Luminescence Studies of Polyelectrolyte Behavior in Solution. 3. Time-Resolved Fluorescence Anisotropy Measurements of the Conformational Behavior of Poly(methacrylic acid) in Dilute Aqueous Solutions[1]

Ian Soutar* and Linda Swanson

School of Physics and Materials, Lancaster University, Lancaster LA1 4YA U.K.

Received August 18, 1993; Revised Manuscript Received April 12, 1994•

ABSTRACT: Time-resolved anisotropy measurements (TRAMS), using synchrotron excitation of fluorescence, have been used to study the conformational behavior of poly(methacrylic acid) (PMAA) in dilute (10^{-3} wt % in polymer) aqueous solution. Copolymerized acenaphthylene (ACE) and 1-vinylnaphthalene (1-VN) were used as fluorescent labels (0.5 mol %). In basic media, segmental relaxation of the polysalt is adequately described by a single exponential model of the fluorescence anisotropy, $r(t)$. However, the dynamics of the acidic form of PMAA are more complex. A minimum of two exponential terms is required for adequate description of $r(t)$. Furthermore, below pH = 4, the relaxation data for PMAA/ACE and PMAA/1-VN become nonequivalent. The rotational correlation time associated with the slower motional process [evident upon dual exponential modeling of $r(t)$] of the label becomes pH independent for PMAA/ACE. In contrast, that for PMAA/1-VN maximizes at *ca.* pH = 4. These differences might have origins in a hindrance upon backbone motion of the PMAA exerted locally by the ACE label at the site of chemical attachment to the chain. Alternatively, the 1-VN label might enjoy greater mobility at lower pH values as a result of "decoupling" of its motion independent of the chain, from that of the macromolecular segments. This might result from a reduction in carboxylate–carboxylic acid interactions at pH values less than that corresponding to the "half-neutralization" point.

Introduction

The solution behavior of water-soluble polymers has proved of interest over many years (see, for example, refs 2 and 3). Environmental considerations have promoted, in recent times, activity in this area which offers considerable degrees of challenge and intrigue to polymer scientists. The versatility of luminescence techniques in interrogation, at the molecular level, of the properties of aqueous solutions of polyelectrolytes, has resulted in a proliferation of reports using this approach within the last 20 years. As examples we cite refs 4–17. Prominent among such studies are those concerning the conformational behavior of polyacids such as poly(methacrylic acid), PMAA, and poly(acrylic acid), PAA. In this paper, we report upon time-resolved fluorescence anisotropy studies of the pH-dependent conformational behavior of PMAA in ultradilute solution. Under such conditions, the properties of the polyelectrolyte are governed by purely intramolecular interactions.

Viscometric[18-20] and calorimetric[21,22] studies are conducive to the belief that, in acid media, PMAA adopts a much more compact form than that evident in PAA. This "hypercoiled" form of PMAA is supposed to originate from the elevated degrees of hydrophobic interaction induced in the acidic form of the polyacid relative to those extant, for example, in PAA. Clearly, the segmental relaxation dynamics of polyacids will be intimately related to the local environment sensed by the relaxing, kinetic unit of the water-soluble polymer. In extremely dilute solutions, the use of labeled polymers offers the only means whereby the local chain dynamics of the water-soluble macromolecule may be assessed. In this context, luminescent species offer distinct advantages over alternative approaches, since they afford high degrees of sensitivity in detection of the transmitted information. However, a primary concern regarding the application of any label or probe as interrogator of a macromolecular assembly is the extent to which the reporter species perturbs the very environment it is supposed to sample. The current work seeks to address this question both in respect of the two fluorescent labels employed and in its relationship to the results of previous investigations.

Luminescence studies of the conformational behavior of aqueous solutions of polyacids such as PAA and PMAA have involved the use both of solubilized probes and covalently-bound labels. The data afforded by these two approaches are complementary. A variety of solubilized fluorescent probes have been employed, including 8-anilino-1-naphthalenesulfonic acid,[23] 9-methylanthracene,[5,6] rhodamine B,[6] and pyrene.[9,10,24] The fluorescence characteristics of the probe which are used to assess the nature of its microenvironment generally involve either emission intensity and/or excited state lifetime data. In addition, certain probes, notably pyrene,[25-27] exhibit a sensitivity of the vibronic structure of their emission spectra to the polarity of the medium in which they are dispersed. Consequently, in studies of the conformational behavior of polyacids, changes in the microenvironments, afforded to a photophysically active *guest* within the relatively hydrophobic coil interiors formed at low pH, are reflected in variations in the fluorescence characteristics of the solubilized probe. Covalent binding of the reporter group, in contrast, allows the microenvironment of the fluorophore to be assessed under conditions wherein a luminescent *probe* would be excluded from preferential solubilization.

Emission anisotropy measurements add a further dimension to the luminescence approach. Photophysical interrogation of guests, occluded in the solubilizing domains created within the coiled forms of polyelectrolytes, yields information regarding the microviscosity apparent in such structures.[28] Covalently-bound labels offer the opportunity, in principle, to examine, *directly*, the segmental relaxational dynamics of the polymeric "host".

---

* Author to whom correspondence may be addressed.

• Abstract published in *Advance ACS Abstracts,* June 1, 1994.

0024-9297/94/2227-4304$04.50/0

Recently, we have observed[1] that the simplest (and *apparently* most direct) approach to such studies, *viz.* that of excitation under photostationary-state conditions[29] suffers from severe limitations, unless a quencher can be found for the reporter chromophore which operates in a truly dynamic fashion over the entire range of pH under investigation. Time-resolved anisotropy techniques, as employed in the current work, do not suffer from these limitations. In addition, the form of the segmental relaxation of the polyelectrolyte under investigation neither *needs* to be presupposed *nor* constrained to that of a simple first-order relaxational behavior.

Valuable information regarding the local environment afforded to a given luminescent probe and/or label, by the polymeric host, can be furnished by studies of the effects of an extrinsic quencher.[9,11,17] The extent to which the quencher can access the luminescent label and/or probe is determined by several factors involving those of polymer chain mobility, local domain hydrophobicity, and intracoil microviscosity. The range of quenchers which have been adopted in such studies includes ionic species, such as Cu^{2+},[11,14] Tl^+,[9,11,17] I^-,[9,11,14,17] and neutral molecules, such as CH_3NO_2.[9,11,14,17] The major problem encountered in such studies concerns the fact that these vicarious samplings of the microenvironments of the luminescent reporter group assume that the quencher itself does not perturb the very domain upon which it is assigned to report. In the area of water-soluble polymers, this premise is questionable, whether ionic or organic quenchers are employed.

Observations[17,24,30-33] that aqueous solutions of water-soluble polymers can sustain room-temperature phosphorescence within solubilized guests and/or copolymerized chromophores has offered a further means whereby the conformational behavior of polyelectrolytes might be examined.[17,32,33] We have shown[17,32] that the phosphorescence induced (*via* an external heavy-atom quencher) in a covalently-bound chromophore can be used to study the pH-induced conformational transition of PMAA. Turro et al.[33] have recently demonstrated the advantages to be gained from the use of a phosphorescing label, bearing its own intramolecular promoter of intersystem crossing, in studies of the conformational behavior of PAA.

In this paper, we employ the method of time-resolved anisotropy measurements (TRAMS) to the study of the segmental dynamics of PMAA in ultradilute ($\leq 10^{-3}$ wt % polymer) aqueous solution. We have previously described the limitations of the steady-state anisotropy approach to the study of this system.[1] Here, we demonstrate the power of the TRAMS technique, as applied under the high-resolution conditions afforded using a high repetition rate excitation source, such as that of the SRS (Synchrotron Radiation Source,[34] Daresbury, UK). In addition, we show the extent to which TRAMS can elucidate the way in which the segmental relaxation behavior of PMAA, in dilute aqueous media, is dependent upon pH. Furthermore, we present data which enhance our understanding not only of the conformational behavior of PMAA itself, but also of the use of fluorescence techniques in evaluation of the physical properties of water-soluble polymers in aqueous media.

Experimental Section

Materials. 1-Vinylnaphthalene, 1-VN, was synthesized from methyl-1-naphthylcarbinol (Koch-Light Ltd.) by dehydration with $KHSO_4$ (BDH). The monomer was purified by fractional distillation under reduced pressure ($\leq 10^{-2}$ Torr) immediately prior to use. Acenaphthylene, ACE (Aldrich), was triply recrystallized from ethanol and triply sublimed. Methacrylic acid, MAA (Aldrich), was prepolymerized (UV radiation) and fractionally, vacuum distilled immediately prior to use. Benzene (BDH) was purified by fractional distillation. Diethyl ether (May and Baker) was fractionally distilled. Methanol (Aldrich, spectroscopic grade) was used as supplied. Water was doubly distilled.

Fluorescently labeled samples of PMAA were prepared by copolymerization of MAA with *ca.* 0.5 mol % of 1-VN or ACE, respectively, in benzene solution (80% by weight of solvent) at 60 °C using AIBN as initiator. The resultant polymers were purified by multiple reprecipitations from methanolic solution into ether.

Characterization. Estimates of the molar masses of the labeled polymers were obtained using size exclusion chromatography (SEC). The sample concentration was 0.1% w/v with an injection volume of 50 μL. The eluent was 0.1 M aqueous $NaNO_3$ using a flow rate of 0.8 mL min^{-1}. Poly(ethylene oxide) standards were used as calibrants. The SEC system incorporated a Waters Associates Model 6000 pump, a Rheodque injector, Waters Associates Ultrahydrogel mixed gel (2 × 30 cm) columns, and a Waters Associates Model 410 RI detector. The molar mass characteristics of the PMAA/1-VN and PMAA/ACE samples were determined as M_n = 56 k (PD = 1.9) and M_n = 620 k (PD = 2.3) respectively. Fluorescence spectroscopy (Perkin-Elmer LS-50) was used to verify the presence of the naphthalene-based labels and the absence of emission from unreacted materials or other species.

Instrumentation. Excitation of fluorescence was achieved using radiation from the Synchrotron Radiation Source, SRS (SERC, Daresbury Laboratory, UK). Excitation wavelengths were selected using a Czerny-Turner 0.75-m medium-resolution monochromator. Fluorescence wavelengths were isolated using interference filters (10-nm band-pass). The SRS, operating in single-bunch mode, delivers pulses of radiation 320 ns apart. Data were resolved, in time, using time-correlated single-photon counting. Using a Philips XP4278b photomultiplier, with small diameter photocathode, results in an instrument response function of <330 ps fwhm.

Fluorescence lifetimes were obtained from analysis of decay data collected at the "magic angle" of 54.7° with respect to that of vertically-polarized excitation.

Fluorescence anisotropy measurements using vertically-polarized excitation, involved collection of the fluorescence intensities transmitted by a polarizer, analyzing in planes parallel, $[I_\parallel(t)]$ and perpendicular $[I_\perp(t)]$ to that of the polarized excitation. A "toggling procedure" was employed in the collection of $I_\parallel(t)$ and $I_\perp(t)$ wherein the analyzer was sequentially altered while memory quarters in the MCA (Inotech 5400) were switched simultaneously. Excitation and emission wavelengths of 290 and 340 nm, respectively, were employed.

Results and Discussion

Fluorescence Decay Data. The fluorescence decay behaviors of PMAA/1-VN and PMAA/ACE in aqueous media have been discussed previously in the context of consideration of both fluorescence quenching[17] and steady-state anisotropy[1] experiments. Consequently, discussion is limited here to a brief description of the salient features of the dependence of fluorescence lifetime upon pH.

At high pH, the fluorescence decays of either label are "largely monoexponential". However, reconvolution analyses using single exponential model decay functions become increasingly less satisfactory as the pH of the system is lowered. In previous reports[1,17] we have associated this increased complexity of decay behavior with a concomitant increase in the heterogeneity of the distribution of the microenvironments experienced by the fluorescent labels within the more compact polyacid conformations adopted at lower pH. Furthermore, as the polymer coil contracts, as a result of the conversion of the polysalt to polyacid form, the average excited state "lifetime" of the fluorescent excited-state increases.[17] (The average lifetime, $\langle\tau\rangle$, of the singlet excited state of a given label is estimated using eq 1 wherein A_i and τ_i represent the preexponential factors

$$\langle \tau \rangle = \sum_i A_i \tau_i^2 / \sum A_i \tau_i \qquad (1)$$

and "lifetimes", respectively, of the parameters afforded by multiexponential fitting to the decay.) Generally the time-resolved fluorescence behaviors of the labeled PMAA samples at low values of pH were adequately described by a dual exponential decay function.

Considering the fluorescence lifetime data, it might be tempting to invoke the model proposed, on the basis of *anisotropy* data, by Ghiggino and Tan[8] to explain the evolution of two decay parameters τ_1 and τ_2 in analyses of the fluorescences obtained from both labels at low pH. Ghiggino and Tan[8] suggested, on the basis of the fluorescence anisotropy behavior of a terminal, dimethylanthryl label of PMAA, that the hypercoiled form of this polyacid consists of compact, globular clusters of polyelectrolyte, interlinked by flexible, relative-extended segments of the macromolecular chain. Within such a model, we could associate the shorter-lived component of the label's fluorescence, τ_1, with species distributed within the more-open, solvent-permeated regimes of the polymer chain: τ_2 would then represent the distribution of chromophores occluded in the compact, hydrophobic domains. Similar interpretations involving the sensing of two broad microenvironments by labels of polyacids have been suggested both by Chu and Thomas[9] and Bednár et al.[35] in rationalization of the fluorescences of pyrenyl[9] and dansyl[35] labels, respectively. However, it is probably an oversimplification to imagine that two, distinct distributions of solubilizing microenvironments exist within the polyelectrolyte coils and that these can be resolved within the lifetime distribution of excited states which are "accommodated" within the coils of the labeled PMAA system. It is more likely that the dual exponential functions which characterize the fluorescence decays of both labels and solubilized probes, merely serve to parameterize complex emission behaviors which, in turn, reflect the widely heterogeneous nature of the microenvironments which exist within the polyacid coils.[17] (This does not negate the proposition that two, broad distributions of *rotationally relaxing* labels exist. Indeed, our TRAMS data indicate, as described below, that such an interpretation has some basis.)

Time-Resolved Fluorescence Anisotropy Data. Use of the SRS as a high repetition rate excitation source has allowed high-resolution time-resolved anisotropy measurements to be made on both the PMAA/1-VN and PMAA/ACE systems over a wide range of pH values. Typical data are shown in Figure 1. The orthogonal components of intensity analyzed in planes parallel, $I_\parallel(t)$, and perpendicular, $I_\perp(t)$, to that of the vertically polarized excitation may be transformed, according to eq 2, into the *observed* anisotropy, $R(t)$

$$R(t) = \frac{I_\parallel(t) - GI_\perp(t)}{I_\parallel(t) + 2GI_\perp(t)} \qquad (2)$$

where G is the correction factor, designed[36] to compensate for instrumental bias in transmission and detection of radiation polarized in different planes. (In the current work G was estimated, in a conventional fashion,[36] by comparison of the relative emission intensities observed in planes parallel and perpendicular to that of *horizontally* polarized excitation. For the experimental conditions adopted, a G value of unity was obtained.)

Direct analysis of the raw anisotropy data, constructed according to eq 2 from $I_\parallel(t)$ and $I_\perp(t)$ data generated at pH 11, using a single exponential decay model of the form

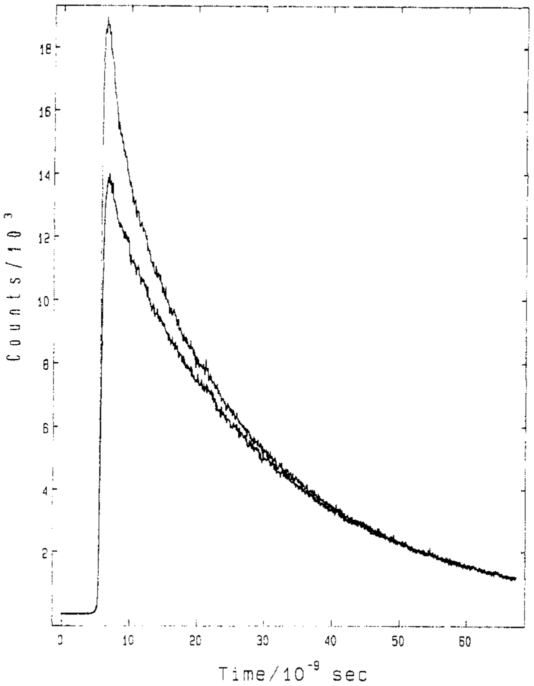

Figure 1. Components of fluorescence intensity, $I_\parallel(t)$ (upper curve) and $I_\perp(t)$ (lower curve), analyzed in planes parallel and perpendicular, respectively, to that of vertically polarized excitation (at 290 nm) for PMAA/1-VN (10^{-3} wt %) at pH = 11.

$$r(t) = r_0 \exp(-t/\tau_c) \qquad (3)$$

yielded estimates of the rotational correlation time, τ_c, for segmental relaxation of the polysalt of 6.7 and 6.5 ns for the PMAA/1-VN and PMAA/ACE systems respectively [*cf.* Table 1]. However, as we have observed[37] previously, there are distinct problems associated with this approach:

(i) It is not possible to derive a value for the intrinsic anisotropy, r_0. This is disadvantageous since the anisotropy is subjected, at early sampling times, to distortions introduced by the finite nature of the excitation pulse, the presence of scattered excitation, timing instabilities, and the general lack of definition of $R(t)$ resultant upon the poor signal to noise ratios extant at early times. The net result is that $R(t)$ does not correspond to the true anisotropy $r(t)$ which we wish to analyze. Consequently, there is considerable uncertainty in the "start channel" i.e. that from which anisotropy decay analysis *should* be initiated. In the current work we have initiated analysis at a channel corresponding to a value of R close to that "expected" for r_0 (for either 1-VN or ACE) on the basis of steady-state fluorescence measurements on other labeled polymer/solvent systems[38-40] and TRAMS upon labeled PMAA in dilute methanol solutions[37] analyzed using the more sophisticated forms of data recovery of impulse reconvolution[41,42] and auto-reconvolution.[37,43]

(ii) The fact that $R(t)$ is not amenable to deconvolution, in a direct fashion, has the consequence that $R(t)$ can *appear* to be complex, even in the instance where $r(t)$ is a simple, single exponential decay function. Reference to Table 1 and Figure 2 reveals that $R(t)$ is not adequately described by a first-order decay law:

(a) The residuals, particularly at early times, are not randomly distributed around zero.

(b) The values of χ^2 exceed the generally accepted limit of 1.3 upon single exponential modeling.

Furthermore, it was found that the value of τ_c recovered in single exponential modeling was dependent upon the choice of channel from which fitting was initiated.

Consideration of these observations might be conducive to the belief that the true anisotropy decay, $r(t)$, is not

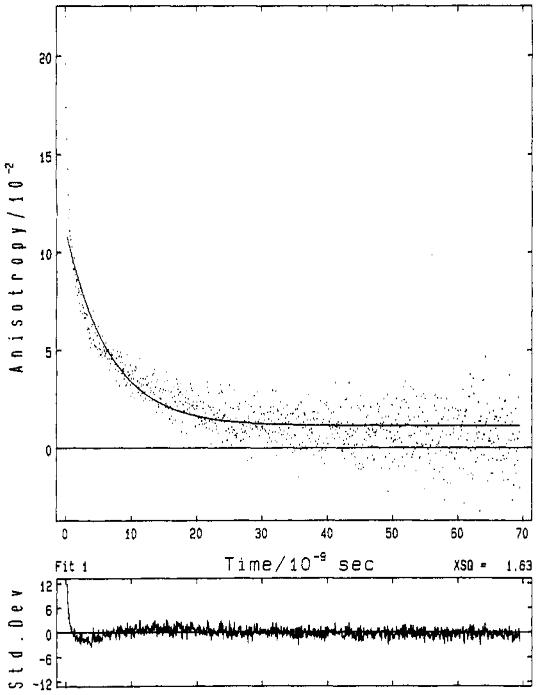

Figure 2. Observed anisotropy, $R(t)$, calculated (using eq 2) from the data shown in Figure 1; single exponential fit afforded by direct analysis of $R(t)$ and distribution of residuals.

Table 1. Correlation Times for Segmental Relaxation of PMAA at pH 11 as Recovered by Direct Analysis of $R(t)$

polymer	τ_{c1}, ns	τ_{c2}, ns	χ^2
PMAA/1-VN	6.7		1.6
PMAA/1-VN	0.8	11.0	1.1
PMAA/ACE	6.5		1.5
PMAA/ACE	2.0	13.0	1.0

capable of representation by a function of the form of eq 3. Indeed, reference to Table 1 reveals that considerable improvement to the quality of fit (as reflected in χ^2) is achieved upon fitting to a dual exponential function. This is not surprising since the assumption of a mathematical form for $r(t)$ of the type

$$r(t) = A_1 \exp(-t/\tau_{c1}) + A_2 \exp(-t/\tau_{c2}) \tag{4}$$

doubles the number of freely variant fitting terms with which to parameterize the decay. The question arises as to whether the resultant parameters have any basis in reality.

As is shown in Table 1, for data acquired at high pH, dual exponential fitting to $R(t)$, over a range similar to that employed for single exponential fits, results in evolution of an apparent longer-lived correlation time, of the order of 11–13 ns, for the two labeled PMAA species and a short-lived component. The presence of the latter component might reflect the existence of a distribution of faster relaxing species within the PMAA/label systems. Alternatively, derivation of a short component might simply be resultant upon the polarizing influence of scattered excitation within the data. [The $R(t)$ data, at short times, certainly exceed the values of r_0 expected on the basis of previous studies[37–40] and approach the theoretical maximum value (of 0.4) for r_o.] However, since the short component is comparable, especially in the case of PMAA/1-VN, to that of the instrument response function, adoption of a fitting procedure which affords a means of deconvolution of the fluorescence information is necessary if the physical significance of the short components, apparent in the analyses of the $R(t)$ data, is to be assessed.

In acid media, the raw anisotropy data, $R(t)$, are poorly described by a single exponential decay function. As described below, this *is* consequent upon a true complexity of the relaxation characteristics of PMAA in acid media. However, determination of correlation times characteristic of the segmental motions of PMAA in the hypercoiled state, requires the use of a data recovery procedure of greater reliability than that of direct analysis of $R(t)$.

In a recent publication,[37] we have considered the relative merits of direct vector reconvolution, impulse reconvolution,[41,42] and auto-reconvolution[43] in recovery of rotational correlation times governing the relaxation behaviors of PAA and PMAA in dilute methanol solutions. The method of direct vector reconvolution can produce problems in data recovery, even in the instance whereby the decays of *both* fluorescence and anisotropy are governed by first-order decay laws.[37,44] That of auto-reconvolution suffers the restriction that the decay law governing the fluorescence behavior should be well understood, and characterized, and the approach is limited in its application if *both* fluorescence and anisotropy require functions of greater complexity than those of a single exponential in description of their decays.[45] In such an instance, impulse reconvolution is the only reliable method, currently available, for the recovery of rotational relaxation data from "complex" luminescence anisotropy information.

In the impulse reconvolution[41,42] approach, the observed sum function, $S(t)$, where $S(t) = I_{\parallel}(t) + 2GI_{\perp}(t)$, is analyzed by a suitable (i.e. statistically adequate) model function. The "best fit" to $S(t)$ is represented by an impulse response function which, in combination with an assumed model for the true anisotropy decay, $r(t)$, may be used in an iterative, least-squares reconvolution procedure to fit the observed difference data, $D(t)$. Optimization of the latter fit allows a judgment to be made regarding how appropriate is the model chosen to represent $r(t)$. Within the limits of the model adopted, the method furnishes rate parameters characteristic of the relaxation process under investigation.

Both impulse reconvolution[41,42] and auto-reconvolution[43] analyses of TRAMS data for the PMAA/1-VN and PMAA/ACE systems in basic media (pH > 6) produced adequate fits when single exponential functions of the form shown in eq 3 were used to model the time dependence of the anisotropy. In addition, the values of the (freely varying) parameters, r_o and t_c, derived for each labeled polymer using impulse reconvolution and auto-reconvolution respectively, were, within experimental error, in agreement. In contrast, the relaxation behavior under acidic (pH < 6) conditions proved more complex. Attempts to model the anisotropy with a single exponential decay were only "successful" if an additional term (descriptive of a residual anisotropy, r_∞) was employed. Consequently, the function of minimal complexity which was found to afford adequate modeling of $r(t)$ is as shown in eq 5.

$$r(t) = A \exp(-t/\tau_c) + r_\infty \tag{5}$$

Typical τ_c data evolved from this form of analysis are shown in Figure 3 with reference to the PMAA/1-VN system. It is worthy of note that the pH dependence of τ_c evolved in this manner is similar to that reported by Bednář et al.[35] upon *direct* analysis of $R(t)$ for dansyl-labeled PMAA. Indeed, at low pH, the actual values of τ_c derived in the current work are, within experimental error, in agreement with those derived by Bednář et al.[35] using a different label. However, before consideration is given to the trends apparent in these data, it is worthwhile questioning whether adoption of a functional form of the

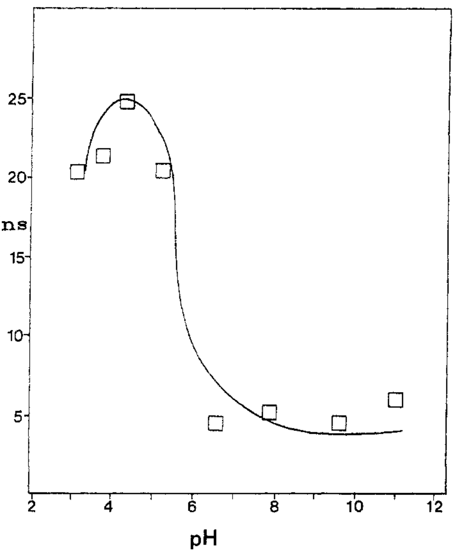

Figure 3. pH dependence of τ_c data for PMAA/1-VN resultant upon impulse reconvolution analyses in which the anisotropy is modeled by a decay function of the form given by eq 5.

type described by eq 5 is realistic, in attempting to characterize the relaxation behavior of the hypercoiled form of PMAA.

Residual anisotropies might reasonably be expected to be observed when the rotation of a fluorophore is hindered due to its location within an anisotropic environment. For example, if the motion of a fluorescent probe is constrained within a restricted angular range, wherein the probe "wobbles within a cone", a limiting anisotropy, r_∞, will be observed. $r(t)$ takes the form of eq 5 wherein $A = (r_0 - r_\infty)$.[46-49] Emission anisotropies which are described by eq 5 evolve when fluorophores such as 1,6-diphenyl-1,3,5-hexatriene are dispersed in anisotropic environments such as lipid bilayers. However, there is little reason to suppose that restrictions upon the segments of PMAA, within its hypercoil, would result in decays of the fluorescence anisotropy of either the 1-VN or the ACE label which would adopt the form of eq 5. Consequently, it is necessary to reconsider our analysis of the fluorescence data obtained at low pH.

Although impulse reconvolution fitting to $D(t)$ using a model for $r(t)$ of the form of eq 5 provides good fits to the data, as judged by the optimizing χ^2 (≤ 1.3), close examination of the distribution of the residuals and the autocorrelation function for the residuals reveal imperfections in the "goodness of fit" [*cf.* Figure 4, parts a and b]. In addition, given that the existence of a residual anisotropy is not expected in PMAA/label systems, it would appear that the quantity r_∞ in eq 5 merely serves as an additional fitting parameter which provides statistically adequate fits that cannot be obtained with a conventional single exponential model alone. Consequently, we have refitted the data obtained from both PMAA/label systems in acid media, using a double-exponential decay function of the form

$$r(t) = A_1 \exp(-t/\tau_{c1}) + A_2 \exp(-t/\tau_{c2}) + r_\infty \qquad (6)$$

to model the anisotropy.

Impulse reconvolution analyses, using eq 6 as model function, in which all five fitting parameters are freely varied, result in fits improved over those furnished using the functional form of eq 5; as is revealed by examination of the residuals and autocorrelation of residuals [*cf.* Figure 5, parts a and b]. The data resultant upon this form of analysis are listed in Table 2. The data provide a degree of confidence in the dual exponential analysis procedure:

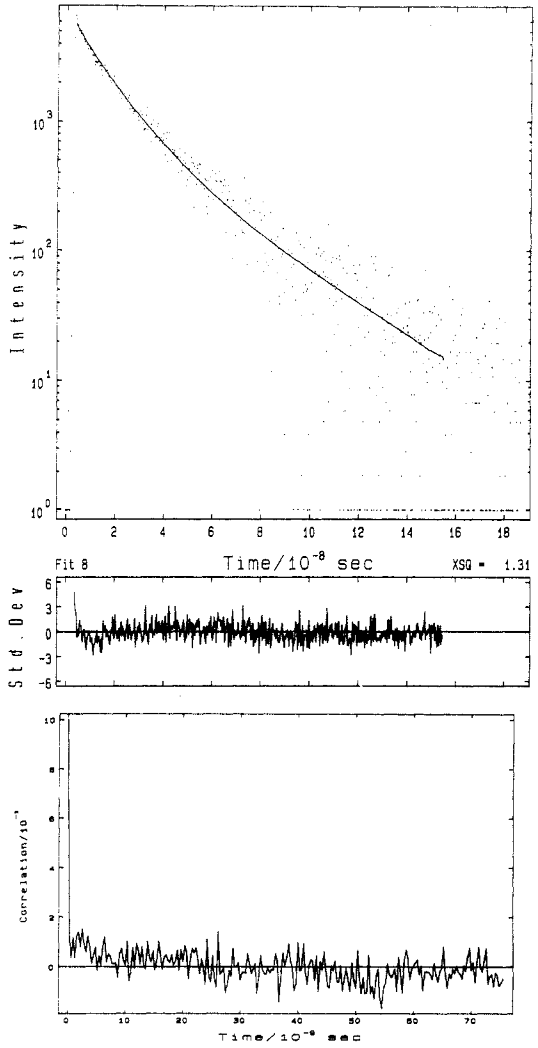

Figure 4. (a) Difference curve, $D(t)$, "best fit" and distribution of residuals and (b) autocorrelation of residuals, following impulse reconvolution fitting to fluorescence data for PMAA/ACE at pH = 4.4, using a single exponential model for $r(t)$ of the form given by eq 5.

(i) Of the two values of τ_c recovered from the analyses of the various data sets, the lower value, τ_{c1}, is relatively constant and, especially for the PMAA/1-VN system, is similar to that obtained in single exponential impulse reconvolution analysis of data obtained at high pH (*cf.* Table 3). Given that the two correlation times are likely to represent averages which seek to parameterize a complex distribution of relaxation processes occurring within the hypercoiled form of PMAA, the current data are supportive of the "interconnected globular structure" of the acidic forms of PMAA, as proposed by Ghiggino and Tan.[8] This aspect of the results is discussed at greater length below.

(ii) Within experimental error, the values of r_∞, evolved in a "fully-varying" fitting procedure, adopting a functional form for the time dependence of the anisotropy as described by eq 6, are negligible; at least in the case of the PMAA/1-VN. [The fact that, even in dual exponential analyses, fitting to the PMAA/ACE data results in evolution of a finite value for r_∞ in acid media, presumably means that the *segmental* (*vide infra*) motion of the macromolecule cannot be described, with total adequacy, by the sum of two exponential terms.]

(iii) The values of r_0 evolved in "free fitting" of the data are, not only self-consistent (within experimental error), but also lie within the range "expected" on the basis of previous studies of anisotropy data obtained under both steady-state[1,37-39] and transient[37,44] excitation conditions from polymers labeled with 1-VN and ACE.

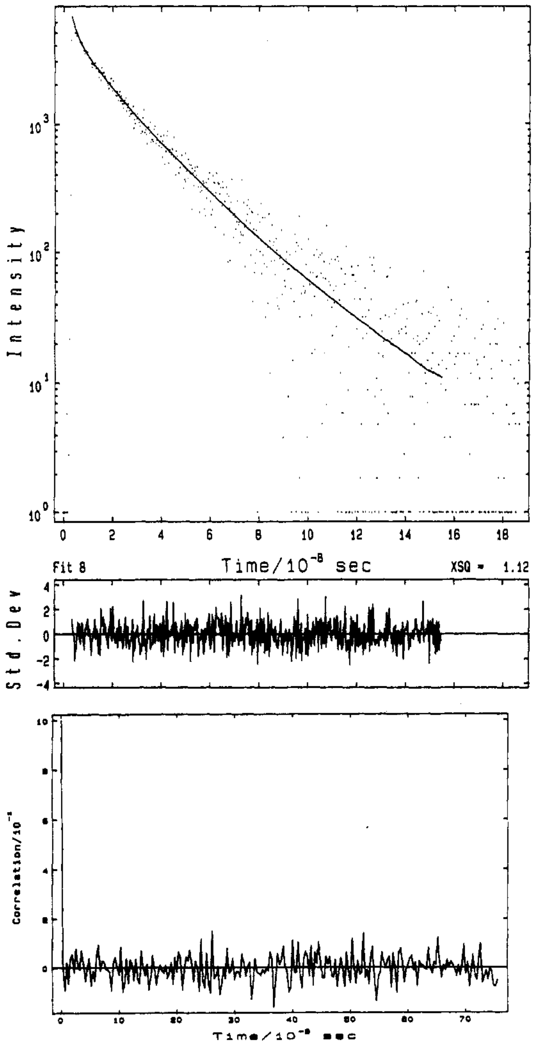

Figure 5. (a) Difference curve, $D(t)$, "best fit" and distribution of residuals and (b) autocorrelation of residuals, following impulse reconvolution fitting to the data shown in Figure 4, using a dual exponential model for $r(t)$ of the form given by eq 6.

Table 2. Relaxation Data Afforded by Dual Exponential Impulse Reconvolution Analysis [Assuming a Functional Form for $r(t)$ as Described by Eq 6, in Text] of PMAA/1-VN and PMAA/ACE in Acid Media

polymer	pH	τ_{c1}, ns	τ_{c2}, ns	$10^3 r_\infty$	r_0	χ^2
PMAA/1-VN	3.2	6.3	39.6	5	0.13	1.0
	3.8	8.9	51.8	3	0.12	1.1
	4.4	6.4	49.1	3	0.13	1.1
	5.3	7.5	48.2	1	0.13	0.9
PMAA/ACE	2.0	7.5	51.9	15	0.12	1.0
	3.0	5.8	53.5	20	0.13	1.0
	3.6	4.5	48.4	15	0.12	1.0
	4.4	3.6	42.2	16	0.13	1.1
	5.7	2.3	19.7	14	0.14	1.0

Table 3. Correlation Times for Segmental Relaxation of PMAA at pH 11 as Recovered by Impulse Reconvolution Using a Single Exponential Model Function for $r(t)$

polymer	τ_c, ns
PMAA/1-VN	8.7
PMAA/ACE	7.9

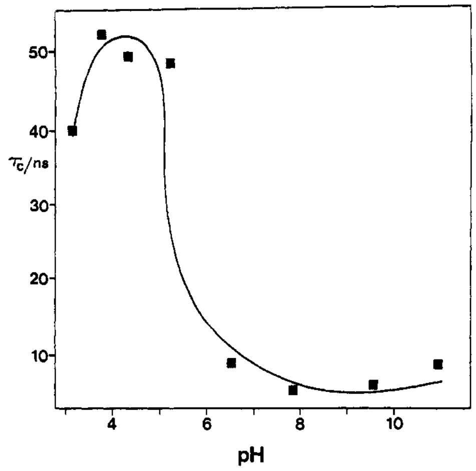

Figure 6. pH dependence of τ_{c2} for PMAA/1-VN.

The data presented in Table 2 would seem to vindicate our proposal that the apparent requirement for inclusion of a limiting anisotropy term (r_∞) in single exponential models [*cf.* eq 5] of $r(t)$, in an impulse reconvolution overview of the global fluorescence anisotropy data sets, is an analytical artifact which reflects the inapplicability of a first-order decay model, for PMAA in acid media. Evolution of two kinetic parameters characteristic of the relaxation behavior of the compact, acidic form of PMAA is in full accord with the observations and predictions of Ghiggino and Tan.[8] The relaxation behavior of the PMAA/1-VN system seems to be in good agreement with the predictions of this model: A shorter-lived component, τ_{c1}, is apparent in impulse reconvolution analyses of the fluorescence data collected from PMAA/1-VN in acid media. This component is well-matched to that derived in single-exponential analyses (of *either* $R(t)$ itself or $D(t)$ *via* impulse reconvolution) of the relaxational characteristics of PMAA/1-VN in basic media [*cf.* Tables 1 and 3]. In this respect, it is tempting to relate the recovery of this short-lived component to the dominance of fluorescences from a distribution of chromophores which are excluded from the hydrophobic domains enjoyed by the majority of the fluorescent labels covalently bound to the PMAA. Following this argument, the longer-lived correlation times, τ_{c2}, would characterize the distribution of chromophores which experience restricted mobilities within the globular structures created in the hypercoiled forms of the polyacid. Given this model, it is appropriate to discuss the pH dependence of the relaxational characteristics of the PMAA/1-VN system, prior to discussing the problems which *might* be apparent in the PMAA/ACE system.

The "interconnected globular structure" model would associate the pH dependence of τ_{c2} with that of the motion of PMAA segments constrained within the confines of the hydrophobic globules of the polyacid. As pH is increased it would be expected that the decay kinetics of $r(t)$ would become less complex, evolving into the simple monoexponential behavior characteristic of the fully neutralized polysalt. The relaxation characteristics of the PMAA/1-VN system appear to conform to the predictions of this model, as shown in Figure 6. As the polyacid is converted into its more flexible, open-coil polysalt form, τ_{c2} is reduced from its maximum value of *ca.* 50 ns to that of *ca.* 9 ns characteristic of the mobile open-chain form of the polyelectrolyte. The general form of this "photophysical titration curve" is similar to that observed by Bednář et al.[35] in direct analysis of $R(t)$ of a dansyl-labeled PMAA. The difference in τ_c obtained in the current work at a given pH within the "regime of acidic behavior" (i.e. at pH values < 6) is readily explained by the different forms of analysis adopted: In direct analysis of $R(t)$, our relaxation data agree very well with those of Bednář et al.[35] in *acid* media. Indeed, Bednář et al.[35] observed that the limiting anisotropy, r_∞, apparent in their analyses was most probably associated with existence of a longer-lived component within the anisotropy data. It was reported[35] that resolution of such a component could not be achieved, with confidence, within the signal to noise ratios obtainable using flashlamp excitation. Such observations are consistent with our findings using a similar experimental approach.[50] However, using the higher repetition rate synchrotron source for excitation, allied to impulse reconvolution analysis, we feel confident that resolution of τ_{c1} and τ_{c2} is possible for data obtained from PMAA/1-VN *in acid media*. Consequently, our data for the PMAA/1-VN system seem to be in general agreement with the behavior observed by Bednář et al.[35] for dansyl-labeled PMAA in acid media. In contrast, there is a marked discrepancy between the values obtained for τ_c at high pH from the differently labeled systems, as discussed below.

At high pH, the value of τ_c recovered in single exponential analyses involving either impulse reconvolution *or* direct analysis of $R(t)$, indicates that the relaxational behavior of PMAA/1-VN is characterized by a correlation time of the order of 6–8 ns (*cf.* Tables 1 and 3). This is in marked contrast to the values of ≤ 1 ns recorded for the dansyl-labeled forms of PMAA.[35] This difference in τ_c is much greater than is explicable on the grounds of differences in analysis procedure (as reference to Tables 1 and 3 indicates). Since the values of τ_c obtained for the PMAA/1-VN and PMAA/ACE systems are (within experimental error) concordant at pH 11, and since the ACE label is "forced", *via* its form of attachment to the polymer chain, to reflect the mobility of the segment to which it is bound (regardless of whether it modifies the behavior of this segment; see below) it must be construed that the differences in τ_c must reflect variations in the degrees of cooperativity between the motions of the labels and those of the chain. We expand upon these observations below, with respect to the acid forms of PMAA. In the interim we noted that, as Bednář et al.[35] have observed, the motion of the dansyl label approaches, at high pH, the rate of relaxation experienced by a model, low molar mass species, in aqueous dispersion. It therefore seems likely that the motion observed in TRAMS studies of the dansyl-labeled PMAA at high pH does not truly reflect that of the macromolecule, but rather records the freedom of movement, independent of the polymer chain, afforded by the flexibility of the covalent mode of attachment of the dansyl label.

In acid media, the flexibility of the PMAA chain, as reflected in the relaxation characteristics of the 1-VN label, is pH dependent. The trend in τ_c values observed for PMAA/1-VN mirrors those observed for dansyl-labeled PMAA samples.[35] The rate of segmental motion of the PMAA appears to achieve a minimum (i.e. a maximum in τ_c is evident) in the region of pH 4. This behavior could be rationalized following Bednář et al.[35] in terms of the restrictions imposed upon the relaxing segment of the PMAA hypercoil by *intramolecular* interactions between carboxylate anions and carboxylic acid groups within the partially neutralized PMAA. However, such a marked pH dependence is not apparent within the relaxation characteristics of the PMAA/ACE system, as is demonstrated in Figure 7. The origin of the differences between the fundamental behaviors of the PMAA/1-VN and PMAA/dansyl compared to that of PMAA/ACE remains to be assessed.

The ACE label is "ideal" in the sense that it *must* record the motion of the polymer segment to which it is attached.[29] In opposition to this consideration is the fact that concern *must* be expressed in any labeling experiment regarding the extent to which the label distorts the very macromolecular behavior *it is designed to study*. The ACE label has proven its worth in the study of macromolecular relaxations in a variety of fluid media[37–40,43,44,50] in comparison with other fluorescent labels including 1-VN. However, caution must be exercised since early dielectric relaxation studies have indicated that copolymerization of relatively low levels of a "similarly restrictive" entity, citraconic anhydride, can markedly effect the relaxation rate of poly(methyl methacrylate) in fluid organic media.[51] Further, in studies of the hydrophobic modification of polyelectrolytes *via* copolymerization of a variety of hydrophobic monomers with both AA and MAA,[28,52] we have shown that copolymerized ACE entities show marked differences in the pH dependence of their photophysical behavior compared to that of 1-VN.[52] It must be noted that in the latter investigations the aromatic species were incorporated at much greater levels ($\geq 10\%$) than in the current labeling studies. Nevertheless, the ACE label has the potential for affecting the local dynamics of the polymer segment to which it is attached in aqueous media and caution must be exercised in interpretation of relaxation data derived using this label.

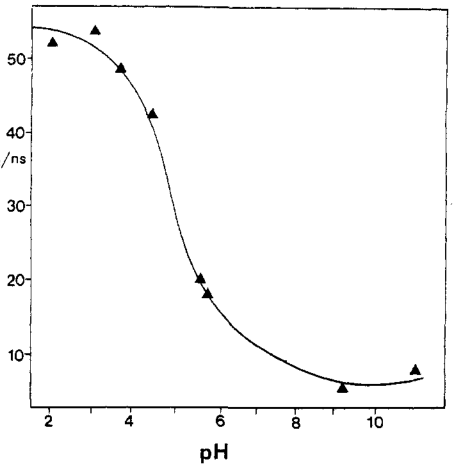

Figure 7. pH dependence of τ_{c2} for PMAA/ACE.

Given that the ACE label has, at least, the potential to distort the kinetic behavior of the polymer segment into which it is incorporated, it might be tempting to rationalize the differences in behavior of the various systems as follows: It might be that, in the hypercoiled form of PMAA, the restrictions imposed by the covalent "bridge-link" binding the ACE to the polyelectrolyte are sufficient to mask the liberation of macromolecular mobility which occurs upon "full acidification" of the PMAA. Consequently, the increased mobility of the polymer chain which results, at pH < 4, from decreasing interactions between carboxylate–carboxylate substituents as the local carboxylate anion concentration is reduced, might not be reflected in the fluorescence behavior of the PMAA/ACE system.

On the other hand, it may be argued that the PMAA/ACE system truly reflects the segmental relaxation behavior of PMAA within the hypercoil and that the information provided by the PMAA/1-VN and PMAA/dansyl label systems is concerned with the onset of a process other than that of segmental relaxation: The polarization characteristics of the fluorescence of naphthalene are complex.[53] Undoubtedly, covalent binding of the naphthyl nucleus (and substitution, in the case of dansyl) will affect the extent of mixing of different electronic states which determines[54–56] the fluorescence anisotropy characteristics of naphthalene derivatives. The fact that the transition vector for fluorescence is not directed solely along the short axis of the molecule means that the 1-VN and dansyl labels have the propensity to reflect, in their emission anisotropies, rotation of the chromophore, independent of the chain backbone, about its bond(s) of attachment to the polymer. Generally, it would appear, in fluid media, on fluorescence time scales, that labels such as ACE and 1-VN provide comparable information upon polymer relaxation[37,38,44] [*cf.* for example, the data obtained herein, at high pH]. At high sampling frequencies the motion of a chain substituent will become cooperative with that of segmental motion. However, at high viscosities if the motion of the chain backbone is sufficiently impeded (as in the hypercoil) it is conceivable that the motions of the 1-VN and the dansyl labels will become decoupled such that independent rotation of the chromophores will be evident in their emission anisotropy characteristics. Certainly, at the lower sampling frequencies employed in *phosphorescence* depolarization studies of the relaxation behavior of polymer solids, 1-VN and ACE labels become "nonequivalent" in their dynamic characteristics.[57]

At present, it is not possible to resolve whether the ACE or the 1-VN label more reliably reports upon the segmental motions within the hypercoiled form of PMAA. It is clear that *some* form of motion is released in PMAA/1-VN at lower pH values as the intramolecular electrostatic bonding between carboxylate and carboxylic acid functions is reduced. It is, of course, possible that *neither* labeled system truly reflects, in its relaxation behavior, that of an unperturbed, unlabeled PMAA: hydrophobic labels such as ACE or 1-VN may well perturb the hydrophobic interactions governing the relaxation behavior of the polyacid. A series of experiments is currently underway employing vinylpyrene as a label of differing hydrophobic dimension to that presented by naphthyl species, in order to try to clarify the situation.

Conclusions

(1) TRAMS offer considerable advantages over their steady-state analogs[1] in studies of the relaxation behavior of water-soluble polymers.

(2) Over the pH range 4–11, correlation times for intramolecular segmental diffusion of the PMAA/ACE and PMAA/1-VN systems are in good agreement. At lower pH values, differences are apparent in the observed behavior. These differences might have origins in a hindrance upon backbone motion of the PMAA exerted locally by the ACE label at the site of attachment to the chain. Alternatively, the 1-VN label might enjoy greater mobility than the ACE label due to the ability of the former to reorient independent of the polymer chain within the hypercoil.

(3) Whatever the root of the differences in relaxation behavior of the PMAA/ACE and PMAA/1-VN systems at low pH, it is evident that some decrease in local, intracoil viscosity occurs upon reduction of the binding induced by carboxylic acid–carboxylate anion attractions which appear to have maximal effect at a pH of about 4.

Acknowledgment. We acknowledge, with pleasure, financial support from The Leverhulme Trust in the form of a Research Fellowship (to L.S.) and the SERC [in provision of "single bunch" beam time at the SRS (Daresbury Laboratory, U.K.)].

References and Notes

(1) Part 2: Soutar, I.; Swanson, L. *Polymer* **1994**, *35*, 1942.
(2) Molyneux, P. *Water Soluble Synthetic Polymers: Properties and Behavior*; CRC Press: Boca Raton, 1983 (Vol I); 1984 (Vol. II).
(3) Shalaby, S. W., McCormick, C. L., Butler, G. B., Eds. *Water-Soluble Polymers: Synthesis, Solution Properties and Applications*; ACS Symposium Series no. 467; American Chemical Society: Washington, 1991.
(4) Anufrieva, E. V.; Gotlib, Yu. Ya. *Adv. Polym. Sci.* **1981**, *40*, 1.
(5) Tan, K. L.; Treloar, F. E. *Chem. Phys. Lett.* **1979**, *73*, 1103.
(6) Snare, M. J.; Tan, K. L.; Treloar, F. E. *J. Macromol. Sci.-Chem. A* **1982**, *17*, 189.
(7) Morawetz, H. In *Photophysical and Photochemical Tools in Polymer Science*; Winnik, M. A., Ed.; NATO Advanced Study Institute Series C; Reidel: Dordrecht, The Netherlands, 1986; Vol. 982, p 85.
(8) Ghiggino, K. P.; Tan, K. L. In *Polymer Photophysics*; Phillips, D., Ed.; Chapman and Hall: London, 1985; Chapter 7.
(9) Chu, D. Y.; Thomas, J. K. *Macromolecules* **1989**, *17*, 2142.
(10) Olea, A. F.; Thomas, J. K. *Macromolecules* **1989**, *22*, 1165.
(11) Arora, K. S.; Turro, N. J. *J. Polym. Sci., Polym. Chem. Ed.* **1987**, *25*, 259.
(12) Chen, H. L.; Morawetz, H. *Eur. Polym. J.* **1983**, *19*, 923.
(13) Bednár, B.; Morawetz, H.; Shafer, J. A. *Macromolecules* **1985**, *18*, 1940.
(14) Delaire, J. A.; Rogers, M. A. J.; Webber, S. E. *J. Phys. Chem.* **1984**, *88*, 6219.
(15) Winnik, F. M.; Winnik, M. A.; Ringsdorf, H.; Venzmer, J. *J. Phys. Chem.* **1991**, *95*, 2583.
(16) Winnik, F. M.; Ringsdorf, H.; Simon, J. *Macromolecules* **1993**, *29*, 371.
(17) Soutar, I.; Swanson, L. *Eur. Polym. J.* **1993**, *29*, 371.
(18) Katchalsky, A. *J. Polym. Sci.* **1951**, *12*, 393.
(19) Arnold, R. *J. Colloid Sci.* **1957**, *12*, 549.
(20) Anufrieva, E. V.; Birshtein, T. M.; Nekrasova, T. N.; Ptitsyn, C. B.; Sheveleva, T. V. *J. Polym. Sci. C.* **1968**, *16*, 3419.
(21) Delben, F.; Crescenzi, V.; Quadrifoglio, F. *Eur. Polym. J.* **1972**, *8*, 933.
(22) Crescenzi, V.; Quadrifoglio, F.; Delben, F. *J. Polym. Sci., Polym. Phys. Ed.* **1972**, *10*, 357.
(23) Treloar, F. E. *Chem. Scr.* **1976**, *10*, 216.
(24) Chen, T. S.; Thomas, J. K. *J. Polym. Sci., Polym. Chem. Ed.* **1979**, *17*, 1103.
(25) Nakajima, A. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 3272.
(26) Nakajima, A. *J. Lumin.* **1976**, *11*, 429.
(27) Kalayanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* **1977**, *99*, 2039.
(28) Ebdon, J. R.; Lucas, D. M.; Soutar, I.; Swanson, L. To be published.
(29) Soutar, I. In *Developments in Polymer Chemistry*; Allen, N. S., Ed.; Applied Science Publishers: London, 1982; Vol. 3, Chapter 3.
(30) Soutar, I.; Swanson, L. *Polym. Commun.* **1991**, *32*, 264.
(31) Soutar, I.; Swanson, L. *Analyst* **1991**, *116*, 671.
(32) Ebdon, J. R. E.; Lucas, D. M.; Soutar, I.; Swanson, L. *Anal. Proc.* **1993**, *30*, 431.
(33) Turro, N. J.; Caminati, G.; Kim, J. *Macromolecules* **1991**, *24*, 4054.
(34) *The SRS*; SERC Daresbury Laboratory: Warrington, UK, 1991.
(35) Bednář, B.; Trnená, J.; Svoboda, P.; Vajda, S.; Fidler, V.; Procházka, K. *Macromolecules* **1991**, *24*, 2054.
(36) Azumi, T.; McGlynn, S. P. *J. Chem. Phys.* **1962**, *37*, 2413.
(37) Soutar, I.; Swanson, L.; Imhof, R. E.; Rumbles, G. *Macromolecules* **1991**, *25*, 4399.
(38) Kettle, G. J.; Soutar, I. *Eur. Polym. J.* **1978**, *14*, 895.
(39) Neish, M. A.; Neves, E.; Soutar, I. Unpublished data.
(40) Swanson, L. Ph.D. Thesis, Heriot-Watt University, Edinburgh, UK, 1989.
(41) Barkley, M. D.; Kowalczyk, A. A.; Brand, L. *J. Chem. Phys.* **1981**, *75*, 3581.
(42) Wahl, P. *Chem. Phys.* **1975**, *7*, 210.
(43) Marsh, A. J.; Rumbles, G.; Soutar, I.; Swanson, L. *Chem. Phys. Lett.* **1992**, *195*, 31.
(44) Christensen, R. L.; Drake, R. C.; Phillips, D.; Soutar, I. To be published.
(45) Crystall, B.; Rumbles, G.; Smith, T. A.; Soutar, I. To be published.
(46) Kinosita, K.; Kawato, S.; Kegami, A. I. *Biophys. J.* **1977**, *20*, 289.
(47) Lipari, G.; Szabo, A. *Biophys. J.* **1980**, *30*, 489.
(48) Szabo, A. *J. Chem. Phys.* **1984**, *81*, 150.
(49) Zannoni, C.; Arcioni, A.; Cavatorta, P. *Chem. Phys. Lipids* **1983**, *32*, 179.
(50) Soutar, I.; Swanson, L. Unpublished data.
(51) North, A. M.; Phillips, P. J. *Trans. Faraday Soc.* **1967**, *63*, 1537.
(52) Bell, S. D.; Culp, T.; Soutar, I.; Swanson, L. To be published.
(53) McClure, D. S. *J. Chem. Phys.* **1954**, *22*, 1668.
(54) Czekalla, J.; Liptay, W.; Döllefeld, E. *Ber. Bunsen-Ges. Phys. Chem.* **1964**, *68*, 80.
(55) Lyle, S. M.; Lim, E. C. *Chem. Phys. Lett.* **1972**, *17*, 367.
(56) Suzuki, S.; Fujii, T.; Ishikawa, T. *J. Mol. Spectrosc.* **1975**, *57*, 490.
(57) Rutherford, H.; Soutar, I. *J. Polym. Sci., Polym. Phys. Ed.* **1980**, *18*, 1021.