

# Fluorescent dyes as model 'hydrophobic modifiers' of polyelectrolytes: a study of poly(acrylic acid)s labelled with pyrenyl and naphthyl groups

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A commercial poly(acrylic acid) (PAA,  $M_v = 150\,000$ ) has been labelled with low levels (0.4 to 3.1 mol%) of either pyrene, naphthalene or both chromophores introduced randomly along the chain. The photophysical properties of the polymers have been investigated by steady-state and time-resolved fluorescence spectroscopy. The pH-behaviour of the polymers has been examined by non-radiative energy transfer (NRET) measurements and by the ratio ( $I_E/I_M$ ) of excimer to monomer intensities. The pH-induced expansion of the pyrene-labelled PAA is accompanied by a sharp decrease in the ratio  $I_E/I_M$ , signalling the disruption of pyrene excimer forming sites. In the case of the doubly-labelled PAA, the extent of NRET, which monitors the increase in the naphthalene to isolated pyrene separation distance, occurs over a wide pH range (3.0 to 4.6). In contrast, the decrease in  $I_E/I_M$ , which reports on the fraction of pyrene groups located in excimer forming sites, takes place sharply at pH 4.5 which is also the polymer  $pK_a$  derived from potentiometric titrations. © 1998 Elsevier Science Ltd. All rights reserved.

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## INTRODUCTION

The applications of fluorescence and phosphorescence spectroscopy as tools to study synthetic polymers have grown in number and scope over the last decades. Originally modelled after studies in biological sciences, the techniques were applied first to study hydrophobic synthetic polymers labelled with hydrophobic dyes<sup>1</sup>. Under these circumstances it could be argued with confidence that a dye, grafted in minute amounts along a macromolecule, would not modify substantially the solution or bulk properties of the material. This assumption, however, becomes questionable when hydrophilic polymers are labelled with hydrophobic chromophores. Indeed, it was observed that pyrenyl groups, for example, undergo ground-state aggregation when linked to polyelectrolytes<sup>2</sup> or neutral water-soluble polymers<sup>3</sup>. This situation could have hampered the use of fluorescence spectroscopy to investigate these important classes of polymers. In fact, the opposite trend took place, in large part as a consequence of the discovery of a new class of water-soluble polymers, known as associating polymers or hydrophobically-modified polymers<sup>4</sup>. These are water-soluble polymers carrying a small number of long alkyl or perfluoroalkyl groups. They act as solution thickeners, highly efficient gelation or viscosification agents in a variety

of industrial fluids<sup>5</sup>. Their unique properties in water are a consequence of the intra- or interpolymeric association of the hydrophobic groups. The hydrophobic aggregates can be disrupted by various stimuli, such as changes in pH and in ionic strength, or the addition of surfactants. When fluorescent dyes are used in lieu of typical hydrophobic substituents, it is possible to monitor changes in their photophysical properties as a function of external stimuli. These can help to understand the molecular basis of the macroscopic properties of their solutions.

Poly(acrylic acid) (PAA) is often cited as the 'textbook' example for synthetic polyelectrolytes<sup>6</sup>. It has been studied in great detail and its solution properties are extremely well characterized. When the uncharged polymer is converted by titration to a chain carrying a large number of ionized groups, the mutual repulsion of the fixed charges leads to chain expansion. The forces between the charges act over relatively long distances and they are much larger than the dispersion forces or the dipole-dipole interactions between uncharged groups. The titration of PAA was examined by Mandel<sup>7</sup> who determined the intrinsic ionization constant of the polymer,  $K^\circ$ , as a function of  $\alpha_i$ , the degree of ionization of the polymer and two empirical parameters. When PAA carries hydrophobic substituents, long-range attractive interactions of the groups attached to the polyion provide powerful resistance to chain expansion. The cohesion of these side-chains, analogous to those at work in the formation of surfactant micelles, may stabilize the globular form even at high charge densities. In addition,

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interpolymeric associations may take place at higher polymer concentrations via aggregation of hydrophobic substituents.

We report here the synthesis and characterization of a series of labelled PAAs, either carrying one dye, pyrene or naphthalene, or two different chromophores capable of undergoing non-radiative energy transfer. The copolymers were prepared by polymer-analogous amidation of a single commercial PAA sample. We have chosen this post-modification approach, in view of the breadth of its scope and its applicability to polymers of industrial significance. Amidation of PAA with dye-substituted *N*-alkylamines offers a simple entry into a family of PAAs of identical molecular characteristics, but labelled with different dyes or dye mixtures. The method was applied first by Audebert and co-workers in their syntheses of hydrophobically-modified PAAs carrying octadecyl side-chains<sup>8</sup>. The same synthetic route allows the preparation of fluorescently-labelled PAAs. Another approach is to introduce the fluorescent dye by copolymerization of acrylic acid and a dye-substituted comonomer, as described by Turro and Arora<sup>9</sup> and Chu and Thomas<sup>10</sup>.

The objectives of the present study are to monitor the photophysical properties of these polymers in solution, examining specifically the influence of the dye structure, of its level of incorporation, and of the length of the alkyl chain linking the dye to the polymer main chain. Tools employed include excimer emission intensity and dynamics and non-radiative energy transfer between two chromophores attached to the same polymer backbone. These techniques were used to monitor the pH-induced uncoiling of the modified PAAs, as they are brought from an acidic to an alkaline medium. The polymer solutions were observed also by conventional techniques, such as quasi-elastic light scattering (QELS) and titrations. Data gathered from the various sources are compared in order to assess the scope of this application of fluorescence spectroscopy to monitor amphiphilic polymers.

## EXPERIMENTAL

### Materials

Poly(acrylic acid) (PAA, nominal molecular weight 150 000, 25% solution in water) was purchased from Wako Chemicals, Japan. Solid PAA obtained by freeze-drying of this solution was used in all syntheses. Water was deionized using a NANOpure water purification system. Reagent-grade solvents were used in all syntheses. 4-(1-Pyrenyl)butylamine hydrochloride was prepared as described previously<sup>11</sup>. 1-Pyrenylmethylamine hydrochloride, 1,3-dicyclohexylcarbodiimide (DCC) and anhydrous 1-methylpyrrolidone were purchased from Aldrich Chemicals Co. 1-Naphthylmethylamine was purchased from Fluka. Dowex 50W-X8, H-form, 20-50 mesh cation-exchange resin was obtained from BioRad. Membranes for dialysis with a molecular weight cutoff (MWCO) of 12 000–14 000 were obtained from Spectrapor.

### Instrumentation

Gel permeation chromatography (g.p.c.) was performed with a Waters 590 programmable h.p.l.c. system equipped with a Waters 410 differential refractometer, a u.v.-visible detector and two Ultrahydrogel columns kept at 30°C and eluted with 0.1 M aqueous NaNO<sub>3</sub> (flow rate 0.7 ml min<sup>-1</sup>). Thin-layer chromatography (TLC) was performed with

silica gel coated glass plates (silica-60 F<sub>254</sub>) obtained from Merck, eluted with acetone/methanol/triethylamine (50/50/1.4, v/v/v). Quasi-elastic light scattering (QELS) measurements were performed on a Brookhaven Instruments Corp. goniometer equipped with a BI 200SM photomultiplier and a BI 9000AT digital correlator using a Lexel 95 (25 mW) argon ion laser operating at a wavelength of 514.5 nm. Data analysis was performed with the software provided by the manufacturer. Titrations were performed on a dual pH meter and Titrimeter (Tanager Scientific Systems Inc. Model#8901). The TANA 58-2 software provided by the manufacturer was used to perform titrations under the following conditions: pH range 3–11; titrant volume 0.2–0.005 ml; time between additions of titrant and recording measurements 600 s; response time 2 s. Four replicate measurements were performed. U.v. absorption spectra were recorded with a Hewlett-Packard 8452A photodiode array spectrometer operated under the HP ChemStation Windows-based software. Steady-state fluorescence spectra were recorded on a SPEX Fluorolog 212 spectrometer equipped with a DM3000F data system. Fluorescence decay were measured on a Horiba NAES 550 time-correlated single-photon counting system consisting of an NFL-111A flash lamp filled with hydrogen to generate excitation pulses (< 2 ns, fwhm) at a frequency of about 10 kHz. Excitation and emission wavelengths were selected with a Horiba SGM-121A and a JASCO CT-10 monochromators, respectively. The decay profiles were fit to either a single- or double-exponential decay function using conventional deconvolution least squares techniques to extract lifetime components of sub-nanoseconds. The goodness of a fit was evaluated by X<sup>2</sup> and the randomness of the residuals distribution. Prior to measurements, sample solutions were deaerated by bubbling with argon for 30 min.

### Synthesis

*Poly(acrylic acid) labelled with 1-pyrenylmethylamine.* A mixture of PAA (1.0 g) in 1-methylpyrrolidone (50 ml) was placed in a 100 ml three-neck flask fitted with a magnetic stirrer, condenser and nitrogen inlet. It was heated to 60°C for 2 hr to allow the polymer to dissolve. A solution of 1-pyrenylmethylamine hydrochloride (0.0956 g, 4.17 × 10<sup>-4</sup> mol) in 1-methylpyrrolidone (5 ml) was added quickly to the resulting solution, followed by triethylamine (65 μl, 4.7 × 10<sup>-4</sup> mol) and a solution of DCC (0.0947 g, 4.59 × 10<sup>-4</sup> mol) in 1-methylpyrrolidone (5 ml). The reaction mixture was stirred at 60°C for 24 hr in the dark. At the end of this period, TLC analysis indicated the absence of unreacted amine. The reaction mixture was cooled in an ice-bath and neutralized by dropwise addition of a concentrated aqueous sodium hydroxide solution, resulting in the precipitation of the sodium salt of the labelled polymer. The solid was separated by filtration and washed three times with hot (60°C) 1-methylpyrrolidone (20 ml) and three times with cold methanol (20 ml). It was isolated by suction filtration and purified further by two consecutive precipitations of an aqueous solution into methanol. The resulting solid was dried in vacuo. It was converted to the acid form by elution through a cation-exchange column. Freeze-drying of the eluent yielded PAA-MePy/32 (200 mg) as a fluffy white solid. A second sample containing a lower amount of pyrene groups was prepared under identical conditions, starting with PAA (1.0 g), 1-pyrenylmethylamine hydrochloride (0.048 g, 2.09 × 10<sup>-4</sup> mol), triethylamine (33 μl, 2.35 × 10<sup>-4</sup> mol) and DCC (0.047 g, 2.30 × 10<sup>-4</sup> mol) and yielding PAA-MePy/73 (300 mg).

*Poly(acrylic acid) labelled with 4-(1-pyrenyl)butylamine.* The procedure described for the preparation of PAA-MePy/32 was applied starting with PAA (1.0 g), 4-(1-pyrenyl)butylamine hydrochloride (0.114 g,  $4.2 \times 10^{-4}$  mol), triethylamine ( $4.2 \times 10^{-4}$  mol) and DCC (0.0947 g,  $4.6 \times 10^{-4}$  mol). The reaction was conducted at 60°C for 48 hr in the dark. The polymer was isolated first as the sodium salt which was converted to the acidic form (pH = 5.3) by means of 0.1 N HCl. Excess salts were removed by dialysis against deionized water until the conductivity in the filtrate reached 2  $\mu$ S. Freeze-drying of the dialysate yielded PAA-BuPy/48 (130 mg) as a fluffy white solid. A sample with lower level of pyrene incorporation, PAA-BuPy/182 (180 mg), was obtained under the same conditions, starting from PAA (1.0 g), 4-(1-pyrenyl)butylamine hydrochloride (0.019 g,  $7.0 \times 10^{-5}$  mol), triethylamine ( $7.0 \times 10^{-5}$  mol) and DCC (0.017 g,  $8.1 \times 10^{-5}$  mol).

*Poly(acrylic acid) labelled with 1-naphthylmethylamine (PAA-N/84).* The procedure described above was applied, starting with PAA (1.0 g), 1-naphthylmethylamine (62  $\mu$ l,  $4.17 \times 10^{-4}$  mol) and DCC (0.095 g,  $5.6 \times 10^{-4}$  mol), yielding the labelled polymer PAA-N/84 (500 mg).

*Poly(acrylic acid) labelled with 1-pyrenylmethylamine and 1-naphthylmethylamine (PAA-Py-N).* A mixture of PAA (1.0 g) in 1-methylpyrrolidone (50 ml) was placed in a 100 ml three-neck flask fitted with a magnetic stirrer, condenser and nitrogen inlet. It was heated to 60°C for 2 hr to allow the polymer to dissolve. 1-Naphthylmethylamine (52  $\mu$ l,  $3.5 \times 10^{-4}$  mol) was introduced into the reaction mixture, followed by a solution of DCC (0.079 g,  $3.8 \times 10^{-4}$  mol) in 1-methylpyrrolidone (5 ml). The reaction mixture was kept in the dark at 60°C for 4 days. At the end of this period TLC analysis showed no unreacted 1-naphthylmethylamine. 1-Pyrenylmethylamine hydrochloride (0.0187 g,  $7.0 \times 10^{-5}$  mol) in 1-methylpyrrolidone (5 ml) was added quickly to the reaction mixture, followed by triethylamine (17  $\mu$ l,  $1.2 \times 10^{-4}$  mol) and a solution of DCC (0.017 g,  $8.2 \times 10^{-5}$  mol) in 1-methylpyrrolidone (5 ml). The reaction mixture was stirred at 60°C for 24 hr in the dark. At the end of this period, TLC analysis indicated the absence of unreacted amine. The reaction mixture was cooled in an ice-bath. The polymer was separated by precipitation into diethyl ether followed by filtration. The precipitate was washed three times with diethyl ether and dried. The solid was dissolved in the minimum amount of water and precipitated into aqueous HCl (0.5 M) in the presence of NaCl (0.5 M). The precipitated polymer was separated by centrifugation (5 min, 5000 rev min<sup>-1</sup>). The polymer was dialysed against water, until constant conductivity was reached in the dialysate (2  $\mu$ S). Freeze-drying yielded PAA-Py-N (630 mg) as a fluffy white solid.

#### Fluorescence measurements

Bandwidths were 0.3 nm (emission) and 1.3 nm (excitation). For the pyrene emission spectra the excitation wavelength was set at 344 nm and the emission range was 360 to 600 nm. Emission spectra were not corrected, except for quantum yield determinations. Excitation spectra were recorded in the ratio mode. They were measured between 250 and 370 nm with emission being monitored at 380 and 485 nm. The excimer to monomer intensity ratio ( $I_E/I_M$ ) was calculated by taking the ratio of the intensity at 485 nm to the half-sum of the emission intensities at 378 and 398 nm, in the case of PAA-BuPy/48. In the case of PAA-MePy/32,

the following wavelengths were selected: 480 nm for  $I_E$ , and 377 and 389 nm for  $I_M$ . For naphthalene spectra the excitation wavelength was set at 290 nm and the emission range was 300 to 560 nm. The excimer to monomer intensities ratio ( $I_E/I_M$ )<sup>N</sup> was calculated by taking the ratio of intensity at 396 nm to that of the intensity at 340 nm. The ratio  $I_P/I_N$  was calculated as the ratio of the half-sum of intensities at 378 and 398 nm to the intensity at 340 nm.

#### Determination of the spectroscopic parameters

Quantum yields were calculated by integration of peak areas of corrected spectra in wavenumber units, using as standard either quinine sulfate in 1 N H<sub>2</sub>SO<sub>4</sub> ( $\Phi = 0.546$ ,  $\lambda_{exc} = 328$  nm, 25°C)<sup>12</sup> or 2-aminopyridine in 0.1 N H<sub>2</sub>SO<sub>4</sub> ( $\Phi = 0.60$ ,  $\lambda_{exc} = 290$  nm, 25°C)<sup>13</sup>. Beer's law corrections were applied for optical density changes at the excitation wavelength. Corrections were made as well for refractive index differences.

#### Solutions for spectroscopic analysis

The amount of pyrene and naphthalene incorporation was calculated from u.v. absorption data of polymer solutions in methanol, using 4-(1-pyrenyl)butylamine hydrochloride ( $\epsilon_{342} = 32800$  l mol<sup>-1</sup> cm<sup>-1</sup>), 1-pyrenylmethylamine hydrochloride ( $\epsilon_{342} = 37500$  l mol<sup>-1</sup> cm<sup>-1</sup>) and 1-naphthylmethylamine ( $\epsilon_{276} = 6800$  l mol<sup>-1</sup> cm<sup>-1</sup>) as model compounds. For the doubly labelled polymer, the pyrene content was determined first; the naphthalene content was then calculated from the absorption at 290 nm, after correction for the pyrene absorption at this wavelength. For fluorescence measurements, stock solutions of polymers (0.6 g l<sup>-1</sup>) were prepared in water of pH 3.0 or 6.0, obtained by addition to deionized water of aqueous HCl (5 M) or NaOH (5 M), respectively. Polymer solutions were allowed to equilibrate for 24 hr at room temperature to ensure complete dissolution. Aqueous solutions were not degassed except for lifetime measurements and for solutions in organic solvents.

#### Titrations

A solution of a polymer aliquot (ca. 0.1 mg) in aqueous sodium nitrate (19.8 ml, 0.1 M) was brought to pH 3 by addition of aqueous HCl (0.2 ml, 0.1 N). The solution was titrated in the absence of CO<sub>2</sub> at 25°C with aqueous NaOH (0.1 M). Titration of a blank solution was performed under identical conditions to determine the amount of titrant required to neutralize excess HCl.

## RESULTS AND DISCUSSION

#### Synthesis and characterization of the labelled poly(acrylic acid)s

Attachment of the labels to the PAA backbone was achieved by reacting PAA with chromophores bearing a short amino-terminated alkyl chain. The reactions were performed in an aprotic solvent, 1-methylpyrrolidone, in the presence of 1,3-dicyclohexylcarbodiimide (DCC). Under these conditions, random attachment of the hydrophobic groups along the macromolecule is favoured over the formation of discreet blocks of contiguous chromophores<sup>8</sup>. Specifically, the polymer was labelled with pyrene by reaction with either 4-(1-pyrenyl)butylamine (BuPy) or 1-pyrenylmethylamine (MePy) and with naphthalene (N) by reaction with 1-naphthylmethylamine. Di-labelled PAAs were obtained by reaction first with 1-naphthylmethylamine,

Preparation of the labelled Poly(acrylic acids)

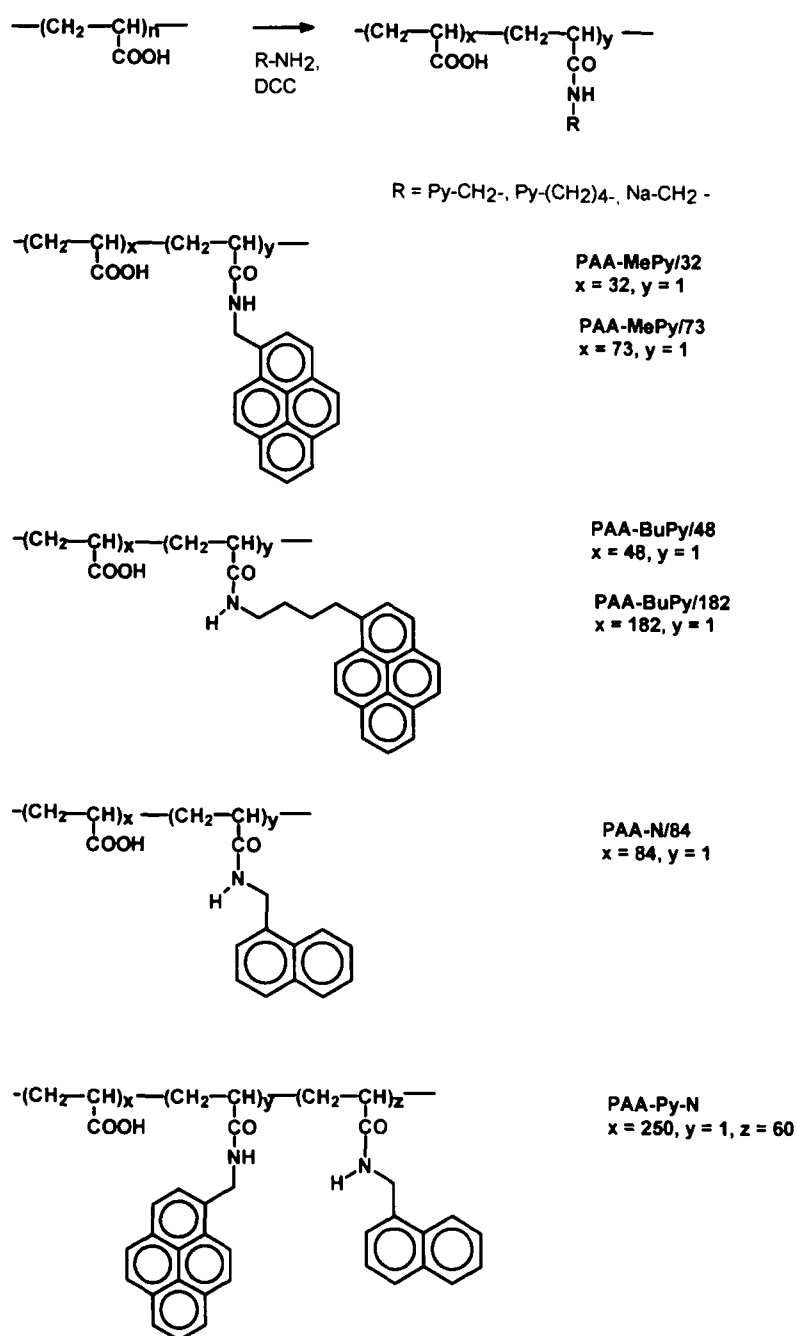


Figure 1 Synthetic scheme for the preparation of the labelled poly(acrylic acid)s and chemical structure of the labelled polymers prepared

then with 1-pyrenylmethylamine. The purified polymers were isolated in the acid form. In all, six polymer samples were prepared (Figure 1). The digits in the polymer designations refer to the average number of acrylic acid units per chromophore, e.g. PAA-MePy/32 has on average 1 pyrene group per 32 carboxylic acid groups, or approximately 60 pyrenes per macromolecule.

Through the use in tandem of a u.v. detector and a refractive index detector for the g.p.c. analysis it was ascertained that the polymers do not contain u.v.-absorbing low-molecular-weight impurities and that the chemical modification did not alter the molecular weight of the PAA. The polymers were characterized by u.v. absorption and <sup>1</sup>H

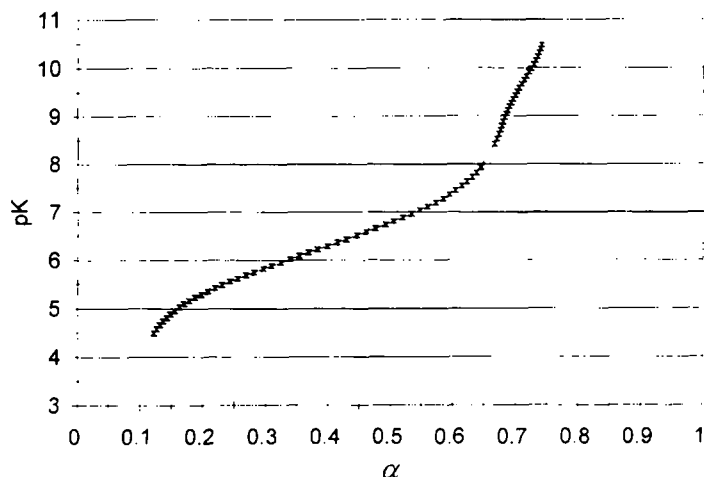
n.m.r. spectroscopy for calculation of the level of labelling (Table 1).

The effective diameter of the sample PAA-BuPy/48 was significantly smaller (10 nm) in aqueous solution of pH 3.0 than unmodified PAA (30 nm). It assumed a larger value (30 nm) in aqueous solutions of pH 6.0, signalling the expansion of the polymer chain. In the presence of salt (0.1 M NaCl), the effective diameter of PAA-BuPy/48 was much smaller (15 nm), indicating a lesser extent of polymer expansion. All the labelled polymers exhibited analogous properties.

Titration of the polymers were conducted by titrating the fully neutralized polymer with sodium hydroxide in 0.1 M

**Table 1** Physical properties of the labelled poly(acrylic acid)s

Polymer sample	Pyrene content			Naphthalene content	
	(mol%) <sup>a</sup>	(mol%) <sup>b</sup>	(mol g <sup>-1</sup> polymer) <sup>d</sup>	(mol%)	(mol g <sup>-1</sup> polymer)
PAA-MePy/73	1.4	–	$1.9 \times 10^{-4}$	–	–
PAA-MePy/32	3.1	2.5	$4.4 \times 10^{-4}$	–	–
PAA-BuPy/48	2.1	2.0	$2.9 \times 10^{-4}$	–	–
PAA-BuPy/182	0.5	0.4	$7.6 \times 10^{-5}$	–	–
PAA-MeN/84	–	–	–	1.2	$1.65 \times 10^{-4}$
PAA-Py-N	0.4	–	$5.64 \times 10^{-5}$	1.7	$2.40 \times 10^{-4}$

<sup>a</sup>From u.v. data (see text)<sup>b</sup>From <sup>1</sup>H n.m.r. data**Figure 2** Titration curve of PAA-Py-N (acidic form) in aqueous sodium nitrate (0.1 M) solution at 25°C**Table 2** Spectroscopic properties of the pyrene-labelled polymers in organic solvents

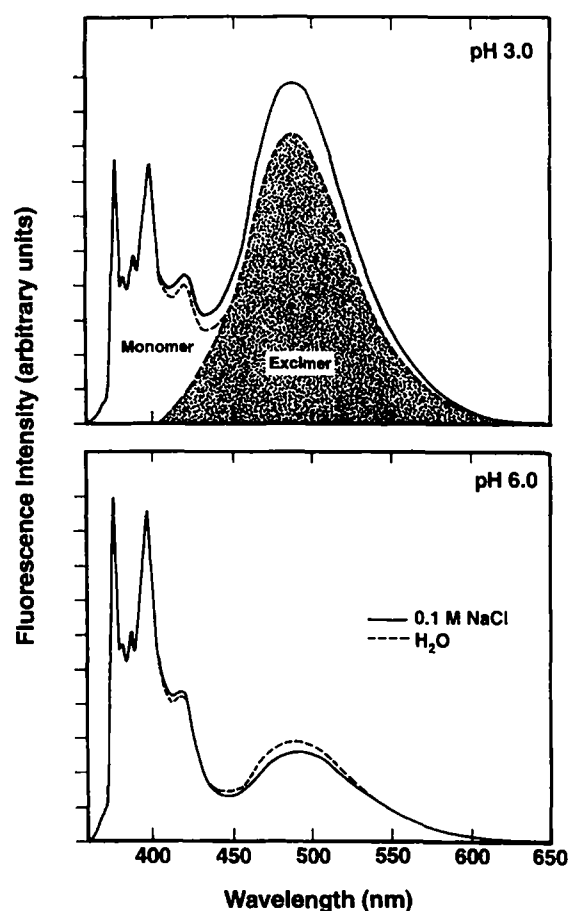
	$\lambda_{\max}$ (nm) <sup>a</sup>	$I_F/I_M$	$I_1/I_3$	$\Phi_T$	$\Phi_E$	$\Phi_M$
<i>PAA-MePy/32</i>						
Ethylene glycol	344	0.08	1.76	0.61	–	0.61
<i>N</i> -Methylpyrrolidone	342	0.07	2.12	–	–	–
Dioxane	344	0.20	1.68	0.60	0.07	0.57
<i>N,N</i> -Dimethylformamide	346	0.15	2.08	0.55	0.08	0.47
Tetrahydrofuran	344	0.44	1.62	0.51	0.18	0.33
Methanol	342	0.54	1.51	0.34	0.15	0.19
<i>PAA-BuPy/48</i>						
Ethylene glycol	344	0.07	–	0.62	–	0.62
<i>N</i> -Methylpyrrolidone	342	0.12	–	–	–	–
Dioxane	344	0.20	–	0.60	0.12	0.48
<i>N,N</i> -Dimethylformamide	344	0.31	–	0.50	0.16	0.34
Tetrahydrofuran	344	0.38	–	0.57	0.21	0.36
Methanol	342	0.87	–	0.28	0.18	0.10
<i>PAA-BuPy/182</i>						
Methanol	342	0.13	–	–	–	–

<sup>a</sup>Wavelength of the pyrene <sup>1</sup>L<sub>2</sub> transition in the absorption spectrum

NaNO<sub>3</sub>. A titration curve obtained by titration of PAA-Py-N with sodium hydroxide in 0.1 M NaNO<sub>3</sub> is given in Figure 2 as a plot of pK versus  $\alpha$ , the degree of ionization of the polymer<sup>7</sup>. The pK<sub>a</sub> values were 4.5 for PAA-Py-N and PAA-MePy/74. These values were in good agreement with those reported for PAA, 4.8 (water) and 4.5 (0.1 M NaCl)<sup>7</sup>.

#### Photophysical properties of the labelled polymers

*Pyrene-labelled polymers.* Emission and excitation spectra of the pyrene-labelled polymers were measured in several organic solvents. In all situations, the polymers show an emission due to locally excited pyrene ('monomer' emission, intensity  $I_M$ ) with the (0,0) band located at 378 nm



**Figure 3** Emission spectra of PAA-MePy/32 in water and in 0.1 M aqueous NaCl, pH 3 (top) and pH 6 (bottom)

and a broad emission centred at 485 nm due to pyrene excimer (intensity  $I_E$ ). The excimer emission intensity increases with decreasing solvent viscosity, at the expense of the monomer emission (Table 2). A plot of the changes in  $I_E/I_M$  as a function of the inverse viscosity follows a linear relationship, as anticipated for dynamic excimer formation following the mechanism originally proposed by Birks<sup>14</sup>. In the case of the polymer bearing 1-pyrenylmethyl

substituents, the monomer emission fine structure is sensitive to the polarity of the chromophore environment. As in the case of pyrene<sup>15</sup>, the ratio  $I_1/I_3$  of the intensity of the (0,0) band to the (0,3) band decreases with decreasing polarity of the solvent (Table 2). This sensitivity is lost in the case of the 4-(1-pyrenyl)butyl substituent.

The emission spectra of the polymers were recorded in aqueous media of pH 3.0 and 6.0, in the absence of added electrolytes and at constant ionic strength, 0.1 M NaCl (Figure 3). The fluorescence quantum yields of the pyrene group are significantly lower for aqueous polymer solutions of pH 3, compared to those measured in all organic media (Tables 2 and 3), suggesting extensive self-quenching of the pyrene emission<sup>16</sup>. Also, the Py emission presents an increased contribution from the excimer, compared to the emission of the respective polymers in organic solvents (Table 3). Moreover, when recording the excitation spectra of PAA-Py solutions in water, we observed that the spectra monitoring the monomer emissions were different from those recorded for the excimer emissions. Their general features are similar, but the former is blue-shifted by about 3 nm. Comparison with the u.v. spectra of aqueous solutions of the polymers reveals that it is the excitation spectrum for the excimer that corresponds to the u.v. absorption spectrum. All these features led us to conclude that the pyrene groups exist predominantly in the form of aggregates in solutions of pH 3, where the polymers are fully protonated<sup>17</sup>. The formation of Py aggregates is driven by hydrophobic association within the polymer coils which are expected to assume a compact conformation.

The occurrence of pyrene aggregates can also account for the results of the time-dependent measurements described next and summarized in Table 4. The most compelling data are the excimer decay profiles, which exhibit no rising component in the nanosecond time-scale of our measurements (Figure 4). This result implies either that all but a very small fraction of the excimer emission arises from ground-state aggregates of pyrene or that the excimers form faster than resolution (1–2 ns) of the measurement. The excimer decay for PAA-BuPy/48 in water can be fitted reasonably well to a single-exponential curve for solutions at pH 2.9 and 6.3, the excimer lifetime being slightly shorter for solutions of pH 6.3. We observed, however, that this was

**Table 3** Spectroscopic properties of the pyrene-labelled polymers in aqueous solutions

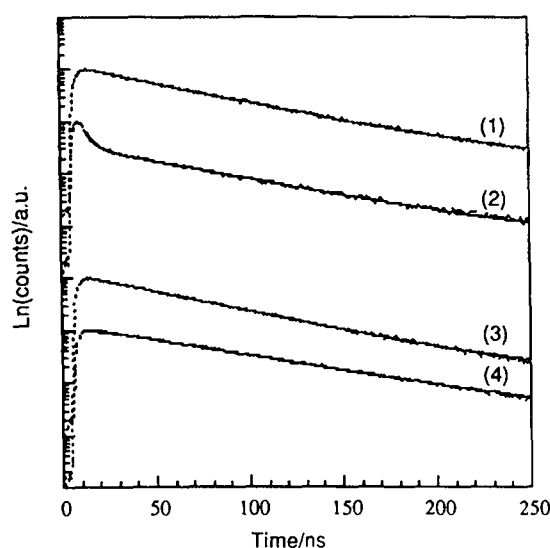
	$\lambda_{\max}$ (nm) <sup>a</sup>	$I_E/I_M$	$I_1/I_3$	$\Phi_T$	$\Phi_E$	$\Phi_M$
<i>PAA-MePy/32</i>						
pH 3	346	1.21	1.63	0.19	0.12	0.07
pH 3, 0.1 M NaCl	346	1.32	1.57	0.27	0.18	0.09
pH 6	342	0.30	1.67	0.21	0.08	0.13
pH 6, 0.1 M NaCl	342	0.28	1.70	0.28	0.10	0.18
<i>PAA-BuPy/48</i>						
pH 3	346	0.52	–	0.24	0.13	0.11
pH 3, 0.1 M NaCl	346	0.55	–	0.33	0.20	0.13
pH 6	344	0.22	–	0.32	0.08	0.24
pH 6, 0.1 M NaCl	342	0.20	–	0.27	0.06	0.21
<i>PAA-BuPy/182</i>						
pH 3	346	0.08	–	–	–	–
pH 6	344	0.05	–	–	–	–

<sup>a</sup>Wavelength of the pyrene <sup>1</sup>L<sub>a</sub> transition in the absorption spectrum

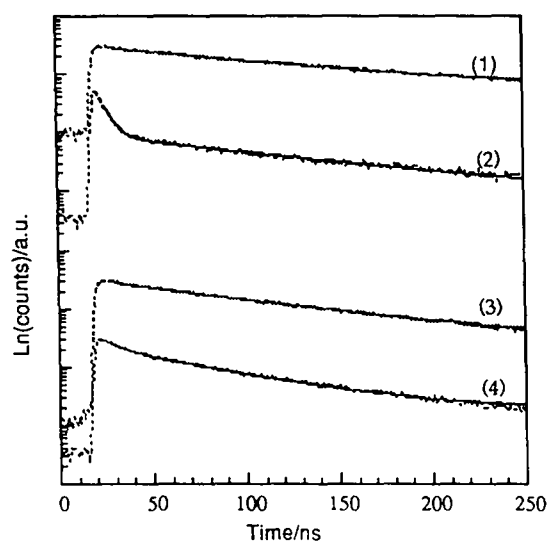
**Table 4** Pyrene monomer and excimer emission decay data for PAA–MePy/32 and PAA–BuPy/48 in water<sup>a</sup>

	$\lambda_{em}$ (nm)	$\tau_1$ (ns)	$a_1$	$\tau_2$ (ns)	$a_2$	$\chi^2$	$\langle \tau \rangle$ (ns)
<i>PAA–MePy/32</i>							
pH 2.9	396	5.10	0.236	112	0.764	1.61	86.8
	480	4.28	0.156	64.7	0.844	1.44	64.0
pH 6.3	396	117	1.000	–	–	1.15	117
	480	36.4	0.183	63.7	0.817	1.37	60.6
<i>PAA–BuPy/48</i>							
pH 2.9	396	7.06	0.060	59.4	0.940	1.83	59.0
	480	71.7	1.000	–	–	1.39	71.7
pH 6.3	396	88.8	1.000	–	–	1.44	88.8
	480	56.4	1.000	–	–	1.39	56.4

<sup>a</sup>Intensities were fit to a sum of exponentials:  $I(t) = \sum a_i \exp(-t/\tau_i)$ ; the angular brackets indicate mean lifetime data



**Figure 4** Pyrene excimer fluorescence decay curves for solutions of PAA–MePy/32 of pH 6 (curve 1) and pH 3 (curve 2) and for solutions of PAA–BuPy/48 of pH 6 (curve 3) and pH 3 (curve 4); polymer concentration  $0.1 \text{ g l}^{-1}$ ;  $\lambda_{em}$  480 nm



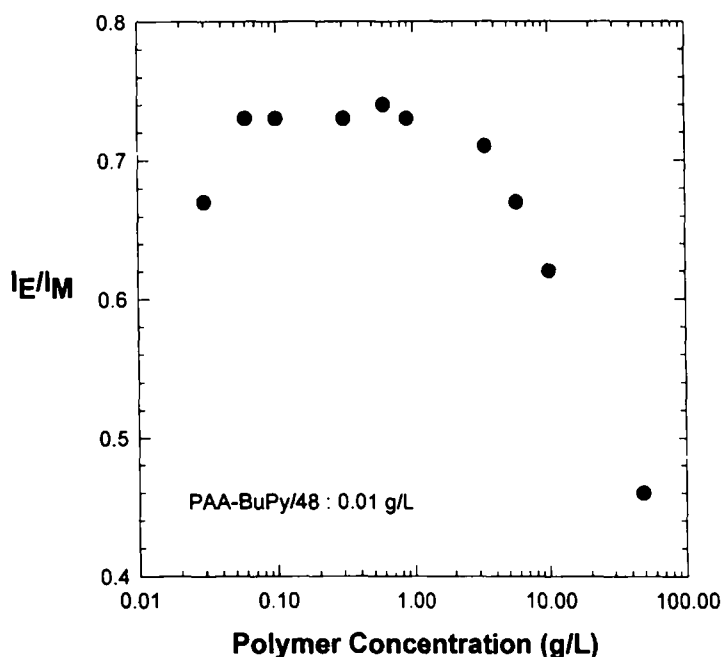
**Figure 5** Pyrene monomer fluorescence decay curves for solutions of PAA–MePy/32 of pH 6 (curve 1) and pH 3 (curve 2) and for solutions of PAA–BuPy/48 of pH 6 (curve 3) and pH 3 (curve 4); polymer concentration  $0.1 \text{ g l}^{-1}$ ;  $\lambda_{em}$  396 nm

not the case for solutions of PAA–MePy/32, the sample in which the chromophore is linked to the polymer main chain by one methylene unit only, not four as is the case in PAA–BuPy/48. Thus, in the case of solutions of PAA–MePy/32 of pH 2.9, the excimer decay profile exhibits two very well resolved components: a short component (4.3 ns, 15% of the total intensity) and a longer component (64.7 ns, 85% of the total intensity) (Figure 4). The monomer decay profile also displays a short-lived component (5.1 ns, 23% of the total intensity) when the polymer is fully protonated (pH 2.9), but the short-lived component disappears completely in the emission of PAA–MePy/32 of pH 6 (Figure 5). This striking difference between the two polymers in solutions of pH 3 and 6 may be accounted for by a difference in the degree of freedom of the local motion of pyrene, depending on the length and flexibility of the spacer group. As there is no rising component in the excimer time-resolved spectrum, the short-lived component in the monomer decay has to be attributed to an  $S_1 \rightarrow S_0$  transition, an electronically weak transition. This pathway results in a shortening of the excited-state lifetime and, consequently, this emission has a lower quantum yield, corroborating the steady-state measurements. The methylene linker in PAA–MePy/32 confines the pyrene groups to a more constraining

microenvironment within the collapsed polymer coil than the butyl chain in PAA–BuPy/48. This effect is amplified in the more acidic solutions.

In order to ascertain that the pyrene aggregates form predominantly among chromophores attached to a single macromolecule, we performed a series of concentration studies. First, we determined  $I_E/I_M$  over a labelled polymer concentration range as wide as possible (5 to 100 ppm). The ratio remained constant over the entire range for solutions of all polymers of pH 2.9 and 6, implying exclusive intrapolymeric micelle formation. Next, we performed a set of competition experiments in which the labelled polymers were mixed with unlabelled PAA. The total labelled polymer concentration was kept constant (10 ppm) and the amount of added PAA was increased from 0 to  $50 \text{ g l}^{-1}$ . As depicted in Figure 6 for PAA–BuPy/48 ( $0.01 \text{ g l}^{-1}$ ) in PAA, the ratio  $I_E/I_M$  remained unaffected until the unlabelled polymer concentration reached a value of approximately  $2 \text{ g l}^{-1}$ . Then the contribution from pyrene excimer emission decreased, signalling the transition from the dilute to the semi-dilute regime characterized by interpolymeric overlap.

*Naphthyl-labelled polymers.* The photophysical properties of a naphthyl-labelled poly(acrylic acid), PAA–N/84,



**Figure 6** Semilogarithmic plot of the ratio of excimer to monomer emission intensities ( $I_E/I_M$ ) for aqueous solutions of PAA-BuPy/48 ( $0.01 \text{ g l}^{-1}$ ) as a function of poly(acrylic acid) concentration

offer the same qualitative features as the pyrenyl-labelled samples. Upon excitation at 290 nm, solutions of the polymer in organic solvents exhibit spectra consisting of two overlapping bands: the major component is a structured band at short wavelengths ( $\lambda_{\text{max}} = 340 \text{ nm}$ ) assigned to naphthalene monomer emission; the minor contribution ascribed to naphthalene excimer emission is a broad, structureless emission centred at  $\lambda_{\text{em}} = 396 \text{ nm}$  ( $(I_E/I_M)^N = 0.13$  in the case of methanolic solutions). The emission of PAA-N/84 in acidic aqueous solutions (pH 3.0) is characterized by a stronger excimer contribution compared to the emission of the polymer in organic solvents.

**Doubly-labelled polymer.** This polymer was prepared to enable the study of the solutions properties of amphiphilic PAAs by the technique of non-radiative energy transfer (NRET). The NRET process originates in dipole-dipole interactions between an energy donor in its excited state and an energy acceptor in its ground state<sup>17,18</sup>. The probability of energy transfer between two chromophores depends sensitively on their separation distance and to a lesser extent on their relative orientation. Naphthalene and pyrene are known to interact as energy donor (N) and energy acceptor (Py) in a non-radiative energy transfer process with a characteristic distance,  $R_0$ , of 29 Å.<sup>†</sup> Therefore, in solutions of the doubly-labelled polymer, PAA-Py-N, if the inter-chromophore separation is within the range of

<sup>†</sup> The characteristic distance is defined in the context of the Förster mechanism of energy transfer as the donor-acceptor separation distance for which the energy transfer efficiency  $E$  is 50% ( $E = R_0^6/(R_0^6 + R^6)$ ) (see<sup>11</sup>).

<sup>‡</sup> Note that in addition there is a weak emission from directly excited Py, since this chromophore has a weak absorbance at 290 nm. This contribution to the spectrum will be taken into account where required. There is one additional concern, if one attempts to draw quantitative information from the NRET experiments: it was observed that the spectra of solutions of the singly-labelled polymer, PAA-N/84, display a significant contribution from naphthalene excimer emission. Unfortunately, in the case of the di-labelled substance this excimer emission (360 to 400 nm) is obscured by the strong Py monomer emission resulting from NRET. Thus, it will not be possible to ascertain whether excitation of naphthalene leads to N excimer formation, in addition to monomer emission.

NRET, excitation at 290 nm will result in emissions from  $N^*$  ( $\lambda_{\text{em}} 310$  to 400 nm) and from  $Py^*$  ( $\lambda_{\text{em}} > 380 \text{ nm}$ ) excited by energy transfer from  $N^*$ .<sup>‡</sup> Excitation at 343 nm detects the emission from directly excited pyrene, with no contribution from naphthalene, since only Py absorbs light of this wavelength.

Figure 7 shows the emission spectra of PAA-Py-N upon excitation at 290 nm in DMF (top) and in aqueous NaCl (0.1 M) at pH 3.0 and 6.0 (bottom). The polymer concentration is the same ( $0.10 \text{ g l}^{-1}$ ) in the three solutions. All spectra exhibit a contribution from  $N^*$  (300–360 nm) and a contribution from  $Py^*$  (360–450 nm). A qualitative measure of the relative extent of energy transfer can be obtained by taking the ratio  $I_{Py}/I_N$  of the intensity of the emission at 390 nm to that of the emission at 340 nm (see Figure 7). In this scale, a larger value reflects an increase in the efficiency of NRET. The largest value was recorded for aqueous solutions of the fully-protonated polymer ( $I_{Py}/I_N = 14$ , 0.1 M NaCl). It was much lower for solutions of the polymer, either at neutral pH ( $I_{Py}/I_N = 4.1$ , 0.1 M NaCl) or in DMF ( $I_{Py}/I_N = 5.8$ ). These values confirm that the polymer adopts a rather open conformation in neutral solution or in DMF and that the polymer coil collapses in acidic pH, rendering the process of non-radiative energy transfer more efficient.

To our surprise, this trend was not reflected by fluorescence decay measurements carried out in aqueous solutions of PAA-Py-N of pH 3.0 and 7.0 (Table 5). The lifetimes of  $N^*$  were quite similar in the two solutions. In either case the decay data were fitted to a double-exponential decay, with a minor short-lived component (ca. 9 ns) and a longer-lived component (ca. 33 ns). The time-dependent profile of  $Py^*$  did not display the expected rising component, but only a decay component with lifetimes of 122 and 110 ns at pH 3.0 and 7.0, respectively. These data, together with the steady-state results, suggest that a large fraction of the Py and N chromophores are located in such close proximity that the NRET takes place on a time-scale too fast to detect with the instrumentation employed. The longer lifetime of Py in solutions of the protonated polymer, compared to those of the partially



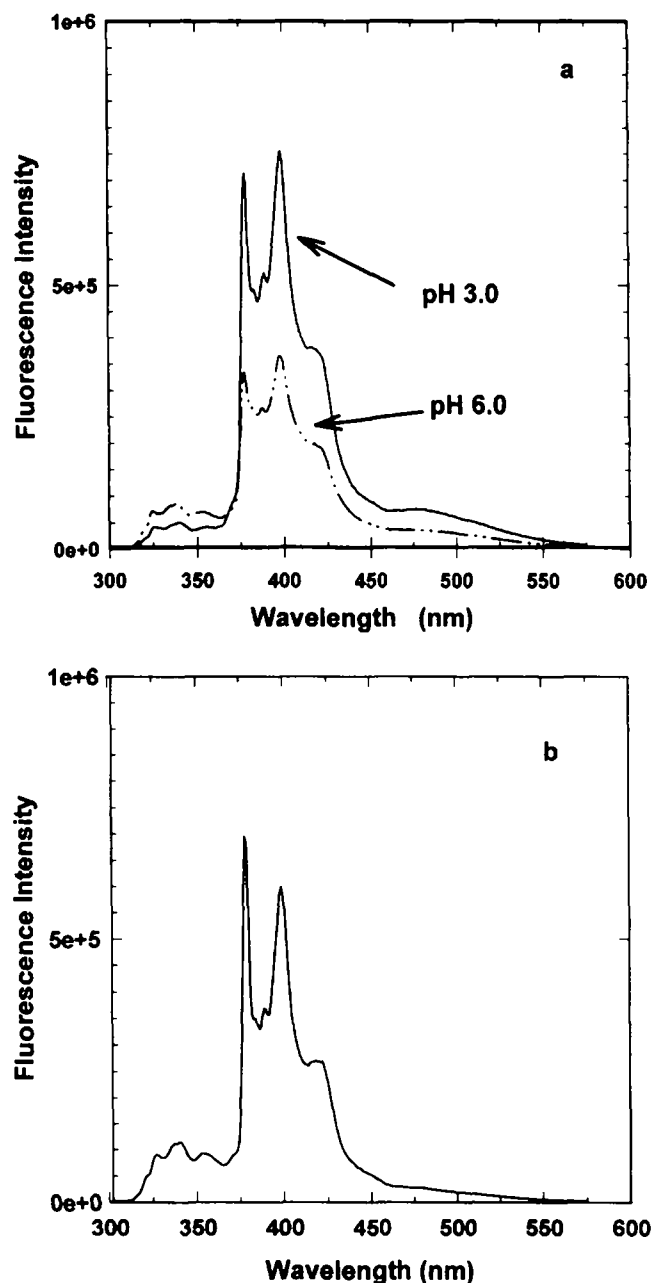
**Table 5** Naphthalene and pyrene monomer emission decay data for PAA-Py-N<sup>a</sup>

	$\lambda_{em}$ (nm)	$\tau_1$ (ns)	$a_1$	$\tau_2$ (ns)	$a_2$	$\chi^2$	$\langle \tau \rangle$ (ns)
pH 3.0	339 <sup>b</sup>	9.24	0.173	34.6	0.827	1.33	30.2
	397 <sup>c</sup>	122	1.000	-	-	1.14	
pH 7.0	339 <sup>b</sup>	10.1	0.091	33.3	0.909	1.68	31.2
	397 <sup>c</sup>	110	1.000	-	-	1.41	

<sup>a</sup> Intensities were fit to a sum of exponentials:  $I(t) = \sum a_i \exp(-t/\tau_i)$ ; the angular brackets indicate mean lifetime data

<sup>b</sup> Naphthalene emission

<sup>c</sup> Pyrene monomer emission



**Figure 7** Fluorescence spectra of the terpolymer PAA-Py-N in: (a) 0.1 M aqueous NaCl, pH 3.0 and 6.0; (b) dimethylformamide. The polymer concentration ( $0.1 \text{ g l}^{-1}$ ) is the same in the three solutions;  $\lambda_{exc} = 290 \text{ nm}$

neutralized sample, is further evidence that most Py are entrapped in the collapsed chain.

#### Photophysical tools as a measure of the pH-induced coil expansion of labelled poly(acrylic acids)

*Singly-labelled polymers.* Spectra of aqueous solutions of PAA-N/84 and PAA-MePy/32 were measured over the

pH range 3 to 12 in water and in 0.1 M NaCl. The ratio  $(I_E/I_M)^N$  increases slightly between pH 3.0 and 4.0 to reach a maximum value of 0.55. The small increase in  $(I_E/I_M)^N$  in acidic medium corresponds to a decrease in monomer emission intensity, while the excimer emission seems unaffected. The ratio  $I_E/I_M$  decreases markedly in solutions of higher pH than 4.0, to reach a constant value in solutions of pH larger than 5.5. Corresponding trends were exhibited by the pyrene-labelled samples investigated in this study, in agreement with results obtained by Turro and Arora in their study of Py-labelled PAA obtained by copolymerization<sup>9</sup>. The increase in pyrene monomer emission at the expense of pyrene excimer emission corresponds to polymer chain expansion. The mid-point of the transitions are listed in Table 6. They correspond remarkably well to the  $pK_a$  of the polymers determined by standard titration (data not shown).

*Doubly-labelled polymers.* Steady-state fluorescence spectra of PAA-Py-N were monitored as a function of pH. A decrease in the N\* to Py energy transfer efficiency was observed as the polymer solution was brought from an acidic to an alkaline pH. An increase of the naphthalene emission intensity takes place, concomitant with a decrease in the intensity of pyrene monomer emission. Fluorescence spectra resulting from excitation at 346 nm were recorded as well, to monitor the pH-induced changes in the emission of directly excited pyrene. As in the case of PAA-MePy/32 discussed previously, the excimer emission decreased sharply in the pH range 4 to 5, in which the polymer chain expansion occurs.

There is an intriguing aspect of the photophysical properties of the doubly-labelled polymer that merits further discussion. The changes in energy transfer efficiency as a function of pH do not occur within the pH range surrounding the  $pK_a$  of the polymer, as do the changes in  $I_E/I_M$  and  $(I_E/I_M)^N$ , but they take place at lower pH values and over a much wider range. This trend is visualized in Figure 8, where we plot as a function of pH the changes in  $I_P/I_N$  and in  $I_E/I_M$  exhibited by solutions of PAA-Py-N, excited at 290 and 343 nm, respectively. The transition in the curve representing the variations in  $I_E/I_M$  is sharp and its mid-point (pH 4.7) corresponds to the  $pK_a$  determined by titration (Figure 2). This may suggest that only a small number of excimer forming sites exist in the doubly-labelled polymer ( $I_E/I_M = 0.07$ ). These sites are stabilized by hydrophobic interactions. They do not dissociate until the polymer chain has expanded to a certain size, then dissociation occurs in a cooperative manner. The NRET phenomena occur predominantly between naphthalene and isolated pyrene sites. Thus they report on the properties of the majority of the Py groups of the polymer chain. The transition in the curve representing  $I_P/I_N$  (mid-point pH 3.8) is diffuse and resembles a typical titration of PAA. The

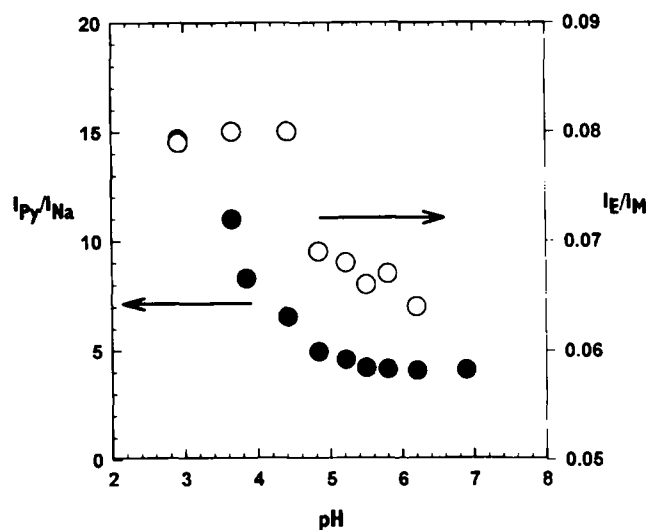
**Table 6**  $pK_a$  values of the labelled polymers evaluated from changes in photophysical parameters

Polymer	Solvent <sup>d</sup>	Fluorescence	Fluorescence
		data	data
		$I_E/I_M$	$I_{Py}/I_N$
PAA-BuPy/48	Water	6.1	—
PAA-BuPy/182	Water	5.7	—
PAA-MePy/32	Water	5.7	—
PAA-MePy/73	Water	5.2	—
	0.1 M NaX	4.9	—
PAA-N/84	Water	5.2	—
PAA-N-Py	0.1 M NaX	4.7	3.8 <sup>b</sup> , 4.7 <sup>c</sup>

<sup>a</sup>In titration measurements, X = NO<sub>3</sub><sup>-</sup>; in fluorescence measurements, X = Cl<sup>-</sup>

<sup>b</sup>From the midpoint of the transition curve

<sup>c</sup>From the end-point of the curve (see text)



**Figure 8** Plots of the ratio of pyrene to naphthalene emission intensities ( $I_{Py}/I_N$ ) and of the ratio of pyrene excimer to monomer emission intensities ( $I_E/I_M$ ) for 0.1 M NaCl aqueous solution of PAA-Py-N as a function of solution pH. Polymer concentration  $0.1 \text{ g l}^{-1}$ ;  $\lambda_{exc} = 290 \text{ nm}$  (for  $I_{Py}/I_N$ ) and  $\lambda_{exc} = 343 \text{ nm}$  (for  $I_E/I_M$ )

levelling-off pH (pH 4.7) of the  $I_{Py}/I_N$  curve is identical to the mid-point in the  $I_E/I_M$  transition (Table 6) and correlates reasonably well with the  $pK_a$  of the doubly-labelled polymer determined by potentiometric titration. This result indicates that the overall conformational reorganization takes place over a wide pH range. When the solution reaches the critical pH corresponding to the sharp transition of  $I_E/I_M$ , the electrostatic repulsive forces between the carboxylate substituent overcome the hydrophobic interactions that keep a small number of hydrophobic groups in close proximity, a thermodynamically unfavourable situation.

## CONCLUSIONS

The present work shows that fluorescently-labelled

poly(acrylic acid)s undergo structural changes with pH that have a profound effect on the environment of the fluorescent groups. The changes in the dyes' photophysical properties can be interpreted on the basis of the well-known unwinding of the polymer chains with increasing pH. This lends support to the insights that can be gained from this study to the understanding of the properties of hydrophobically-modified polymers. Particularly powerful are the experiments performed with a doubly-labelled polymer, where two different techniques can monitor phenomena involving different populations of chromophores.

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