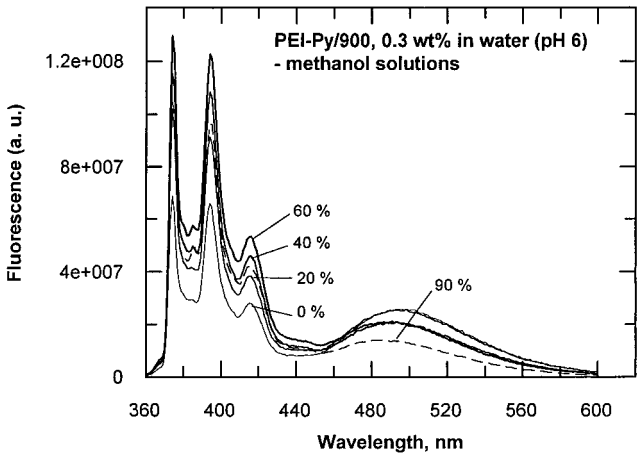


Figure 12. Fluorescence spectra of 0.3 wt % PEI−Py/900 in different water (pH 6)−methanol mixtures. The numbers on the plot refer to the volume fraction of methanol in each solution.

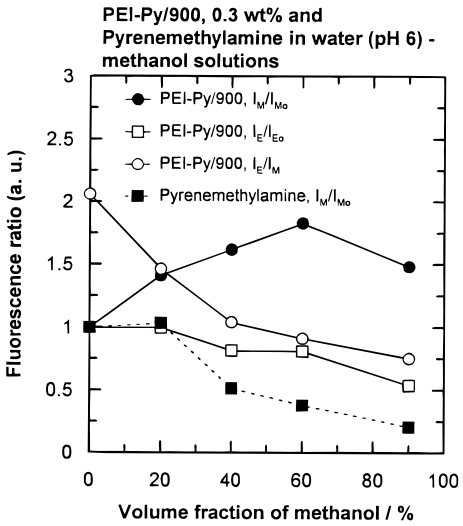


Figure 13. Effect of the volume fraction of methanol in water (pH 6)−methanol mixtures on I_E/I_M, and on relative changes in I_M and I_E, for a 0.3 wt % solution of PEI−Py/900. The plot also shows the corresponding relative changes in I_M for pyrenemethylamine (dashed line) in media of similar composition.

Effect of Methanol on Pyrene Fluorescence. Many pyrene-labeled polymers exhibit very different fluorescent properties in organic solvents than in aqueous solutions.[48,49] These changes are particularly pronounced when a cosolvent such as methanol disrupts hydrophobic association. For example, pyrene-labeled poly(*N*-isopropylacrylamide) (b-PNIPAM−C_{18}Py) exhibits strong excimer emission in water, whereas the excimer emission in methanol is very weak.[49] The authors of ref 49 argue that in aqueous solution, the pyrene groups are in close proximity, presumably within the core of micellar aggregates formed by the C_{18} hydrophobic substituents. In solvents such as methanol, these micellar structures do not form.

In Figure 12, we show emission spectra of PEI−Py/900 in methanol−water (pH 6) mixtures of different compositions. The changes in I_M and I_E and in I_E/I_M obtained from these spectra are summarized in Figure 13. In this figure, we also show the relative changes that occur in I_M for a model compound, pyrenemethylamine, in the same solutions. As can be seen in Figures 12 and 13 for the pyrene-labeled polymer, the monomer

emission increases in intensity up to 60% methanol and then decreases a little above this point. The excimer emission shows a very small decrease over this range and as a consequence, the I_E/I_M ratio shows a pronounced decrease with increasing volume fraction of methanol in these water−methanol mixtures.

It is interesting to compare the monomer emission intensity to the emission from pyrenemethylamine, which decreases under the same set of conditions. This intensity decrease can be explained entirely by increases in oxygen concentrations, since oxygen solubility increases with the increasing content of methanol in water−methanol mixtures. Despite the quenching effect of oxygen, the I_M of the PEI−Py/900 increases. We attribute this, at least in part, to a decrease in the effectiveness of amine quenching of pyrene monomer emission. We anticipated that methanol would promote dissociation of pyrene aggregates. However, even at 90% methanol, the PEI−Py/900 solution exhibits a remarkably intense excimer emission. One of the reasons why pyrene excimer emission remains prominent at high concentrations of methanol in the water−methanol mixtures is that methanol is not a good solvent for the branched PEI.[29] Thus the PEI−Py system we examine is different than hydrophobically modified b-PNIPAM−C_{18}Py, where methanol is a good solvent for the polymer backbone.[49]

Our results can be contrasted with those of Parker and Joyce[50] who measured the fluorescence polarization of dansyl conjugates of PEI in aqueous and ethanolic solutions. They concluded that there are no significant changes in the conformation of PEI in these solutions.

Conclusions

We synthesized pyrene-labeled poly(ethylenimine) by reductive amination of PEI with 1-pyrenecarboxaldehyde with $NaCNBH_3$ in aqueous methanol at pH 6.0. The fluorescence of the PEI−Py is characterized by both pyrene monomer and excimer emission. The excitation spectra showed that the excimer emission of the PEI−Py originates from preassociated ground-state pyrene aggregates. As expected, the excimer-to-monomer ratio I_E/I_M decreases with the decreasing average content of Py moieties on the PEI macromolecule. We find that both monomer (I_M) and excimer (I_E) emission intensities increase with the decreasing pH of the solution. The changes in I_M and I_E, however, are very different. As the solution is acidified from pH = 10 to pH (1.5, I_M increases approximately 100-fold, whereas I_E increased just 2−3-fold. As a consequence, the I_E/I_M ratio decreases with decreasing pH. This is caused by quenching of pyrene fluorescence by PEI amino groups. Excimer emission is much less sensitive to quenching than monomer emission. The extent of quenching depends on the degree of protonation of the amino groups of the PEI. Model experiments in aqueous solutions with pyrenemethanol as a fluorescent probe and triethylamine, diethylamine, and ethylamine revealed that the pyrene monomer fluorescence is quenched by tertiary amino groups approximately 20 times more rapidly than by secondary amino groups. Addition of methanol to solutions of the polymer in water at pH 6.6 leads to an increase in I_M and a smaller decrease in I_E. The increase in I_M can be attributed primarily to a decrease in the effectiveness of amine quenching of pyrene emission.

When acid is added to the solutions, the changes observed in I_M for the pyrene-labeled PEI occur over a different range of pH values than for pyrenemethanol (compare Figures 8 and 3). The Py groups on the PEI macromolecule are quenched by unprotonated tertiary or secondary amino groups on the PEI. This quenching takes place within a microenvironment in the neighborhood of each Py, and not all amino groups participate in the Py quenching. In contrast, all quencher molecules are translationally equivalent in the bimolecular quenching of pyrenemethanol in solution. More important, the degree of protonation of the PEI amino groups differs from that of amino groups in solution because of local electrostatic repulsion between protonated amino groups on the polymer. As a consequence, the amount of neutralizing agent (HCl) added to the PEI in order to obtain the same degree of the protonation of the charged amino groups must be larger than that added to the triethylamine or diethylamine in the solution.

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