Water-soluble copolymers: 26. Fluorescence probe studies of hydrophobically modified maleic acid–ethyl vinyl ether copolymers

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Pyrene fluorescence spectra and lifetimes have been employed to study the pH-induced conformational transitions of maleic acid–ethyl vinyl ether copolymers that have been hydrophobically modified with varying amounts of 4-butylaniline (4-BA). Results indicate that those copolymers containing up to 50 mol% 4-BA (relative to the total number of maleic acid groups) undergo a transition from a highly collapsed 'hypercoil' conformation at low degrees of ionization (α) to an open, hydrated conformation at high α. In contrast, a copolymer containing 70 mol% 4-BA moieties forms compact, hydrophobic microdomains over the entire range of α. Fluorescence quenching of the pyrene probe by Tl^+ and nitromethane indicates that the accessibility of these microdomains is related to α and/or the degree of hydrophobe incorporation. Diffusion coefficients and effective coil diameters of these polymers as determined via dynamic light scattering measurements indicate an increasing degree of intramolecular association with increasing hydrophobe content at high α, while fluorescence measurements indicate no such interactions. It is suggested that, though the pyrene probe may associate with the hydrophobic regions of these polymers, it is not adequately 'protected' from the bulk aqueous solution for fluorescence enhancement to occur.

(**Keywords: copolymers; fluorescence spectroscopy; conformation; maleic acid; ethyl vinyl ether; hydrophobic modification**)

INTRODUCTION

In recent years, considerable interest in microdomain-forming polyelectrolytes or 'polysoaps' has arisen. Owing to the presence of such microdomains, these polymers are capable of solubilizing large, water-insoluble hydrocarbons in much the same way as surfactant micelles. One class of polysoaps consists of alternating maleic anhydride/alkyl vinyl ether copolymers. Strauss and others demonstrated that for alkyl groups of length n (where $3 < n < 10$), at low degree of ionization α, the observed fluorescence intensity of a dansyl label is large, indicating that the label molecules are surrounded by non-polar alkyl groups[1–5]. As α is increased, the fluorescence intensity drops sharply, indicating a transition from a collapsed 'micelle-like' conformation to an expanded coil in which the probe is surrounded by a polar aqueous environment. Furthermore, as alkyl group size increases, the relative hydrophobicity within the coil at low α is increased as is the degree to which the polyacid must be ionized in order to destabilize the compact conformation[5–9].

We have conducted a photophysical study of the effects of the relative hydrophobicity of a polysoap on the stabilization of the collapsed polymer coil at high α. The polysoaps were 4-butylaniline-modified copolymers of maleic acid–ethyl vinyl ether (MA–EVE), with aqueous solution behaviour typical of a polyelectrolyte[1–3]. In this paper, we report preliminary results on the utility and apparent limitations of pyrene as a fluorescence probe to study changes in the conformation of the polysoaps with increasing α.

EXPERIMENTAL

Synthesis

The preparation of a series of hydrophobically modified MA–EVE copolymers was carried out in a three-step process. First, an alternating parent polymer of maleic anhydride and ethyl vinyl ether was synthesized. Next, the parent polymer was derivatized to varying degrees with a long-chain, primary amine. Finally, these derivatized copolymers were hydrolysed to form the corresponding water-soluble polymers.

Maleic anhydride-co-*ethyl vinyl ether* (*MA—EVE*). Maleic anhydride (12.26 g, 0.125 mol), ethyl vinyl ether (18.02 g, 0.250 mol) and 2,2-azobisisobutyronitrile (0.022 g. 1.25×10^{-4} mol) were dissolved in 250 ml of benzene and degassed with N_2 bubbling for 30 min. The solution was then heated to 65°C. After approximately 30 min, the polymer had begun to precipitate. After 16 h, both the precipitated polymer and the benzene solution were poured into 1 litre of ethyl ether to yield a white, powdery precipitate. The precipitate was redissolved into 100 ml acetone, reprecipitated into 750 ml ethyl ether, and dried under vacuum at 50°C; yield 19.35 g (91.0%).

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Methyl esterification of MA–EVE. Esterification of the copolymer for molecular-weight studies was carried out as follows. The copolymer was slurried in $\sim$50 ml of methanol. Upon complete dissolution of the copolymer, 0.02 vol% of concentrated H_2SO_4 was added and the mixture heated to 50°C for 8 h. The esterified polymer was purified by repeated precipitation from benzene into ether, and finally dried under vacuum at 40°C.

Modification of MA–EVE with 4-*butylaniline.* Maleic anhydride-*co*-ethyl vinyl ether (3.5 g, 2.06×10^{-2} mol of anhydride units) was dissolved in 100 ml of ethyl acetate. The solution was cooled to 0°C with an ice bath and degassed with N_2 for 20 min. An appropriate amount of freshly distilled 4-butylaniline (4-BA) was dissolved in 50 ml of ethyl acetate and placed in an addition funnel. With continued N_2 bubbling, the aniline solution was added dropwise to the polymer solution, keeping the temperature below 5°C. The nitrogen purge was removed, and the solution was heated to 65°C for 10 h, at which time the polymer was precipitated into 600 ml of ethyl ether. The white, powdery precipitate was dissolved in 75 ml of acetone, reprecipitated into 600 ml of ethyl ether, and dried under vacuum at room temperature.

Hydrolysis of hydrophobically modified MA–EVE. The modified MA–EVE copolymers (2.0 g) were slurried in 50 ml of 1.0 N aqueous KOH. This mixture was stirred at room temperature until complete dissolution of the polymers occurred. The polymers were precipitated into 700 ml of methanol and immediately redissolved in water. The solutions were dialysed against deionized water (Spectra/Por 4 membranes, *MW* cut-off 12 000–14 000) and freeze-dried. The degree of 4-butylaniline incorporation in each of the copolymers was determined via u.v. absorption methods using the extinction coefficient determined for a model compound in water ($\varepsilon = 11\,800\ \mathrm{M}^{-1}\ \mathrm{cm}^{-1}$ at 250 nm). The resulting compositions are summarized in *Table 1.*

Characterization

From the molecular-weight estimate of the parent polymer and a calculated average molecular weight per repeat unit (u.v. compositional studies), molecular weights of each polymer were calculated such that the concentration of each modified copolymer in solution could be maintained at approximately 6.3×10^{-7} mol l^{-1}.

Molecular-weight determination of esterified polymer. A qualitative molecular-weight estimate of 3.17×10^5 (approx. $DP = 1450$) was made from gel permeation chromatography (g.p.c.) measurements on a Waters system employing a bank of 100, 500, 10^4 and 10^5 Å μ-Styragel columns and a differential refractometer. Monodisperse poly(methyl methacrylate) standards (Scientific Polymer Products) were used to establish the calibration curve.

Table 1 Composition of 4-BA copolymers

Polymer	4-BA[a] (mol%)
4-BA-10	9.3
4-BA-25	22.1
4-BA-50	46.9
4-BA-70	71.1

[a] As determined via u.v. absorption

Fluorescence emission studies of pyrene probe. Solutions of modified MA–EVE copolymers were 'doped' with pyrene by adding microlitre quantities of a concentrated pyrene/methanol solution to the polymer solution. Typically, 1 μl of a 10^{-2} M pyrene solution was added to 10 ml of polymer solution to give a final pyrene concentration of 10^{-6} M. Samples were degassed by bubbling with helium.

Pyrene steady-state emission spectra were recorded with a SPEX Fluorolog-2 fluorescence spectrometer. The spectra were corrected for the wavelength dependence of the detector response using an internal correction function provided by the manufacturer. All samples were excited at 324 nm, and the intensities of the first and third vibronic bands were measured at approximately 372 and 382 nm, respectively.

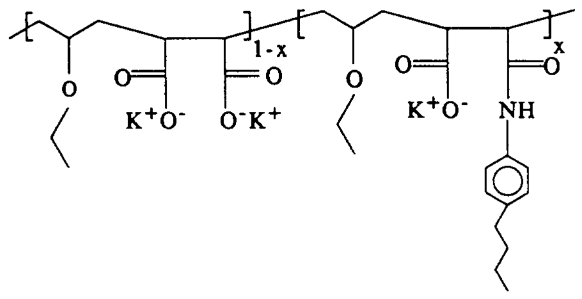
Fluorescence decay curves were measured with a Photochemical Research Associates single-photon counting instrument using an N_2-filled 510-B flashlamp. An IBM-PC was employed along with PRA software to fit the decay profiles using the non-linear iterative deconvolution technique. In those cases where non-exponential decays were observed, only the long-lived portion of the decay curves was fitted.

Dynamic light scattering. Photon correlation spectroscopy studies were conducted using a Brookhaven model BI-DS with a Spectra-Physics 127 laser operating at 653.6 nm. The sample chamber was in a thermostated index-matching bath (toluene) and a Brookhaven model BI-2030AT autocorrelator and associated software were used to process signals. All data presented are for a 90° scattering angle at 25°C.

Much care was taken during sample preparation in order to remove all traces of dust. Deionized, filtered water was used for initial sample preparation. Solutions were then filtered in line for several hours using Gelman Acrodisc filters (0.45, 1.2, or 3.0 M pore size). Contaminated samples were easily identified by their erratic non-reproducible scattering signals.

RESULTS AND DISCUSSION

The 'parent' polymer of maleic anhydride-*co*-ethyl vinyl ether (MA–EVE) was prepared by free-radical polymerization in benzene ($M_n = 317\,000$, $DP = 1450$). Each of the hydrophobically modified polymers was prepared by reaction of the parent copolymer with an appropriate amount of 4-BA and subsequent hydrolysis in dilute aqueous KOH. U.v. compositional analysis was employed to determine the mole percentage of maleic anhydride units that had been derivatized; e.g. 4-BA-50 denotes a copolymer in which one-half of the total available maleic anhydride units were derivatized with 4-BA. The general structure of 4-BA-modified MA–EVE copolymers is shown below:



The low water-solubility and long lifetime (200 ns in water and 400 ns in organic solvents) of pyrene make it an excellent probe of micelles or microdomain-forming polymers[10]. The fluorescence spectrum and lifetime of the pyrene molecule yield information about the polarity of the local environment (micropolarity) and the change in polarity when the medium is modified. The ratio of the third and first vibronic peaks of the steady-state emission spectrum of pyrene (I_3/I_1) and the fluorescence lifetime serve as a sensitive indicator of the polarity of the microenvironment. Higher values of I_3/I_1 indicate a more hydrophobic environment.

Effects of pH

Figure 1 depicts the dependence of I_3/I_1 on pH in aqueous solutions of the hydrophobically modified 4-BA–maleic acid–ethyl vinyl ether copolymers. For copolymers containing 10–50 mol% 4-BA, changes in I_3/I_1 are qualitatively similar to those observed for poly(methacrylic acid) (PMA)[11–13] and maleic acid–alkyl vinyl ether copolymers where the alkyl group size is less than 10 carbons[1–3]. This implies the presence of a conformational transition from a collapsed hydrophobic coil to one that is highly expanded and hydrated with increasing pH. Also, as the degree of incorporation of the hydrophobic group in the copolymers is increased, the degree to which the polymer must be ionized in order to induce this transition also increases. These results are also similar to those previously reported for maleic acid–alkyl vinyl ether copolymers in which the relative hydrophobicity of the polymer coil is varied by the length of the hydrophobic alkyl chain rather than by the degree of hydrophobe incorporation[4]. Furthermore, it is worth noting that for 4-BA-50, I_3/I_1 at high pH is significantly larger than in pure water but is still low enough to indicate a strong hydrophilic environment. This behaviour suggests that, though the coil is highly expanded and hydrated, the pyrene probe continues to interact with the hydrophobic butyl-phenyl side groups rather than remaining in the bulk aqueous phase. This point will be addressed further in the discussion of the fluorescence quenching data.

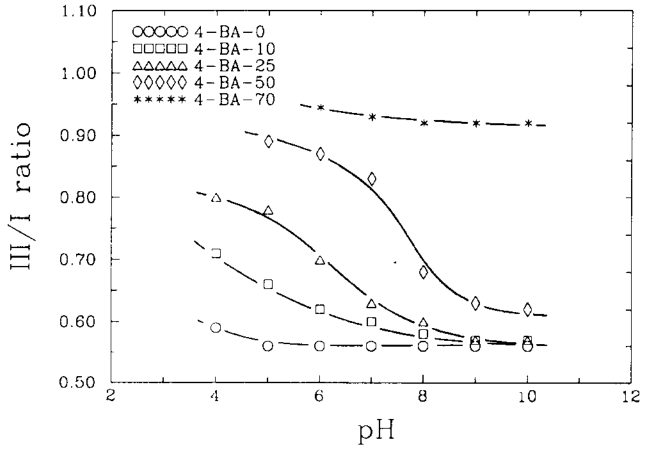


Figure 1 Effect of pH on I_3/I_1 for pyrene probe solubilized in 4-BA copolymers

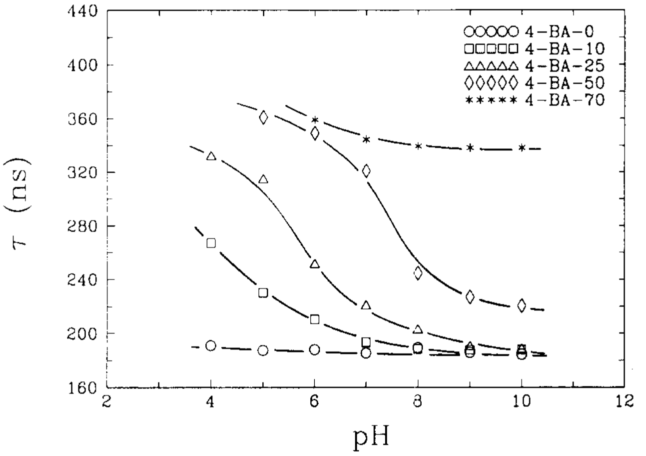


Figure 2 Effect of pH on the fluoresence lifetime τ for pyrene probe solubilized in 4-BA copolymers

In 4-BA-0, a low I_3/I_1 (about 0.56) reflects an essentially aqueous environment and is invariant with changes in pH. This behaviour suggests a relatively expanded conformation for the polymer over the entire pH range. In contrast, a high ($\sim$0.93) and relatively pH-insensitive I_3/I_1 of pyrene in solutions of 4-BA-70 reflects the presence of microdomains even at high degrees of ionization of the polymer. These data indicate stabilization of the collapsed polymer coil via hydrophobic interactions between 4-BA moieties and are once again similar to those observed in analogous systems.

The effects of pH on the pyrene fluorescence lifetime τ are depicted in *Figure 2*. At this time, it should be pointed out that only the fluorescence decay curves of pyrene in those solutions whose I_3/I_1 indicate a totally aqueous environment could be satisfactorily fitted to a single exponential. Numerous efforts to fit the remaining decay curves to a sum of exponentials (either two or three) proved to be unsuccessful. Thus, values of τ were estimated by fitting the long-lived portion of these decay curves to a single exponential. In the interest of consistency, the same number of data channels were fitted for each decay. Nonetheless, for each of the 4-BA copolymers, τ changes with pH in very much the same way as does I_3/I_1, thus confirming the role of hydrophobic interactions in forming and maintaining the collapsed hypercoil structures of these polymers.

The non-exponential character of the pyrene fluorescence decay in non-aqueous environments could possibly be affected by the formation of a non-emissive exciplex between pyrene and the acetanilino group of the 4-BA copolymers and/or a distribution of hydrophobic host sites for the pyrene probe. Using model compounds, an inefficient ($k_q = 3 \times 10^6\ M^{-1}\ s^{-1}$), but nonetheless significant, quenching of pyrene fluorescence in methanol by a small-molecule acetanilide model compound occurs: no emissive complex is formed. A slight deviation from exponential decay is observed for pyrene in the presence of the model compound, suggesting that a non-

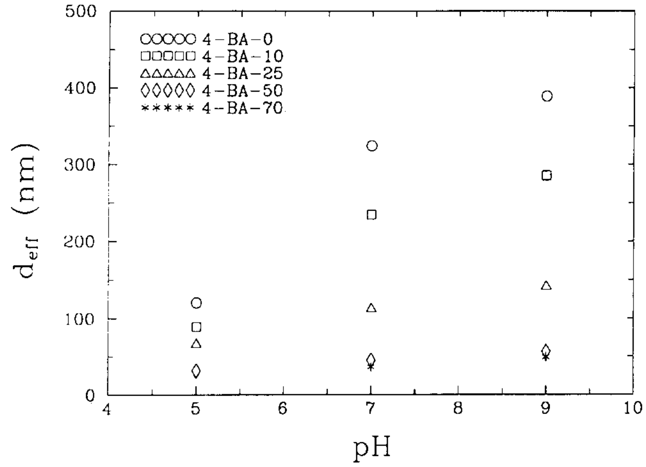


Figure 3 Effect of pH on the effective diameter of the polymer coil d_{eff} for 4-BA copolymers

fluorescing reversible complex could be present. However, since the deviation from non-linearity is minimal, complex formation, if occurring, could be only a minor contributing factor in the non-exponential decays observed for pyrene in solutions of the 4-BA copolymers. Perhaps more applicable to the present case is the fact that the rate of emission decay of the pyrene probe becomes multiexponential when the probe molecules are partitioned between/among different environments[13]. If a continuous distribution of microenvironments exists within the polymer coil, the fluorescence lifetimes of the probes associated with these different environments should be described by a function that comprises the sum of several exponential decay functions. Therefore, it is suggested that the hydrophobic characters of the discrete heterogeneous microenvironments associated with the 4-BA copolymers are non-uniform throughout the solution and that the fluorescence lifetimes (*Figure 2*) as measured describe the most hydrophobic regions of the polymer coil.

The effects of pH on the effective diameter of the polymer molecules d_{eff} as measured by quasielastic light scattering are shown in *Figure 3*. Assuming minimal degradation of the copolymers on hydrolysis, the degree of polymerization of each of the copolymers is the same ($DP \approx 1450$). Therefore, any variations of d_{eff} that occur within the copolymer series may safely be considered to be due to hydrophobic interactions between 4-BA groups rather than due to differences in molecular weight. As might be expected, as the degree of hydrophobic substitution increases, the degree to which the polymer coil expands on increasing pH is dramatically decreased. Furthermore, a decrease in d_{eff} with increasing 4-BA substitution at each value of pH indicates that these associations are largely intramolecular in nature. Apparently, a minimum value of d_{eff} is reached at high hydrophobe content (>50 mol%), indicating that the polymer has assumed the smallest coil dimensions possible without macroscopically phase-separating. These data are again indicative that stabilization of the collapsed coil at high degrees of ionization occurs via hydrophobic aggregation along the polymer chain.

An interesting phenomenon is observed on comparison of the effective diameters of the copolymers containing 0, 10 and 25 mol% 4-BA groups with the fluorescence data, both steady-state and transient, at pH 9.

Fluorescence measurements for each of the three copolymers indicate that the pyrene probe is exposed to an aqueous environment, i.e. there are no hydrophobic aggregates in the solution. However, significant decreases in d_{eff} as the mole percentage of 4-BA is increased indicate that intramolecular hydrophobic interactions are indeed present in the system. We have obtained similar results in numerous efforts to employ fluorescence probe techniques to study a number of other water-soluble polymer systems in our laboratories. Though solution methods such as viscometry and light scattering indicate that hydrophobic interactions are indeed a factor in observed polymer solution properties[14–17], such interactions are not always reflected by pyrene probe photophysics. Polymer solution characteristics may be influenced considerably by the presence of hydrophobic interactions within the solution; however, these interactions or associations may not be in the form of aggregates of sufficient size or propensity to 'protect' a probe molecule from the bulk aqueous solution. This also suggests that, though the pyrene probe is a very effective tool in the study of hydrophobic interactions in water-soluble polymer systems, caution must be exercised in interpreting the absence of fluorescence enhancement of a probe molecule as a complete absence of hydrophobic associations.

Fluorescence quenching

The bimolecular quenching rate constants, k_q, for quenching of pyrene steady-state fluorescence intensity in water and the various copolymer systems are presented in *Table 2*. The values of k_q obtained from linear Stern–Volmer intensity quenching with the neutral quencher nitromethane in the 4-BA copolymer solutions are lower than those observed in water, suggesting that, even in the case of 4-BA-0, penetration of the quencher molecule is inhibited by the main chain of the host polymer. At low pH, this restrictive effect is particularly sensitive to the degree of incorporation of hydrophobe in the copolymer; increased hydrophobe content leads to a more highly collapsed coil, which in turn slows diffusion of the quencher to the probe and lowers k_q. As with I_3/I_1 and τ, the quenching rate constants at high pH for 4-BA-0, 4-BA-10 and 4-BA-25 are similar, again suggesting the absence of hydrophobic interactions capable of sequestering a pyrene probe from the aqueous environment.

Table 2 Fluorescence quenching rate constants[a] for pyrene in aqueous solutions of 4-BA copolymers

Quencher	Polymer	pH 8.0	pH 5.0
CH_3NO_2	Water	8.5×10^9	8.4×10^9
	4-BA-0	5.2×10^9	6.2×10^9
	4-BA-10	5.1×10^9	2.8×10^9
	4-BA-25	5.1×10^9	3.7×10^8
	4-BA-50	1.9×10^9	1.4×10^8
	4-BA-70	1.2×10^8	insoluble
$TlNO_3$	Water	6.1×10^9	5.9×10^9
	4-BA-0	7.9×10^9	7.8×10^9
	4-BA-10	7.9×10^9	3.7×10^9
	4-BA-25	8.1×10^9	1.8×10^9
	4-BA-50	2.8×10^9	1.1×10^9
	4-BA-70	1.4×10^9	insoluble

[a] Changes in fluorescence intensity measured at 382 nm

The electrostatic binding of the cationic quencher Tl^+ to the negatively charged 4-BA copolymers results in relatively higher quenching efficiencies, perhaps due to static quenching, than those observed for the non-interactive quencher nitromethane. This phenomenon is particularly prominent for the 4-BA-70 copolymer. Similar decreases in k_q, but less substantial than those observed for quenching with nitromethane, with increasing hydrophobe content also suggest that the degree to which the polymer coil collapses at a given pH is related to its degree of hydrophobicity. An interesting feature of the Tl^+ quenching experiments at high pH involves the three copolymers for which pyrene shows no appreciable hydrophobic aggregation, 4-BA-0, 4-BA-10 and 4-BA-25. As with nitromethane, the k_q values for each of the three copolymers are approximately equivalent, indicating an open, hydrated structure. However, unlike nitromethane, the k_q values for Tl^+ quenching are higher (slightly) than in water. Since it is well known that cationic quenchers interact strongly with anionic polymers such as the 4-BA series, these data suggest that the pyrene probes may also be associated with the more hydrophobic regions of these three copolymers, regions which, as previously mentioned, apparently do not form micelle-like domains capable of protecting the pyrene molecule from the aqueous phase.

CONCLUSIONS

Photophysical studies of the pyrene probe molecule solubilized by the 4-BA copolymers indicate that these polymers are capable of forming hydrophobic microdomains in aqueous solution. The stability and relative hydrophobicity of these domains are related to both the degree of ionization and the extent of hydrophobic modification of the polymer. Direct comparison of photophysical characteristics of the pyrene probe (steady-state and transient) with effective molecular size of the copolymers as determined by dynamic light scattering as a function of pH suggests that hydrophobic interactions that may alter the solution characteristics of a polymer rather dramatically may not significantly enhance the photoproperties of a probe molecule. Fluorescence quenching measurements indicate that, in the absence of microdomains (high α, low hydrophobe content), the pyrene probe nonetheless associates with the hydrophobic segments of the expanded polymer coil. It is suggested that, while these segments are capable of hydrophobic interactions with each other as well as with the pyrene probe, they do not form discrete microdomains that are capable of 'protecting' the probe molecule from the bulk aqueous phase.

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