Fluorescence depolarization and quenching studies of acenaphthalene-labelled poly(acrylamide) in water*

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Acenaphthalene was copolymerized with acrylamide in water to form poly(acrylamide-*co*-acenaphthalene) [P(AAm-*co*-ACE)] with molecular weight $\approx 2 \times 10^6$ g mol^{-1}. A variety of fluorescence experiments were carried out at 0.8 wt% polymer concentration. Time-resolved fluorescence depolarization measurements gave a mean rotational correlation time for backbone motion of 30 ns. Time-resolved and steady-state fluorescence quenching experiments were carried out using acrylamide as the quencher. In the case of polymer-bound chromophore, we were able to follow the transient effect in the diffusion-controlled quenching process. The mutual diffusion coefficient D characterizing this reaction is a factor of six slower than the self-diffusion coefficient $D_{AAm} = 9.15 \times 10^{-6}$ cm^2 s^{-1} for acrylamide in water, measured by pulsed gradient n.m.r. We interpret this result to mean that acrylamide diffusion is significantly slower inside the P(AAm) coil than it is in bulk water.

(Keywords: depolarization; fluorescence; poly(acrylamide))

INTRODUCTION

Polymerization of acrylamide (AAm) in water occurs rapidly to yield high molecular weight polymer. Many factors that affect this polymerization are only poorly understood. In addition, several unusual observations about this polymerization have never adequately been explained. In order to gain further insight into these issues, we prepared polyacrylamide (P(AAm)) labelled with a fluorescent dye and studied this polymer in water with a variety of fluorescent techniques. The P(AAm) was copolymerized with acenaphthalene (ACE), to form P(AAm-*co*-ACE) containing 1 to 10 ACE groups per 10^6 molecular weight. Fluorescence depolarization experiments were carried out as a means of estimating backbone mobility in this polymer.

Of particular interest was the susceptibility of the naphthalene unit in the copolymer to fluorescence quenching by AAm[1-3]. This quenching normally occurs under diffusion control. The kinetics of this process for the polymer-bound chromophore provides information about the interaction of AAm with preformed P(AAm), which is an important issue in the polymerization process. In fact this quenching was found to be slower than anticipated. To gain further insights into the problem, the diffusion coefficient of AAm in water was measured directly by pulsed gradient spin-echo (p.g.s.e.) n.m.r. Fluorescence studies were restricted to polymer concentrations at the low end of the semidilute regime, where most measurements of fundamental kinetics are performed. In principle, however, there is no reason why the basic methods used here cannot be applied to gain information at higher concentrations.

ACE monomer was chosen as the fluorescent label for a number of reasons, including: (i) ACE is readily quenched by AAm; (ii) the reactivity ratios for AAm and ACE ($r_1 = 0.033$ and $r_2 = 0.086$, approximated using Q and e values[4]) favour random copolymer production; (iii) ACE did not severely reduce polymer molecular weight through chain transfer as is usually the case for such labelling reactions[5]; and (iv) copolymerized ACE has the additional advantage of being bonded in such a manner that motion independent of the polymer chain is not possible. Kettle and Soutar[6] have already elaborated on the importance of this last point for fluorescence depolarization studies.

The initial concern of this study was the so-called instability effect of aqueous P(AAm), which has been extensively reviewed by Kulicke and co-workers[7]. This effect is reported to manifest itself over a period of weeks as a decrease in hydrodynamic volume V_H of P(AAm) whose molecular weight exceeds 1.5×10^6 g mol^{-1}.

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Table 1 Recipes and properties of P(AAm-*co*-ACE) samples

Sample	[AAm] (wt%)	[Initiator] (M)	[Bound ACE][a] (M)	[Bound ACE][a] (units per chain)	$M_w \times 10^{-6}$ ($g\,mol^{-1}$)
ACE2	8	4×10^{-2}	3×10^{-5}	9	0.92
ACE3	20	4×10^{-2}	2×10^{-5}	0.5	0.51
ACE4	50	4×10^{-2}	3×10^{-6}	0.6	1.4_5
ACE5	50	1×10^{-2}	7×10^{-6}	0.7	1.9
ACE6	60	5×10^{-3}	5×10^{-5}	13	2.1
ACE7	60	5×10^{-3}	0	0	2.1
ACE10	60	5×10^{-3}	2×10^{-5}	8	2.1
ACE11	60	5×10^{-3}	1×10^{-5}	4	2.1

[a] It was assumed that the value of the molar absorption coefficient for aqueous P(AAm-*co*-ACE) was equivalent to that of aqueous ACET, measured by u.v.-visible spectroscopy to be 6500 $M^{-1}\,cm^{-1}$

Obviously such instability in the polymer coil conformation cannot be ignored when attempting to determine the segmental motion of the polymer backbone. Hence the first task in this study was to investigate the severity of this effect, as a function of time, within the molecular weight and concentration range used here. This was done by monitoring the relative change in V_H using size exclusion chromatography (s.e.c.) and the degree of segmental mobility using steady-state fluorescence depolarization.

EXPERIMENTAL

Reagents and synthesis

ACE (Aldrich, 85% and 15%) was separated from its byproduct, acenaphthene (ACET), by column chromatography, using silica gel (70–230 mesh, 60 Å) as support and a 4:1 ratio of hexane to ethyl acetate as solvent. The separated product, ACE, and ACET (Aldrich, 99%) were recrystallized from a 4:1 ratio of hexane to ethyl acetate and the AAm (Aldrich, >99%) was recrystallized from ethyl acetate. Aqueous hydrogen peroxide (Aldrich, 30%) was used as received. Distilled water, further purified by passing through a Milli-Q filter system (Millipore), was employed throughout.

Prior to polymerization, the reaction solutions were subject to filtration using an aqueous filter (Millipore, Millex-HA, 0.45 μm). Polymerizations were initiated with hydrogen peroxide, and the reaction solutions subsequently maintained at 35°C with an excess of ACE crystals and high purity argon gas bubbling to eliminate oxygen. Once these solutions began to thicken, they were transferred to dialysis tubing (Spectra/Por Membrane 7 [MWCO 50 000]). Dialysis of the polymer product was ultimately carried out over a period of 1 week; however, samples were withdrawn from dialysis after about 1 day for the coil stability study. The cleaned polymer typically had a solids content of 0.8%. The recipes and particular properties of each polymer product are listed in *Table 1*.

Proof that the ACE had copolymerized was given by: (i) the parallel size-exclusion chromatograms traced from a refractive index (r.i.) and a u.v. detector (set at 290 nm) (*Figure 1*); and (ii) a u.v. peak absorption shift of ACE from 322 nm (unreacted) to 290 nm (polymerized). The fluorescence excitation and emission spectra of P(AAm-*co*-ACE) are shown in *Figure 2*. Absence of a peak around 450 nm in the emission spectrum indicates that there was no excimer formation (i.e. transient excited

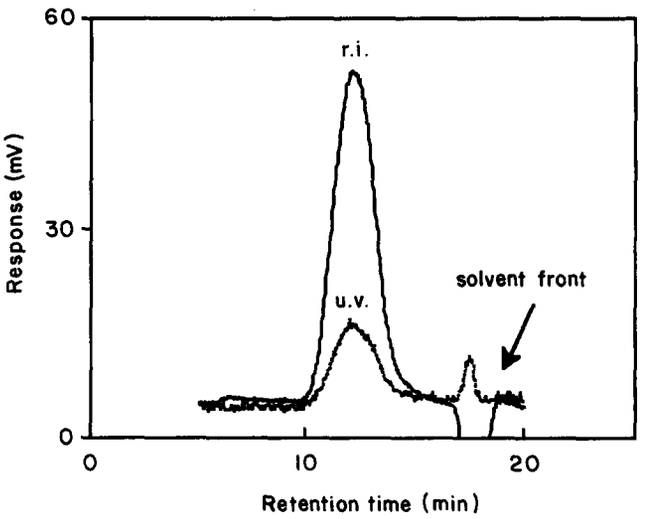

Figure 1 Example of a size-exclusion chromatogram of P(AAm-*co*-ACE) (sample ACE6) monitored by an r.i. (——) detector and a u.v. (···) detector

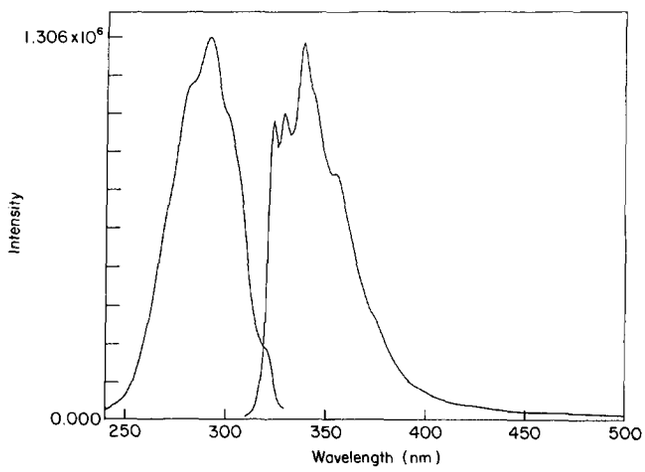

Figure 2 Examples of fluorescence excitation (monitored at 339 nm) and emission (excited at 290 nm) spectra of P(AAm-*co*-ACE) (sample ACE6)

dimers). Moreover, intramolecular association between naphthalene units was ruled out since polymer solutions containing on average less than one ACE unit per polymer chain displayed equivalent anisotropy to that containing an average of 13 ACE units per polymer chain.

Methods

Polymer molecular weights and relative values of V_H were determined by s.e.c. at ambient temperature on a Waters M-6000A chromatograph equipped with a linear-ultrahydrogel column (Waters), an R401 refractive index detector (Waters) and a UV-50 detector (Varian). Poly(ethylene oxide) (PEO) standards (Pressure Chemicals) were employed in conjunction with the universal calibration procedure, using Mark–Houwink parameters: at 25°C, for PEO[8], $K=17\times10^{-3}$ cm^3 g^{-1}, $\alpha=0.75$; for P(AAm)[9], $K=4.9\times10^{-3}$ cm^3 g^{-1}, $\alpha=0.80$.

Steady-state intensity measurements were performed at ambient temperatures on a SPEX DM3000 spectrofluorimeter, fitted with a single and a double monochromator at the excitation and emission ends, respectively. Because naphthalene displays self-absorption of its fluorescence radiation (due to its small Stokes loss), the optical density of all samples[10] (for a 1 cm pathlength) was kept below 0.15. All samples were de-oxygenated in 1 cm × 1 cm quartz sample cells by 15–30 min of high-purity argon gas bubbling.

Steady-state depolarization experiments were carried out with a crystal polarizer (32% transmittance at 290 nm) and a polymer film polarizer (20% transmittance at 339 nm) at the excitation and emission positions, respectively. The fluorescence intensity was represented as an average of emission intensity between wavelengths 329 and 349 nm. The excitation wavelength was set at 290 nm. Confidence in the instrumental performance for polarization studies was tested by measurement of the polarization value p (defined in equation (3)) for fluorescein (Aldrich, 98%) in water (10^{-5} mol dm^{-3}, pH 7 adjusted with NaOH, excitation $\lambda=470$ nm and emission $\lambda=516$ nm). The result was $p=0.019\pm5\%$, which agrees adequately with the literature (e.g. 0.017 and 0.018 measured by Soutar and co-workers[6,11]).

For the steady-state quenching study, the excitation wavelength was 290 nm and the emission monitored at 339 nm. Correction of fluorescence intensity was necessary for this study since the quencher, AAm, absorbs at the excitation wavelength, 290 nm. The standard Beer's law correction factor was used for emission intensity:

$$c=\left(1+\frac{\varepsilon'_F[Q]}{\varepsilon'_F\varepsilon_F}\right)\frac{\{1-\exp(-l\varepsilon'_F[F])\}}{\{1-\exp(-l\varepsilon'_F[F]+l\varepsilon'_Q[Q])\}} \quad (1)$$

where Q and F denote quencher and fluorophore, respectively, l is the cell pathlength, the molar absorption coefficients $\varepsilon'=2.303\varepsilon$, ε_Q was measured as 0.504 M^{-1} cm^{-1} for AAm between 0 and 0.7 mol dm^{-3} and $\varepsilon_F=6500$ M^{-1} cm^{-1} was assumed for P(AAm-*co*-ACE) (see footnote of *Table 1*).

Time-resolved fluorescence (single photon counting) was performed at ambient temperature on two types of spectrometers: a deuterium flash-lamp (Edinburgh, 199F) apparatus and a pulsed laser (Coherent) apparatus. The latter apparatus was used to determine rotational relaxation times, since its relatively powerful photon source was able to compensate for the poor transmission resulting from use of polarizers. The laser apparatus employed 1024 channels of a multi-channel analyser, and the lamp apparatus employed 512 channels. Procedures such as deoxygenation and precautions with naphthalene self-absorbance were carried out as already described for the steady-state measurements. The excitation and monitored emission wavelengths were 292 and 339 nm, respectively. To minimize light scattering from the polymer samples, a 320 nm cut-off filter was employed. All non-single exponential fluorescence decay profiles represent a total of $\sim2\times10^4$ counts in the maximum channel, whereas the single exponential decays consist of $\sim10^4$ counts. The reconvolution and fitting procedures have already been described elsewhere[12,13]. Briefly, a model decay function was reconvoluted with the excitation profile using the mimic technique and a reference compound (2,5-diphenyloxazole, with a characteristic excited lifetime τ of 1.39 ns). Minimization of the parameter χ^2 was used as a criterion of fit.

The diffusion coefficient of AAm in D_2O was determined by the p.g.s.e. method. A magnetic resonance imaging (MRI) probe (Doty Scientific, Columbia, SC, USA) was installed in a Chemagnetics CMX 300 n.m.r. spectrometer operating at 300 MHz for protons. A standard p.g.s.e. sequence was used[14]. P.g.s.e. experiments were performed at 23°C and the gradient pulse was applied to the z-direction only. The gradient strength (0.019 T m^{-1}) was calibrated by using the diffusion coefficients of water ($D_{H_2O}=2.35\times10^{-5}$ cm^2 s^{-1}) and 2 vol% H_2O in D_2O ($D=1.9\times10^{-5}$ cm^2 s^{-1})[15]. The precision of the measured D values is estimated as better than 5%.

INTERPRETATION OF DATA

Steady-state polarization

The degree of polarization p was calculated using the standard expression:

$$p=\frac{I_{VV}-GI_{VH}}{I_{VV}+2GI_{VH}} \quad (2)$$

where $G=I_{HV}/I_{VV}$, which is the correction factor for the detection system for vertically and horizontally polarized light. The subscripts represent the relative orientation of polarizers along the lightbeam path before and after the sample cell.

Rotational relaxation time

The rotational relaxation time ($\rho=3\phi$, where ϕ is rotational correlation time) was extracted from the shape of the time-dependent fluorescence intensity decay $I(t)$ using[16]:

$$I_i(t)=A_1\exp\left(\frac{-t}{\tau}\right)+A_2\exp\left[-t\left(\frac{1}{\tau}+\frac{1}{\phi}\right)\right] \quad (3)$$

where the subscript i denotes the relative orientation of the polarizers (in this case parallel or perpendicular), τ is the sample characteristic excited lifetime and A_1 and A_2 are the amplitudes corresponding to the biexponential decay. This equation assumes a spherical rotor and hence can only be regarded as a first-order approximation.

The values of p (obtained from steady-state depolarization) and ϕ enable the calculation of the intrinsic polarization p_0 through the Perrin equation, which also assumes a spherical rotor:

$$\left(\frac{1}{p}-\frac{1}{3}\right)=\left(\frac{1}{p_0}-\frac{1}{3}\right)\left(1+\frac{\tau}{\phi}\right) \quad (4)$$

Steady-state quenching

For a dynamic collisional quenching process without a static or transient component, the variation of

fluorescence intensity I and lifetime τ are related to the quencher concentration [Q] by the Stern–Volmer equation:

$$\frac{I_0}{I}=\frac{\tau_0}{\tau}=1+k_q\tau_0[Q] \quad (5)$$

where the subscript 0 refers to fluorescence characteristics in the absence of quencher, and k_q is the quenching constant. When quenching is diffusion controlled:

$$k_q=4\pi N'_A\sigma D \quad (6)$$

where N'_A is the Avogadro constant per millimole, σ is the reaction radius and D is the mutual-diffusion coefficient. Equation (6) describes the long-time behaviour of a diffusion-controlled quenching process.

The complete Smoluchowski equation for diffusion-controlled reactions contains a transient term[7]:

$$k_q=4\pi N'_A\sigma D+4N'_A\sigma^2(\pi D)^{1/2}t^{-1/2} \quad (7)$$

In the Smoluchowski model[17], one assumes that when F* and Q approach to within a distance σ, quenching is instantaneous. In the Collins and Kimball model[17], one allows the reaction at distance σ to occur at a finite rate in competition with diffusion apart. This has the effect of replacing σ in equation (7) with $\sigma_{eff}\leqslant\sigma$. In this more realistic model, σ_{eff} is an 'effective capture radius' whose magnitude depends upon the diffusion rate of the reactants, with $\sigma_{eff}\rightarrow\sigma$ as $D\rightarrow 0$.

To a good approximation, the transient term in equation (7) can be incorporated as a static component in the Stern–Volmer equation:

$$I_0/I=\exp(N_A V[Q])(1+k_q\tau_0[Q]) \quad (8)$$

to account for the quencher molecules being adjacent to the fluorophore at the moment of excitation. The volume of reaction is defined as $V=4/3\pi\sigma^3$.

Time-resolved quenching

The decay law governing a simple dynamic quenching process is a single exponential:

$$I(t)=A\exp(-t/\tau) \quad (9)$$

Modification of equation (9) to incorporate transient quenching leads to[18]:

$$I(t)=A\exp(-at-2b\sqrt{t}) \quad (10)$$

where

$$a=(1/\tau_0)+4\pi N'_A\sigma D[Q] \quad (10a)$$

$$b=4\sqrt{\pi D}\sigma^2 N'_A(Q] \quad (10b)$$

Thus, given a system displaying transient quenching behaviour, it is possible to obtain the variables σ and D independently.

RESULTS AND DISCUSSION

Coil stability of P(AAm)

The coil stability of P(AAm) was monitored over a 2 week period by s.e.c., which gave a relative measure of V_H, and by following the steady-state value of polarization, equation (2). Measurements were made intermittently, typically every day for 3 days then every 3–5 days thereafter. Monitoring of polarization was delayed for the first day after polymerization due to the necessity of dialysing out the unreacted residual AAm (a fluorescence quencher) and ACE. Diagnostic information was not expected to be lost over this initial day, since the instability effect when reported has ensued over a period of days and even weeks. Neither the values of V_H (for samples ACE2 to ACE7) nor the values of p (for samples ACE2 to ACE6) indicated any significant change over the 2 week period for 0.8 wt% polymer with molecular weight range 0.5×10^6–2×10^6 g mol^{-1}.

Other workers have observed that for freshly prepared P(AAm) of molecular weight $\approx 3.3\times10^6$, the values of η_{sp}/c (specific viscosity/concentration of polymer) and V_H decrease by approximately 10 and 25%, respectively, over the course of 1 week[19,20]. The latter percentage indicates that our s.e.c. experiments (error $\sim$10%) were sufficiently sensitive to detect such changes in V_H.

Eliassaf and Silberberg[21] have shown that 5 and 10% ethanol addition to aqueous P(AAm) (which is well below the concentration required for precipitation) reduces η_{sp}/c by 7 and 12%, respectively. The magnitude of these changes is similar to that observed for ageing P(AAm). Hence the sensitivity of the quantity p was tested by adding similar quantities of ethanol to a 2-week-old P(AAm-*co*-ACE) sample. The following outlines this experiment. Values of p were determined for sample ACE5 initially without ethanol, then with subsequent additions of 5 then 10% ethanol. These three solutions were deoxygenated by bubbling of argon gas which had first passed through 0, 5 and 10% aqueous ethanol, respectively. This was done to preserve the initial quantities of ethanol added to the polymer samples. The samples were then allowed to equilibrate for less than 1 h before measurement. The resultant value of p without ethanol was about 0.037. This value increased 70 and 370% for 5 and 10% ethanol additions, respectively. Thus it appears that the fluorescence depolarization experiments were highly sensitive for detecting mobility changes in P(AAm).

Segmental mobility of P(AAm)

Time-resolved depolarization measurements were used to determine the rotational relaxation time of the ACE unit copolymerized in the P(AAm) backbone. Before presenting these results, however, it is important to be aware of the state of the polymer coil in solution. The coil stability section of this paper has already established that peculiar polymer conformations previously suspected are unlikely within the molecular weight range and concentration we are concerned with, namely $\leqslant 2.1\times10^6$ g mol^{-1} and 0.8 wt%, respectively. Another important factor that may influence the rotational relaxation time is polymer coil overlap: at sufficiently high concentration, i.e. above the overlap concentration commonly designated c^*, the coils will interpenetrate one another and hence restrict local segmental movement. The concentrations used in these measurements were of the order $c^*=2.5/[\eta]=0.4$ wt%. At this low level of overlap, we expect the effects on segmental motion to be minimal.

Table 2 summarizes the results of the time-resolved depolarization study for the P(AAm-*co*-ACE) sample ACE11. The value of τ was determined with the polarizers set at 54.7° (the so-called magic angle). The resultant fluorescence decay is presented in *Figure 3a*. Rotational relaxation times were determined with the polarizers set parallel and perpendicularly to one another. By way of

Table 2 Results of the time-resolved fluorescence depolarization study for polymer sample ACE11

Polarizer orientation	τ (ns)	$(1/\tau+1/\phi)^{-1}$ (ns)	χ^2	$1/p_0$	ρ (ns)
Magic angle (54.7°)	33.5	–	1.2	–	–
Parallel	33.3	6.6	1.3	5.6	25
Perpendicular	33.4	8.6	1.2	7.2	35

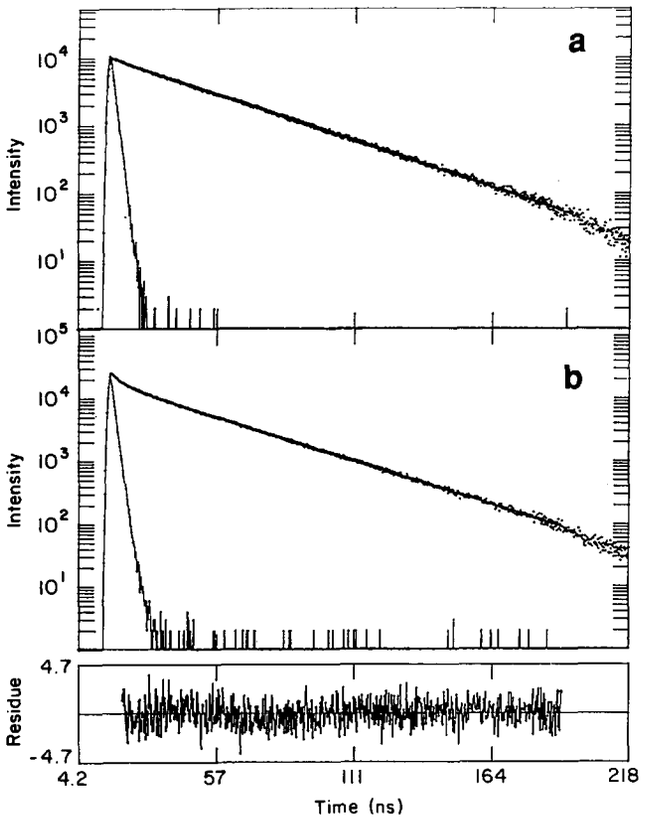

Figure 3 (a) Fluorescence decay profile for P(AAm-*co*-ACE) with polarizers set at 54.7° (magic angle). (b) Fluorescence decay profile and residuals for P(AAm-*co*-ACE) with polarizers set in parallel

example, the resultant fluorescence decay profile for the polarizers set in parallel is presented in *Figure 3b*. The values of $1/p_0$ (*Table 2*) were calculated using equation (4) and $p=0.037$, as given by steady-state fluorescence. These values of $1/p_0$ provide confidence in the present study since they agree reasonably well with the value of 7.2 observed by Kettle and Soutar[6] for poly(methyl methacrylate-*co*-ACE). The discrepancy in the values of ρ (and consequently $1/p_0$) between the two polarizer configurations reflects the inadequacy of the spherical rotor model described by equation (3). Because most previous workers have also assumed the same model for other polymer systems, these values, although semiquantitative, are at least useful for the purpose of comparison.

Nishijima *et al.*[22] have reported that the value of ρ for a fluorescent label attached to the terminal position of P(AAm) is between 3 and 6 ns. From the results presented in *Table 2*, this indicates that the rotational freedom of the terminal position of P(AAm) is approximately six-fold greater than that of an internal position. This observation is consistent with similar studies performed by Soutar and co-workers[6,11] who found that there was a five-fold difference for poly(methyl methacrylate) and polystyrene. It is also interesting to note that the ρ values for the organic solvent systems reported by Soutar *et al.* are generally significantly lower (half an order of magnitude) than those for aqueous P(AAm). Presumably the relatively rigid motion of the P(AAm) backbone is due to H-bonding interactions with water.

Mobility of AAm in aqueous P(AAm)

Fluorescence quenching experiments involving diffusion-controlled quenching provide a measure of diffusive mobility of the reactants. Here we examine three systems which involve quenching of the naphthyl chromophore by AAm. Of most interest is the quenching of the polymer-bound fluorophore by AAm. For comparison, we also examine quenching of acenaphthene by AAm, in pure water and in the presence of 0.8 wt% P(AAm). Details of the experiment and our notation are presented in *Table 3*.

The fluorescence decay profiles for both ACET and P(AAm)+ACET solutions in the presence of AAm could be fitted to simple exponential decays (*Figure 4*). For the

Table 3 Code and composition of fluorescence solutions used in fluorescence quenching study to determine diffusion coefficients

Code	Solutes (aqueous)
ACET	Acenaphthene [10^{-5} M]
P(AAm)+ACET	P(AAm) [0.8 wt%] + acenaphthene [10^{-5} M]
P(AAm-ACE)	Poly(AAm-*co*-acenaphthalene) [0.8 wt%]

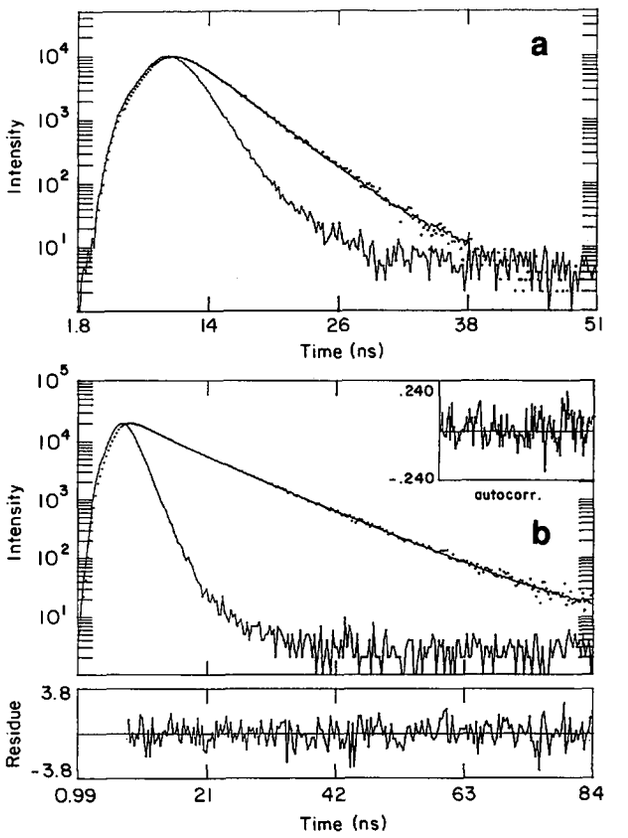

Figure 4 (a) Example of a fluorescence decay profile representing the quenching of the ACET solution (containing 0.047 M AAm). (b) Example of a fluorescence profile curve and residuals representing the quenching of the P(AAm-ACE) solution (containing 0.045 M AAm)

Table 4 Results of time-resolved fluorescence quenching study

System	[AAm] (M)	τ (ns)	χ^2 Eqn (9)	χ^2 Eqn (10)	σ (Å)	$D \times 10^{-6}$ ($cm^2\ s^{-1}$)	$k_q \times 10^9$ ($M^{-1}\ s^{-1}$)
ACET	0	26.1	1.2	–	–	6.7[a]	5.4[b]
ACET	0.010	10.7	1.1	–	–		
ACET	0.047	3.4	1.3_5	(2.2)	–		
P(AAm)+ACET	0.0092	10.8	1.2	–	–	7[a]	6[b]
P(AAm-ACE)	0	33.4	1.2	–	–	–	–
P(AAm-ACE)	0.045	–	(5.9)	1.0	10.4	1.6	1.3[c]
P(AAm-ACE)	0.068	–	(7.0)	1.5	10.8	1.4	1.1[c]

[a] Calculated from k_q using equation (6) and $\sigma = 10.6$ Å
[b] Calculated by linear regression from a plot of τ_0/τ *versus* [AAm], with $\tau_0 = 26.1$ ns, see equation (5)
[c] Calculated from D and σ values using equation (6)

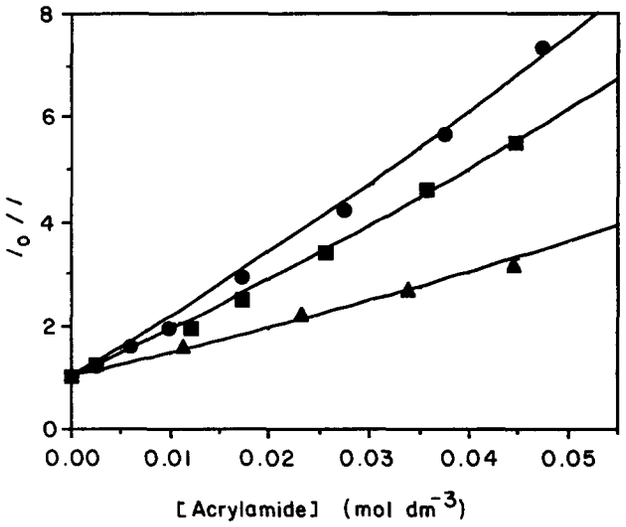

Figure 5 Stern–Volmer plots for the AAm quenching of the fluorescence solutions: ●, ACET; ■, P(AAm)+ACET; ▲, P(AAm-ACE) (sample ACE6). The solid lines represent best fits to equation (8)

former set of solutions, a plot of τ_0/τ *versus* AAm concentration (equation (5)) was linear, and with the value of $\tau_0 = 26.1$ ns, yielded a value for $k_q = 5.4 \times 10^9\ M^{-1}\ s^{-1}$. For P(AAm)+ACET solutions, we measured the decay time for only a single AAm concentration (*Table 4*) from which a value of $k_q \approx 6 \times 10^9\ M^{-1}\ s^{-1}$ can be estimated. The uncertainty in this number is large, and it only serves to indicate that the presence of 0.8 wt% P(AAm) does not have a large effect on the quenching of ACET* by AAm in water. We return to this point in the discussion of the steady-state fluorescence quenching results.

Experiments involving polymer-bound fluorophore [P(AAm-ACE) and AAm] gave non-exponential fluorescence decays in the presence of AAm (*Figure 4b*). A likely origin of this behaviour is the transient effect in the diffusion-controlled quenching process. Eftink and Ghiron[1], and Lakowicz *et al.*[2] have observed a transient component in the quenching of indole fluorescence by AAm, and Gryczynski *et al.*[3] have made similar observations for AAm as a quencher of Y_t-base fluorescence. We therefore chose to fit our decay profiles to equation (10). Reasonable fits were obtained, and the parameters determined from these fits yielded realistic values for the mutual diffusion coefficient D and the interaction radius σ. In this case, k_q values were calculated from D and σ using equation (6). These data are collected in *Table 4*. The values here of $1.2 \times 10^9\ M^{-1}\ s^{-1}$ are a factor of four

Table 5 Results of steady-state fluorescence quenching study

System	τ_0 (ns)	$D \times 10^{-6}$ ($cm^2\ s^{-1}$)	$k_q \times 10^9$ ($M^{-1}\ s^{-1}$)
ACET	26.1	5.2	4.1
P(AAm)+ACET	26.1	4.1	3.2
P(AAm-ACE):			
ACE6	33.4	1.4	1.1
ACE10	33.4	1.5_5	1.2

smaller than those for ACET, indicating that polymer-bound ACE is quenched less effectively by AAm than is the free chromophore in solution.

Steady-state fluorescence intensities were measured for each of the above solutions. Stern–Volmer plots (see equation (5)) of the data are presented in *Figure 5*. Each plot shows a small upward curvature. This type of curvature is expected in diffusion-controlled quenching reactions where transient effects are important. The transient effect manifests itself as an apparent static quenching contribution from fluorophores that happen to be close to quenchers at the moment of excitation. It is interesting that the curvature in the data seen so easily in *Figure 5* for ACET and P(AAm)+ACET does not lead to a measurable deviation from an exponential decay profile in the fluorescence decay measurements. These results are not inconsistent. They simply point to the different sensitivities of the two experiments.

The steady-state fluorescence quenching data were analysed in terms of equation (8). At this point we do not distinguish σ and σ_{eff}. By setting $\sigma = 10.6$ Å, values for k_q and D could be calculated. These data are presented in *Table 5*. It is clear from *Figure 5* that AAm is a somewhat less effective quencher of ACET in the presence of 0.8 wt% P(AAm) than in its absence. This result could not be discerned from our more limited lifetime studies. The decrease in k_q occurs because the polymer slows down the diffusion of the reactants in the solution by about 20%. For ACET, the steady-state fluorescence quenching experiments and the fluorescence decay results are in reasonable agreement. For the case of the polymer-bound chromophore, the two sets of data are in excellent accord.

To interpret these results, we must keep in mind that for samples of P(AAm-ACE) values of D and σ were obtained directly as parameters from analyses of fluorescence decay profiles for samples in the presence of AAm. For all other samples, including the steady-state measurements on P(AAm-ACE), D values in

Tables 4 and *5* were obtained from k_q by assuming that $\sigma = 10.6$ Å. Our value for σ is about 25% larger than the values of 7.1 Å and 8 Å obtained by Lakowicz and co-workers[2,3] in their study of AAm quenching fluorescence from indole[2] and Y_t-base[3], respectively. While we initially felt that this difference in σ values reflected differences between laboratories in obtaining and fitting non-exponential fluorescence decay curves, we now believe this difference to be real and to reflect the influence of D on the magnitude of the effective reaction radius. We return to this point in more detail below.

Our most important result is that D for the case of the polymer-bound chromophore is substantially smaller than that in the case of the free chromophore. For these types of experiment, it is normally asserted that:

$$D = D_F + D_Q \tag{11}$$

where D_F is the diffusion coefficient of the fluorophore and D_Q is that of the quencher. Since AAm is smaller than ACET, one might also expect that $D_{ACET} < D_{AAm}$. In the case of the polymer-bound chromophore, the ACE mobility is restricted by being anchored to the chain. If we set $D_F = 0$ in equation (11), we obtain $D_{AAm} = 1.5 \times 10^{-6}$ cm^2 s^{-1}.

To probe more deeply into the question of AAm mobility in water, we used p.g.s.e. n.m.r. to measure directly the diffusion coefficient of AAm in D_2O. We obtained values of $D_{AAm} = 9.15 \times 10^{-6}$ cm^2 s^{-1} at AAm concentrations of both 2.9 wt% and 11 wt% at 23°C. This is a factor of six larger than that obtained from analysis of the fluorescence quenching kinetics of polymer-bound ACE by AAm. Comparison of these values indicates that diffusion of AAm is slowed considerably *inside the polymer coil.*

Consistent with this result is the influence of added P(AAm) on quenching of ACET fluorescence by AAm. The steady-state experiments (*Figure 5* and *Table 5*) indicate a significant decrease in k_q in the presence of a relatively small amount of added polymer. Even though the effect is small, it suggests that the effect of P(AAm) concentration on k_q and D_{AAm} could be an interesting topic for future experiments.

The values of D reported in *Table 4* for quenching ACET by AAm are inconsistent with the value of $D_{AAm} = 9.15 \times 10^{-6}$ cm^2 s^{-1} determined by p.g.s.e. n.m.r. Since $D = D_{ACET} + D_{AAm}$, and D_{ACET} should be on the order of 4×10^{-6} cm^2 s^{-1} in a solvent with a viscosity comparable to that of water[23], we conclude that these D values in *Table 4* are too small. These values were calculated by assuming that $\sigma = 10.6$ Å, an assumption consistent with the Smoluchowski model[17] of diffusion-controlled reactions. From the point of view of the more realistic Collins and Kimball model[17], data analysis in terms of equations (7) and (10) yields values of σ_{eff}, an effective capture radius, whose magnitude should decrease with increasing D. Thus we find $\sigma_{eff} = 10.6$ Å for the case of polymer-bound ACE + AAm, where $D = 1.5 \times 10^{-6}$ cm^2 s^{-1}, and anticipate a smaller σ_{eff} for ACET + AAm where k_q and D are significantly greater. If we take the Lakowicz[2,3] value of $\sigma_{eff} = 7$ Å for the quenching of indole fluorescence by AAm and use it to calculate D from k_q, we find $D = 1 \times 10^{-5}$ cm^2 s^{-1} for ACET + AAm. This value is nicely consistent with all other parameters obtained in these experiments, and lends substance to the idea that in these experiments σ_{eff} does in fact decrease with increasing D.

SUMMARY

We have examined several aspects of the fluorescence of poly(acrylamide) containing a very small amount of acenaphthyl groups. These groups are rigidly anchored along the polymer backbone. Fluorescence depolarization studies provide a measure of polymer segment mobility and yield a mean rotational relaxation time of 30 ns. We found no indication of the slow coil relaxation, over periods of days and weeks, reported by Kulicke *et al.*[7] for high molecular weight P(AAm) in water.

Fluorescence quenching studies were carried out on both ACET and on polymer samples containing the ACE chromophore using AAm as quencher. Stern–Volmer plots showed upward curvature, consistent with diffusion-controlled quenching with an increasing contribution of transient effects at the higher AAm concentrations. In the case of the polymer-bound chromophore in the presence of quencher, this transient effect could be measured directly with the nanosecond fluorescence decay experiments, and yielded values of the mutual diffusion coefficient $D = 1.5 \times 10^{-5}$ cm^2 s^{-1} and the reaction radius $\sigma = 10.6$ Å. This D value is nine times smaller than the diffusion coefficient D_{AAm} measured independently for acrylamide in water, and indicates that acrylamide diffusion inside the P(AAm) coil is significantly smaller than in bulk water.

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