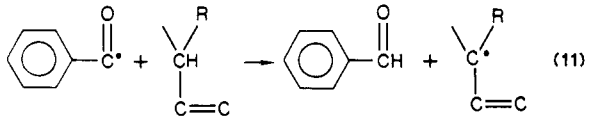
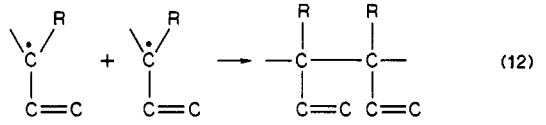
This means that for every radical formed one benzoyl radical is necessary:



(11)

Cross-linking may only occur when two allylic radicals recombine:



(12)

Hence, for every cross-link to be formed, two initiator molecules are necessary.

Conclusions

From studies with model compounds, it is concluded that macroradicals are formed when photocross-linking EPDM rubbers are mainly of the allylic type, which are formed by hydrogen abstraction by benzoyl radicals. The cross-links are formed when these allylic radicals combine.

However, rubbers containing 1,4-hexadiene as a third unsaturated comonomer are cross-linked more slowly. This is explained by the fact that the relatively stable benzoylheptenyl radical is formed which cannot combine with itself. This radical can probably act as a trap for the reactive benzoyl radical which would otherwise give cross-linking.

This latter mechanism may also explain the poor results achieved when polyisoprenes or polybutadienes have been cross-linked with hydroxyalkylacetophenone-type photoinitiators.

Acknowledgment. This work was supported by STU, the Swedish Board for Technical Development, which is gratefully acknowledged by the authors.

Registry No. ENA, 2146-40-9; 2H-DCPD, 4488-57-7; *cis*-2-heptene, 6443-92-1; *trans*-2-heptene, 14686-13-6; benzil, 134-81-6; 1-hydroxycyclohexyl phenyl ketone, 947-19-3; pentamethylnitrosobenzene, 65594-36-7.

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Fluorescence Polarization Study of the Poly(acrylic acid)/Poly(ethylene oxide) Interpolymer Complex in Aqueous Solution

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ABSTRACT: Fluorescence polarization studies of acenaphthylene (ACE) labeled poly(acrylic acid) (PAA) have demonstrated that the complexation between PAA and poly(ethylene oxide) (PEO) results in a tightly packed, sterically restricted conformation in which the mobility of the PAA segments is inhibited. Complexation is maximized when the PAA and PEO repeat unit concentrations are equal, while neutralization of <10% of the carboxylic acid groups restores the initial (uncomplexed) fluorescence polarization of the ACE probe. Time-resolved measurements indicate that the motion of the polymer-bound ACE probe is anisotropic, and information concerning the conformational dynamics of the PAA/PEO complex at various extents of association has been obtained.

Introduction

In nature, interactions between macromolecules play an important role in determining the structures and functions of biological systems.[1] However, the inherent complexities of biological systems make their direct investigation difficult. It is for this reason that synthetic polymers of known structure have been widely used as simple models for biological macromolecules.[2] Particular interest has been directed at synthetic water-soluble polymers, with polyelectrolytes being an important class of such polymers that have been studied by many researchers.[3-5]

One of the most studied systems is the hydrogen-bonded interpolymer complex formed in aqueous solution between poly(ethylene oxide) (PEO) and the polyelectrolyte poly(acrylic acid) (PAA). The bulk properties of this system have been well characterized.[6-9] Complex formation is known to result in a rise in solution pH and a decrease in solution viscosity.[6,9] The significant dependence of the solution properties of the complex on the chain length of the interacting polymers suggests a cooperative complexation process occurs in this system.[7]

Recently, a number of researchers have used fluorescence probe techniques to study the complexation between PAA and PEO and also other related systems.[10-15] The advantage of fluorescence probe measurements is that information about the behavior of the polymers on a molecular level can be obtained, as compared to the bulk properties determined by nonspectroscopic methods.[16]

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A fluorescence probe technique that has not been extensively applied to this type of system is fluorescence polarization analysis. When a collection of fluorophores is illuminated by plane-polarized light there is selective excitation of those fluorophores that have a component of their absorption dipole moment in the plane of polarization of the excitation light. Because of the initial random orientations of the probe molecules in the sample, the resulting fluorescence emission will be depolarized to some extent even if no rotation of the fluorophore occurs during fluorescence. If the fluorophore rotates during its fluorescence lifetime, further depolarization of the fluorescence will result. By binding a fluorophore to the polymer chain, the observed degree of depolarization of the fluorescence will reflect the mobility of the polymer segments attached to the fluorophore.[17-22]

The objective of this work was to use steady-state and time-resolved fluorescence polarization techniques to study the PAA/PEO complexation by incorporating an acenaphthylene (ACE) fluorescent probe in the PAA chain backbone. The advantages of using the ACE probe in polarization studies of polymers have been described previously by other workers.[23,24]

Theory

When a fluorescent sample is illuminated by plane polarized light under steady-state conditions, the degree of polarization (P) of the fluorescence is defined as[25,26]

$$P = \frac{I_V - I_H}{I_V + I_H} \tag{1}$$

where I_V and I_H are the fluorescence intensities measured through vertical and horizontal polarizers when using vertically polarized excitation radiation. The larger the value of P, the smaller the extent of the depolarizing motions of the fluorophore during its fluorescence lifetime. The steady-state degree of polarization can be related to the average rotational correlation time (τ_c) of the probe by using the Perrin equation:[27,28]

$$\left(\frac{1}{P} - \frac{1}{3}\right) = \left(\frac{1}{P_0} - \frac{1}{3}\right)\left(1 + \frac{\tau_f}{\tau_c}\right) \tag{2}$$

where τ_f is the fluorescence lifetime of the fluorophore and P_0 is the limiting P in the absence of depolarizing motion of the probe. The average rotational correlation time is related to the rotational diffusion coefficient (D) of the probe ($\tau_c = (6D)^{-1}$).

The temporal decay of fluorescence recorded through vertical and horizontal polarizers ($I_V(t)$ and $I_H(t)$) following excitation by a short duration light pulse can be used to calculate the time-resolved emission anisotropy function $r(t)$:

$$r(t) = \frac{I_V(t) - I_H(t)}{I_V(t) + 2I_H(t)} = \frac{D(t)}{S(t)} \tag{3}$$

where $S(t)$ and $D(t)$ are the sum and difference decay functions. In the case of an isotropically rotating sphere, $r(t)$ will decay by a single exponential with a time constant equal to τ_c. If the probe rotates with spherical polymer clusters of different sizes, $r(t)$ is described by a sum of exponential terms with different rotational correlation time (τ_c) parameters.[29] For more complex molecular motions of the probe (e.g., nonspherical geometry) other functions for $r(t)$ have been proposed.[2,23,29]

Experimental Section

Materials. Ferrous sulfate (AJAX), cuprous chloride (AJAX), sodium hydroxide (B.D.H.), methanol (M&B), diethyl ether (M&B), THF (AJAX), acetonitrile (AJAX), and 1,4-dioxane (Hopkins and Williams) were used as supplied without further purification.

Acrylic acid (Hopkins and Williams) was purified of inhibitors by vacuum distillation by using ferrous sulfate to decompose peroxides and cuprous chloride to inhibit polymerization. Acenaphthylene (T.C.I.) was purified by multiple recrystallizations from methanol, 2-butanone (AJAX) was purified by distillation, and 2,2'-azobis(isobutyronitrile) (AIBN) (T.C.I.) was purified by multiple recrystallizations from acetonitrile/water mixtures before use. Water was obtained from a Milli-Q (Millipore) water reagent system.

The PEO samples used in this work are listed in Table I. The viscosity-average molecular weights given were determined as described elsewhere.[30]

Table I
Sources and Molecular Weights of PEO Samples Used in This Work

source	supplied $\langle M \rangle$	designation	actual $\langle M \rangle$[a]
ICI Aust.	6000	PEO-6	6900
Union Carbide	100000	PEO-100	93000

[a] Viscosity-average molecular weight.

Polymerization. Acenaphthylene (ACE) labeled poly(acrylic acid) (PAA-ACE) was synthesized from acenaphthylene and acrylic acid monomers by AIBN initiated free radical polymerization in 2-butanone solvent under an Ar atmosphere. The polymerization conditions were maintained at 60 °C for 90 min. The crude polymer was washed several times with diethyl ether then dissolved in water prior to gel permeation chromatography (using Sephadex G-50) to remove low molecular weight material, including unreacted ACE. Solutions of the polymer underwent repeated dialysis against water and were then freeze-dried.

Characterization of Polymer. The viscosity-average molecular weight of the PAA-ACE was determined by viscosity measurements in 1,4-dioxane at 30 °C. The viscosity-average molecular weight was calculated to be $(5.5 \pm 0.5) \times 10^5$ g/mol by use of the Mark–Houwink equation constants 8.5×10^{-4} (100 mL/g) and $a = 0.50$.[12]

The degree of labeling of ACE in the PAA-ACE was determined to be 1 ± 0.2 ACE units per 2×10^4 repeat units in the polymer chain (i.e., less than 1 ACE label per polymer chain), by comparing the PAA-ACE absorption spectrum with the absorption spectrum of a solution of known concentration of acenaphthene in THF. The absorption spectra were measured by using a Hitachi Model 150-20 spectrophotometer/data processor system.

Preparation of Solutions. Standard aqueous solutions of PEO and PAA-ACE were prepared. Solutions of different repeat unit mole ratio R

$$R = [\text{PEO}]/[\text{PAA}] \tag{4}$$

were prepared by using appropriate volumes of the standard solutions, where [PEO] and [PAA] are expressed in terms of the repeat unit concentrations of the polymers. The concentration of PAA was 0.020 repeat unit mol/L in all prepared solutions, so that [PEO] was changed to vary R between solutions.

A potentiometric pH titration of a 0.020 repeat unit mol/L PAA-ACE solution by a standard 0.50 mol/L sodium hydroxide solution was performed to accurately determine the number of carboxylic acid groups in the PAA-ACE solution. Solutions with required degrees of neutralization (α) of the PAA acid groups were prepared by using appropriate volumes of sodium hydroxide solution as determined from the pH titration.

Steady-State Fluorescence Polarization Measurements. The steady-state fluorescence apparatus used in this work was based upon the design of Bashford et al.[31] The excitation source is a 150-W Xe arc lamp (Cathodeon). The excitation beam was focused onto the entrance slit of a Jobin-Yvon H-10 holographic grating monochromator, and the 295-nm excitation wavelength selected passed through a polarizer to select the excitation polarization before illuminating the sample cell. The two photomultipliers located at right angles to the direction of the excitation beam observed the fluorescence through polarizers set parallel and perpendicular to the excitation polarization. The temperature of the sample was maintained at 25 °C by using a water circulation system.

Table II
Dependence of the Average Rotational Correlation Time (τ_c) of PAA-ACE, Uncomplexed and Complexed with PEO-100, on the Degree of Neutralization (α) of the PAA Acid Groups

	τ_c (±0.3 ns)	
α, %	R = 0.0	R = 1.0
0	5.1	35.3
4	2.9	5.2
6	2.5	4.5
8	2.8	3.5
10	3.0	3.0

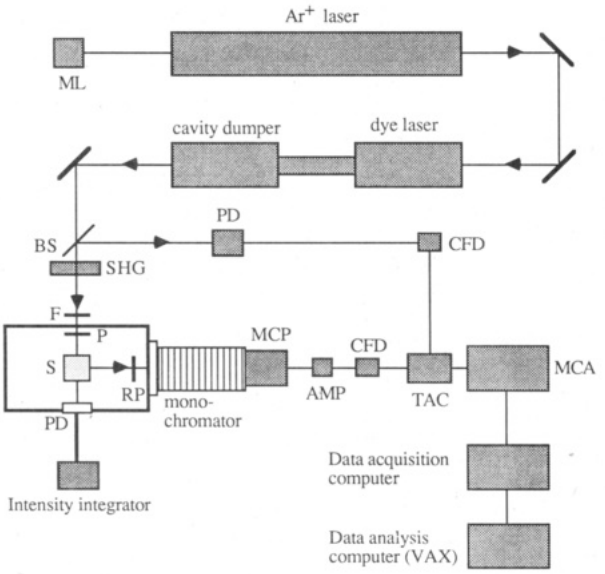

Figure 1. Time-resolved fluorescence apparatus: ML, mode-locker; BS, beam splitter; PD, photodiode; SHG, frequency doubling crystal; F, Corning filter; P, polarizer; RP, rotatable polarizer; S, sample cell; MCP, multichannel plate; CFD, constant fraction discriminator; AMP, amplifier; TAC, time-to-amplitude converter; MCA, multichannel analyzer.

Time-Resolved Fluorescence Polarization Measurements. Fluorescence decay measurements were performed by time-correlated single photon counting techniques,[2,32-34] using a mode-locked, cavity-dumped, synchronously pumped dye laser (Spectra Physics) as the excitation source, as shown in Figure 1.[35-37]

The system consists of a mode-locked 12W Ar^+ laser (Spectra Physics 171) which synchronously pumps a Rhodamine 6G jet stream dye laser (Spectra Physics 375). A cavity dumper (Spectra Physics 454) provides pulses of ca. 5-ps duration at a repetition rate of 4 MHz. The 585-nm output of the dye laser was frequency doubled by using a temperature-tuned ADA crystal, to give vertically polarized light pulses of 292.5 nm for excitation of the acenaphthylene chromophore. Residual undoubled light was removed by a Corning 7-54 filter. The fluorescence at right angles to the direction of the excitation beam passed through a rotatable polarizer and was focused into a Jobin-Yvon H-20 holographic grating monochromator where 340-nm emission was selected for observation. The fluorescence was detected by a proximity-type multichannel plate photomultiplier tube (Hamamatsu R1564U-01) which was connected to time-correlated single photon counting electronics.[36] Anisotropy decays were corrected for the polarization bias of the detection system, determined by measuring the polarization of the very fast rotor, acenaphthylene, in THF.

For fluorescence lifetime measurements, the emission polarizer was set at 54.7° with respect to the vertically polarized excitation light, to correct for any polarization effects on the fluorescence. For emission anisotropy measurements, the emission polarizer was set at 0° and 90° to the excitation polarization, to measure the vertical and horizontal fluorescence decays, respectively.[23,28] Data were collected in the memories of the multichannel analyzer (MCA) and then transferred to a microcomputer for long-term storage. The data were transferred to a VAX 11/780 computer for analysis. Deconvolution of the instrument response function from the experimental decays was determined to be unnecessary due to the very short duration (184 ps fwhm) of the instrument response function compared to the long fluorescence decays observed in this work.

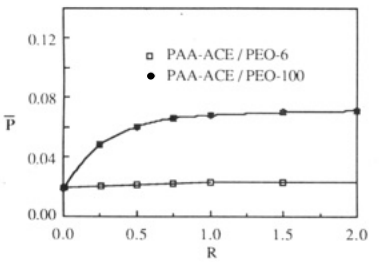

Figure 2. Steady-state polarization behavior of PAA-ACE upon complexation with PEO-100 and PEO-6 in aqueous solution.

The fitting of the experimental decays to sums of exponentials utilized a nonlinear least-squares (NLLS) iterative procedure based on the algorithm of Marquadt.[39] The "goodness of fit" of the sums of exponentials to the observed decays was assessed by considering the randomness of the weighted residuals and autocorrelation function, the reduced χ-square value, and the Durbin-Watson fitting parameter.[2,35,36]

Results

Steady-State Fluorescence Polarization Study. Figure 2 shows the experimentally determined relationship between the steady-state degree of polarization (P) of ACE in PAA-ACE and the repeat unit mole ratio R for PAA-ACE complexed with PEO-100 and PEO-6 separately.

In the PAA-ACE/PEO-100 system, there is a clear rise in P as R is increased toward unity, indicating that the addition of PEO-100 inhibits the depolarizing motions of the ACE probe attached to the PAA chain. When R exceeds unity there is very little further increase in P, suggesting that the mobility of the ACE probe is most restricted when the concentrations of the PAA and PEO repeat units in solution are equal. In the PAA-ACE/PEO-6 system the effect upon the ACE mobility by the addition of the lower molecular weight PEO (PEO-6) is much smaller than for the PEO-100 case.

The stability of the complexation between PAA-ACE and PEO upon neutralization of the PAA acid groups by added base was investigated for the PAA-ACE/PEO-100 system. The average rotational correlation times (τ_c) were calculated from the polarization data by using eq 2 and a P_0 value of 0.139.[24] The fluorescence lifetime of the ACE probe bound to PAA was determined to be between 29 and 40 ns, depending on the conditions of the system. The results are summarized in Table II.

The neutralization of uncomplexed PAA is known to cause the uncoiling of the polymer chain in aqueous solution, due to electrostatic repulsions between the ionized acid groups.[27] For uncomplexed PAA-ACE (R = 0), the observed decrease in the average rotational correlation time upon neutralization indicates that the probe, and the PAA chain to which it is attached, becomes more mobile. For the complex (R = 1), the average rotational correlation time before neutralization is much larger than the value for uncomplexed PAA-ACE, although it can be seen that the values converge as the degree of neutralization of the

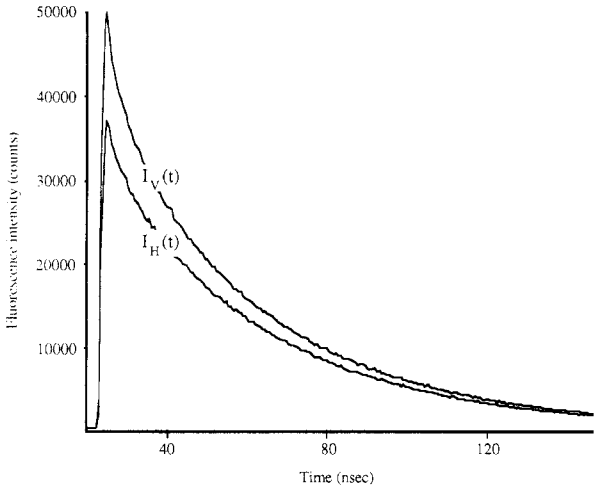

Figure 3. Fluorescence decay profiles collected through vertical ($I_V(t)$) and horizontal ($I_H(t)$) polarizers, for the R = 1 PAA-ACE/PEO-100 unneutralized (α = 0) system.

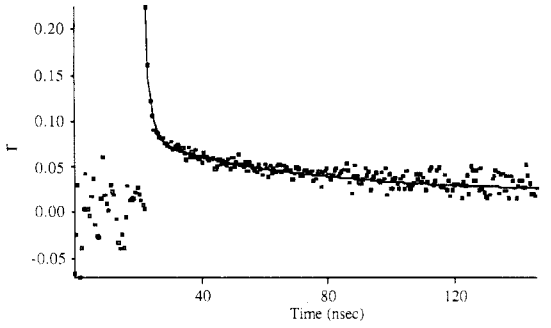

Figure 4. $r(t)$ calculated from the data in Figure 3: squares, data points; solid curve, simulated anisotropy profile with two rotational correlation times τ_{c1} 1.8 ns and τ_{c2} 46.0 ns, determined as described in the text.

PAA-ACE acid groups is increased. The average rotational correlation times are equal by 8–10% neutralization, indicating that the complex has broken-up by this degree of neutralization, since the PEO-100 has no effect upon the PAA-ACE chain mobility. It should also be noted that the greatest increase in the mobility of the ACE probe (and the PAA chain) occurs within the first 4% neutralization.

Time-Resolved Fluorescence Anisotropy Study. For the ACE probe used in this work, $I_V(t)$ is initially larger than $I_H(t)$ when using vertically polarized excitation, but as shown in Figure 3, the intensity profiles converge at longer times where the probe rotation randomizes the orientation of the emission dipoles. The corresponding fluorescence anisotropy decay, $r(t)$, calculated from eq 3 is shown in Figure 4. The low signal-to-noise ratios for the $r(t)$ decay profiles lead to significant uncertainty in the rotational correlation times for the ACE probe extracted from fitting $r(t)$ directly. However, τ_c values can also be determined from fitting the difference decay function $D(t)$ to sums of exponentials of the form

$$D(t) = I_V(t) - I_H(t) = \sum_j C_j e^{-t/\tau_{dj}} \quad (5)$$

The decay time parameters (τ_{dj}) obtained from such a fit are related to the rotational correlation times (τ_{cj}) of the probe by eq 6, where τ_f is the fluorescence lifetime of the ACE probe.

$$\frac{1}{\tau_{dj}} = \frac{1}{\tau_f} + \frac{1}{\tau_{cj}} \quad (6)$$

The higher signal-to-noise ratios of the $D(t)$ profiles allow more accurate determination of the rotational correlation times, and therefore, in this work τ_c values were obtained by using eq 5 and 6. As an example, in the case of the data in Figure 3, a single-exponential function was inadequate to describe $D(t)$ and a double-exponential function was required (cf. Table II). The solid line through the $r(t)$ data in Figure 4 is a reconstructed profile using the τ_c parameters obtained from analysis of the corresponding $D(t)$ curve, overlaid on the $r(t)$ data.

Table III
Dependence of the Fluorescence Lifetime (τ_f), Difference Decay Lifetimes (τ_d), and Rotational Correlation Times (τ_c) of ACE upon the Repeat Unit Mole Ratio R for the PAA-ACE/PEO-100 System

R	τ_f, ns	τ_{d1}, ns	τ_{c1}, ns	τ_{d2}, ns	τ_{c2}, ns
0.0	33.61 ± 0.05	1.75 ± 0.05	1.85 ± 0.03	7.2 ± 0.5	9.2 ± 0.6
1.0	39.50 ± 0.05	1.72 ± 0.03	1.80 ± 0.02	21.3 ± 0.7	46 ± 4
2.0	39.20 ± 0.06	2.6 ± 0.3	2.8 ± 0.4	23.1 ± 0.7	56 ± 7

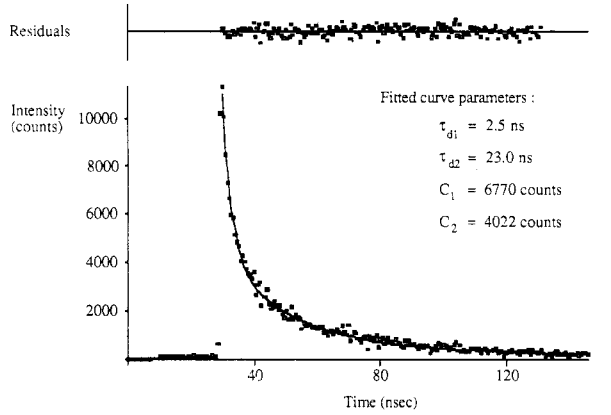

Figure 5. Double-exponential fit to $D(t)$ for the PAA-ACE/PEO-100 system when R is equal to 2.0: squares, data points; solid line, numerical fit.

The PAA-ACE/PEO-100 system was investigated as a function of the repeat unit mole ratio of the two polymers, with the results obtained summarized in Table III. When PAA-ACE is uncomplexed, and when R equals 1 and 2, $D(t)$ is well described by a double-exponential function (as shown in Figure 5 by the random residuals obtained), indicating that a model including two rotational correlation times is adequate to describe the ACE motion under these conditions. However, when $0 < R < 1$ (i.e., when the repeat unit concentration of PEO-100 was less than the PAA-ACE repeat unit concentration) $D(t)$ could not be described by either single- or double-exponential decay functions (as shown in Figure 6 by the nonrandom residuals obtained for a double-exponential fit).

The effect of neutralization of the PAA acid groups upon the motion of the ACE probe was investigated for the complexed (R = 1) PAA-ACE/PEO-100 system. The results are summarized in Table IV. Over the 0–4% neutralization range studied (i.e., where the steady-state fluorescence polarization measurements indicate the greatest decrease in the average rotational correlation time for the complex occurs), $D(t)$ was well described by a double-exponential function, indicating two rotational correlation times were sufficient to describe the ACE motion as the complex was neutralized, although the values of τ_{c2} decreased as the degree of neutralization increased.

Discussion

The labeling of PAA by ACE in PAA-ACE is ideal in the sense that depolarizing motion of the ACE probe in-

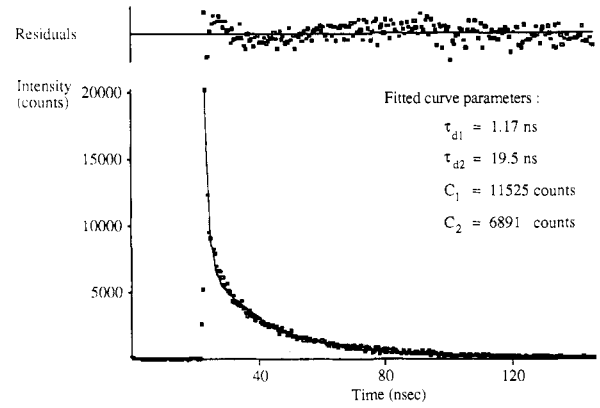

Figure 6. Double-exponential fit to $D(t)$ for the PAA-ACE/PEO-100 system when R is equal to 0.5: squares, data points; solid line, numerical fit.

dependent of the polymer backbone is impossible, so that only motion involving the polymer chain will result in depolarization of the fluorescence of the probe.[24] Thus the ACE probe can be employed to yield information regarding the motions of the PAA backbone under different complexation conditions.

The increase in P as R approaches unity (Figure 2), where the PEO and PAA repeat unit concentrations are equal, indicates that the addition of PEO restricts the depolarizing motions of the ACE probe and therefore also inhibits the mobility of the PAA chain. This can be explained as being the result of the formation of a tight, compact structure upon complexation, as has been suggested by the work of other researchers.[7-9] Such a tight structure of hydrogen-bonded PAA and PEO chains would inhibit the motion of the PAA chain and therefore inhibit the probe motion. The reasonably constant P values after R exceeds unity indicate that the additional PEO-100 in solution has no further significant effect upon the PAA chain mobility. This result suggests that complexation is complete when the repeat unit concentrations of PAA and PEO are equal in solution, in contrast to the behavior noted by some other workers, where further complexation has been reported after R exceeds unity when other techniques and polymer molecular weights are used.[9-12]

It has been reported previously that the behavior of the PAA/PEO complex varies considerably depending on the molecular weights of both PEO and PAA used.[9,12,14] In this work the effect of altering the PEO molecular weight was investigated by using PEO-6 in place of PEO-100 to complex with PAA-ACE. The observed behavior (Figure 2) indicates that PEO-6 has only a minor influence upon the mobility of the PAA chain backbone, implying that complexation is much less extensive in this system, compared to the effect resulting from the use of much higher molecular weight PEO (PEO-100).

This result agrees with the chain length dependence of the complexation found by other techniques.[9] The chain length dependence suggests that complexation is a cooperative process.[6-9] The hydrogen bonds formed between PAA and various chain length PEO polymers will be of approximately the same strength, which does not explain the chain length dependence. However, there is structuring of water about the polymer chains in aqueous solution which will be released upon complexation as the complex contracts to its compact structure. The longer the PEO chain, the greater the amount of structured water released when the PEO associates with a PAA chain, and therefore the greater the entropy driving force for the complexation. The increased hydrophobic character of the associated chains complements the entropy driving force, resulting in further contraction of the chains and more complete complexation. Polarization data cannot yield information concerning the fraction of the PAA acid groups that are involved in hydrogen-bonding to PEO, but the pH behavior of the polymer solutions can be used to estimate the degree of binding.[9] For the PAA-ACE/PEO-100 system pH measurements show that when maximum complexation is achieved, only about 80% of the PAA acid groups are actually involved in hydrogen bonding to PEO, in agreement with previous studies.[9] This result indicates that there must be steric constraints upon the alignment of the PAA and PEO chains, which require some of the PAA acid groups and PEO oxygens to remain unbound. The term "cooperative" was used by Baranovsky et al.[40] to describe the hydrogen-bonding interactions in uninterrupted sequences of monomer residues, but the pH measurements referred to above suggest that such uninterrupted sequences are unlikely to be present in the complex, as has been argued by other workers.[14] The term "cooperative" is used in the present work to describe the combination of factors (entropy/hydrophobic interactions) which favor complex formation.

The stability of the complexation between PAA and PEO should be sensitive to the degree of neutralization (α) of the PAA acid groups, since the association between PAA and PEO chains requires the formation of hydrogen bonds involving carboxylic acid group protons. Deprotonation of these groups by the addition of base to solution should therefore gradually destroy the complex by reducing the number of hydrogen bonds. In addition, changes in the conformation of the PAA following ionization may not be conducive to association of the polymers.

The complex stability was investigated by studying the decrease in the steady-state average rotational correlation time of the probe upon neutralization of the PAA-ACE/PEO-100 complex. The data in Table II indicate that the probe (and the PAA chain to which it is attached) become more mobile as the complex is destroyed. The results show that 8–10% neutralization of the PAA acid groups is sufficient to destroy effectively all traces of complexation, as detected by the mobility of the ACE fluorescent probe in the backbone of the PAA chain. A similar value of 13% has been reported by Iliopoulos and Audebert, using pH measurements and viscometry.[9] The greatest increase in the mobility of the PAA chain occurs in the first 4% neutralization. Thus it would seem that very few of the hydrogen bonds need to be broken to seriously destabilize the complex. The reason for this behavior may be found in the cooperative nature of the complex formation. The removal of a few hydrogen bonds not only results in decreased binding between the polymers but also leads to the formation of negatively charged hydrophilic carboxylate groups. The net effect is to destabilize the complex through Coulombic repulsions, by decreasing the hydrophobic nature of the complex and by reducing the number of hydrogen-bond linkages between the polymers. The fluorescence polarization technique is thus a sensitive method for detecting the inhibition of the PAA motion upon complexation and for following the breakdown of the complex upon neutralization.

The rotational correlation time (τ_c) expected for the rotation of the PAA-ACE polymer as a single unit can be calculated from eq 7, where η is the solvent viscosity, $[\eta]$

$$\tau_c = \gamma M \eta [\eta] / 3RT \tag{7}$$

is the intrinsic viscosity of the polymer, M is the polymer molecular weight, and γ is a coefficient dependent upon the selected chain model (e.g., $\gamma = 1.2$ for spherical impermeable particles and $\gamma = 2$ for Gaussian coils).[29] For

the Gaussian coil conformation of PAA-ACE in aqueous solution at 25 °C, with experimental values of $M = 5.5 \times 10^5$, and $[\eta] = 0.635$ (100 mL/g), the expected τ_c value is 7.4 μs.

It is apparent that the rotational correlation times obtained from time-resolved measurements of PAA-ACE (Table III) are much smaller than the value expected for the rotation of the polymer as a whole, indicating that the rotation of smaller polymer clusters is responsible for the fluorescence depolarization observed. A single-exponential function is insufficient to describe $D(t)$ for the polymer-bound probe under all conditions. One possible explanation for the two τ_c values required when $R = 0$, 1, and 2 is that there are two different depolarizing motions of the ACE probe attached to the PAA. The dependence of the long rotation time τ_{c2} upon the repeat unit mole ratio R suggests that τ_{c2} is due to the slower motion of the probe and a substantial amount of polymer surrounding it, since for different extents of complexation the amount of polymer closely packed about the probe will vary and therefore so will the rate at which the probe and the surrounding packed polymer can rotate. The similarity of the τ_{c2} values obtained when $R = 1$ and $R = 2$ suggests that complexation reaches completion when R is close to unity, in agreement with the behavior determined by steady-state fluorescence measurements.

In contrast, the much smaller τ_{c1} is reasonably independent of R, indicating that it represents faster motion that is not significantly affected by changes in the mobility of the PAA chain as a whole. Space-filling molecular models of PAA-ACE and PEO suggest that when complexation occurs, the presence of the bulky ACE group prevents the acid groups in the vicinity of the probe from hydrogen bonding to the PEO chain. The ACE and adjacent PAA segments would have sufficient freedom to undergo a fast local "wobbling" motion, which, although restricted in not allowing complete rotation of the ACE, would allow sufficient motion to lead to some fast depolarization of the fluorescence. This motion would be reasonably independent of the overall extent of complexation of the PAA and PEO chains and would also be much faster than the rotation of the probe and surrounding packed polymer.

When the concentration of PEO is insufficient to ensure maximum complexation of all the PAA chains in solution (i.e., when $0 < R < 1$), the difference decay $D(t)$ of the ACE could not be adequately fitted to a double-exponential function. In this region of incomplete complexation, it would seem a more complex model than that provided by two rotational correlation times is required to describe the motion of the ACE probe.

The PAA-ACE sample has 3.5 times as many repeat units per chain as PEO-100, and it might be expected that when the concentration of PEO is insufficient to ensure maximum complexation of all the PAA chains, the solution would contain PAA-ACE chains bound to between zero and four PEO-100 chains. For such a nonuniform complexation there would be a number of different environments of the ACE probe, each undergoing different rotational behavior. Under these conditions $D(t)$ would be expected to be complex.

The break-up of the PAA-ACE/PEO-100 complex was studied over the 0–4% neutralization range (i.e., where steady-state fluorescence polarization measurements indicate that the greatest breakdown of the complex occurs). The difference decay $D(t)$ was well described by a double-exponential function in this region. τ_{c1} was again reasonably independent of the extent of complexation of the PAA chain. The τ_{c2} results (Table IV) indicate that as the degree of neutralization of the PAA acid groups is increased, the rotating units of the polymer/ACE probe become smaller, reflecting uncoiling and increased mobility of the polymer chain. The complex appears to break down uniformly throughout solution, otherwise the different environments of the ACE probe might have been expected to lead to a more complex $D(t)$ than the double-exponential function observed.

Table IV
Dependence of the Rotational Correlation Times (τ_c) of ACE upon the Degree of Neutralization (α) of the PAA Acid Groups in the $R = 1$ PAA-ACE/PEO-100 System

α, %	τ_{c1}, ns	τ_{c2}, ns
0.0	1.82 ± 0.03	46 ± 4
0.5	2.18 ± 0.04	31 ± 3
1.5	3.1 ± 0.1	23 ± 2
4.0	1.12 ± 0.05	6.8 ± 0.7

Conclusions

Fluorescence polarization studies indicate that the association of PAA and PEO in aqueous solution results in the formation of a complex with a structure that restricts the mobility of the PAA chain. Steady-state fluorescence polarization measurements of the complex have shown that maximum association is achieved when the repeat unit concentrations of the two interacting polymer chains are equivalent. Complexation is favored for higher molecular weight PEO, while ionization of only a small percentage (<10%) of the PAA carboxylic acid groups is sufficient to break up the complex and restore the initial (uncomplexed) mobility of the polymer-bound ACE probe. These results confirm the cooperative nature of the complexation process. Time-resolved fluorescence polarization measurements indicate that the motion of the ACE probe is anisotropic and provide some insight into the conformational mobility of the hydrogen-bonded PAA/PEO complex.

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Registry No. PAA–PEO (complex), 52284-08-9; (AA)(ACE) (copolymer), 117687-75-9.

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Fluorescence Studies of the Conformational Changes of Poly(methacrylic acid) with pH

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ABSTRACT: Steady-state and pulse laser studies of the fluorescence of pyrene hosted by poly(methacrylic acid) (PMA) in aqueous solution are described. Studies on the effect of pH on the fluorescence lifetime and the quenching of the fluorescence by O_2, Tl^+, and CH_3NO_2 are used to describe the conformational changes induced in the polymer by pH. At least two hydrophobic guest sites are suggested in PMA at pH around 5 for noncharged molecules such as pyrene. The data are actually interpreted in terms of two sites. The photophysical studies suggest the following concepts to describe the pH-induced conformational transition of PMA. The pH-induced change is not a homogeneous ionization of PMA with increasing pH, as only one site would be observed. Increasing the pH ionizes a number of COOH groups on PMA which leads to a hydrophobic core of PMA surrounded by a more hydrophilic mantle or ionized section of the polymer. Both sites act as hosts for pyrene, and the ionized site also binds cationic quenchers such as Tl^+ which increase the rate of decay of excited pyrene at this location. Further increase in pH leads to a decrease in the ratio of hydrophobic PMA core to ionized PMA "mantle". Eventually at high enough pH the polymer is extensively ionized, uncoils, and does not act as a host for pyrene.

Introduction

For many years, much effort has been dedicated to the study of macromolecules carrying ionized, or ionizable, groups in the side chain. These synthetic polyelectrolytes are important in many industrial applications and also as simplified models of natural polyelectrolytes. Thermodynamic and kinetic studies of synthetic polyelectrolytes can be used to gain relevant information of the physicochemical properties of biomolecules such as nucleic acids, polysaccharides, etc. Poly(methacrylic acid) (PMA) shows a marked pH-induced conformational transition. This process has been studied by different techniques: potentiometric titration,[1-3] viscometry titration,[4-6] calorimetry,[7-9] Raman spectrometry,[10] pH jump,[11,12] fluorescent probing,[13-16] etc. The data suggest that at low pH the macromolecule adopts a hypercoiled form in order to minimize the hydrophobic interactions. At a high degree of ionization (higher pH) and in the absence of electrolytes, the PMA chain stretches to a rodlike form. The conformational transition between the two states takes place at pH 4–6. However, the nature of this transition is still open to controversy. Some authors suggest that the transition is highly cooperative and occurs in one step,[5] while data from Raman spectroscopy indicate a multiplicity of structures.[10]

Some light may be thrown on this problem by use of fluorescent probes incorporated into the hydrophobic microdomain of PMA. The probes should exhibit photophysical properties that are strongly dependent on the medium. This approach has been employed previously,[12-15] but the authors have always monitored a variable that is a result of an average over the whole system. With pyrene, for example, spectral characteristics such as fluorescence intensity, ratio III/I, ratio monomer/excimer, etc. contain contributions from all Py* independent of its location. Thus, if there is more than one structure in the transition region, it would be impossible to distinguish between them, and these kind of measurements as a function of pH would show abrupt changes. An alternative procedure is to employ a property that could be uniquely separated from the overall sum of all contributions. The natural choice is the rate of emission decay which becomes multiexponential when there are various species with different lifetimes.

In this paper we report a study of PMA solutions in the region of conformational transition using pyrene as a fluorescent probe, together with quenchers to enhance the fluorescent lifetime differences in the different structures.

Experimental Section

Two kinds of poly(methacrylic acids) were used: one purchased from Polysciences, I, and the other synthesized with AIBN as initiator, II. The viscosity-average molecular weights were 120 000 and 250 000 respectively. These values were estimated from the intrinsic viscosity in 0.002 M HCl solution, using the Mark-

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