# Synchrotron-Generated Time-Resolved Fluorescence Anisotropy Studies of the Segmental Relaxation of Poly(acrylic acid) and Poly(methacrylic acid) in Dilute Methanolic Solutions

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ABSTRACT: The segmental relaxation of poly(acrylic acid), PAA, and poly(methacrylic acid), PMAA, in dilute methanolic solutions has been studied using time-resolved fluorescence anisotropy. The anisotropy data, as analyzed by a variety of data retrieval procedures, indicate that a single exponential function is sufficient in description of the relaxational behavior of these systems. The segmental motions of PAA and PMAA in methanol are characterized by rotational correlation times of ca. 1.3 and 4.2 ns, respectively.

## Introduction

Measurement of the anisotropy of the luminescence observed from a suitably labeled macromolecule, following polarized photoselection, is a powerful means of acquiring information regarding polymer dynamics and relaxation mechanisms. (For example, see refs 1–5.) In this context, steady-state emission anisotropy measurements constitute a relatively rapid and simple (given that due care is paid to optical and scattering distortions of data) method for the study of macromolecular dynamics. The resultant data frequently provide unique insights into polymer relaxation mechanisms. However, the steady-state approach is constrained, in general, to the "force-fitting" of the anisotropy to a first-order decay law. In principle, adoption of time-resolved techniques allows a more rigorous examination of the decay kinetics of the anisotropy function descriptive of the label dynamics and thereby (with careful design) those of the polymer to which the label is attached.

The behavior of polyelectrolytes in aqueous media is the object of much current study. Viscometric[6–8] and calorimetric[9,10] studies lead to the belief that poly(acid)s such as poly(methacrylic acid), PMAA, and poly(acrylic acid), PAA, exist, at low pH, in compacted forms relative to those observed in alkaline media. Furthermore, it is evident that the additional hydrophobic interactions encountered in PMAA compared to PAA lead, in acid media, to a "hypercoiled" structure for PMAA of considerably reduced hydrodynamic volume relative to that of PAA.[6–10]

As the degree of neutralization of the polyacid is increased, repulsions between the carboxylate anions result in an expansion of the polymer coil. The conformational transition, from compact to loosely coiled form, has been the subject of several studies using fluorescence spectroscopy (see e.g. refs 11–13 for reviews of the topic). In the majority of these investigations, information regarding the conformational characteristics of the poly(acid)s have been inferred from changes in the fluorescence spectra, intensities, or lifetimes of either occluded probes or covalently bound labels. Arguably, fluorescence polarization measurements upon labeled poly(acid)s offer the best opportunity for the study of macromolecular behavior during conformational changes of the polyelectrolyte since the technique reports *directly* upon the relaxation kinetics of the polymer segment containing the label. However, relatively little use has been made of luminescence anisotropy measurements,[11,13] particularly under time-resolved conditions, for the study of polyelectrolytes. Recently, reports have appeared which used time-resolved fluorescence anisotropy to study the pH-controlled conformational behavior of both PMAA[14] and PAA[14,15] and the complexation between poly(ethylene oxide) and PMAA[16] or PAA.[15]

We also have studied the relaxation behavior of PMAA and PAA as a function of pH using time-resolved anisotropy measurements (TRAMS). Our results will be published in the near future.[17] However, the complex hydrophobic interactions between poly(acid) and water and the influence of intramacromolecular H-bonding at partial degrees of neutralization[14] complicate the fluorescence behavior. Both the fluorescence *and* anisotropy decays are rendered nonexponential as a result of the variety of microenvironments sampled by the fluorescent labels[14–17] (or indeed by solubilized probes).[13,18,19] In such circumstances, the problems of retrieval of reliable relaxation data from the fluorescence information is exacerbated. Consequently, we resolved to study the relaxation behavior of PMAA and PAA in the simpler situations afforded by methanolic solutions, in order that our approaches to data retrieval might be tested prior to attempted examination of the polyelectrolytes in aqueous media. The results of TRAMS experiments on methanolic solutions of PMAA [bearing fluorescent labels based upon copolymerized 1-vinylnaphthalene (1-VN) and acenaphthylene (ACE)] and of PAA [labeled with 1-VN] are presented below.

## 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 ($10^{-2}$ Torr) immediately prior to use.

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Table I
Molar Mass Data

| polymer | $M_n/k$ | $M_w/k$ |
|---|---|---|
| PAA/1-VN | 164 | 302 |
| PMAA/1-VN | 56 | 105 |
| PMAA/ACE | 620 | 1400 |

Acenaphthylene, ACE, (Aldrich) was recrystallized from ethanol and triply vacuum sublimed. Acrylic acid (Koch Light) and methacrylic acid (Aldrich) were prepolymerized (UV radiation) and fractionally vacuum distilled immediately prior to use. Benzene (BDH) was purified by fractional distillation. Methanol (Aldrich, spectroscopic grade) was used as supplied. Fluorescently labeled samples of poly(methacrylic acid) (PMAA) and poly(acrylic acid) (PAA) were prepared by copolymerization of the appropriate monomer 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 reprecipitation from methanolic solutions into diethyl ether.

**Characterization.** Molar masses of the labeled polymers were determined using size exclusion chromatography (SEC). The sample volume 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 $min^{-1}$. Relative molar masses were estimated using poly(ethylene oxide) calibration standards. The SEC system incorporated a Waters Associates Model 6000 pump, a Rheodque injector a Waters Associates Ultrahydrogel mixed gel (2- × 30-cm) columns, and a Waters Associates Model 410 RI detector. The resultant molar mass data are listed in Table I. Fluorescence spectroscopy (Perkin-Elmer MPF3L instrument) was used to verify the presence of "naphthyl" labels and the absence of emission from unreacted materials or other species.

**Instrumentation.** Excitation of fluorescence was achieved using synchrotron radiation from the SRS, Daresbury, U.K. 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 the single bunch mode, delivers pulses of radiation separated, in time, by the orbit period of 320 ns. Time-resolved data were obtained using the method of time-correlated single photon counting. Use of a Philips XP2020Q photomultiplier in the detection system produces an instrument response function of fwhm of 600 ps (compared to the 200-ps pulses produced by the synchrotron).

Fluorescence lifetimes were estimated from decays analyzed at the "magic angle" of 54.7° with respect to that of the vertically polarized excitation.

Fluorescence anisotropy measurements involved collection of $I_\parallel(t)$ and $I_\perp(t)$ using a "toggling" procedure in which the analyzer was rotated sequentially through 90° with simultaneous switching of memory quarters in the MCA (Inotech 5400). Excitation and emission wavelengths of 295 and 340 nm, respectively, were employed.

**Analysis Procedures.** With the exception of direct analyses of "raw" anisotropy data, time-resolved fluorescence response curves were "deconvoluted" using a suite of computer programs (IBH Consultants, Ltd., Glasgow, U.K.) using an iterative, nonlinear, least squares reconvolution procedure. "Goodness of fit" was assessed using a variety of statistical criteria: reduced $\chi^2$, randomness of residuals, serial correlation coefficient, and autocorrelation of residuals. Details of these functions may be found elsewhere (e.g. ref 20 and references therein). Procedures for the recovery of relaxation parameters from TRAMS data are discussed, in detail, in the Results and Discussion.

## Results and Discussion

A major problem which can be encountered in the application of TRAMS concerns the retrieval of reliable relaxation data from the experiment. In particular, it is important, when the applicability of a given model for the anisotropy function is to be tested (especially at short times following excitation), that a reliable means of deconvolution be adopted to compensate for the distortions of the fluorescence introduced by the instrumental response and the finite width of the excitation pulse.

Table II
Correlation Times for Segmental Relaxations As Recovered by Direct Analysis of $R(t)$

| polymer | $\tau_r$/ns | $\chi^2$ |
|---|---|---|
| PAA/1-VN | 1.3 | 1.1 |
| PMAA/1-VN | 3.4 | 1.1 |
| PMAA/ACE | 3.6 | 1.3 |

The time-resolved fluorescence experiment involves analyzing the orthogonal components of intensity, $I_\parallel(t)$ and $I_\perp(t)$, in planes parallel and perpendicular, respectively, to the plane of vertically polarized excitation. The *observed* anisotropy $R(t)$ is then given by

$$R(t) = \frac{I_\parallel(t) - I_\perp(t)}{I_\parallel(t) + 2I_\perp(t)} = \frac{D(t)}{S(t)} \quad (1)$$

$R(t)$, constructed according to eq 1, not only contains the reorientational information which we seek but also reflects the distortions inherent in the intensity functions $I_\parallel(t)$ and $I_\perp(t)$. Unfortunately, reconvolution analysis does not apply to the anisotropy function. Therefore other approaches must be adopted in elucidation of the true form of the anisotropy, $r(t)$. Some of the options are discussed below.

**(1) Direct Analysis of $R(t)$ (DA).** Direct analysis of the raw $R(t)$ data can yield reliable estimates of relaxation times which are long compared to the width of the instrument response function or "prompt". The method offers a rapid, direct means of analysis but can be totally misleading in the treatment of data in which instrumental distortions are significant. For example, failure to account for such distortions can result in an *apparently* complex functional form for $R(t)$ when that of the true anisotropy, $r(t)$, is simple[5] (e.g. a single exponential).

Relaxation data for the various PMAA and PAA systems resultant upon iterative fitting to $R(t)$ using a single exponential model function are listed in Table II. The fits are adequate (as judged by the values of $\chi^2$ and the randomly distributed residuals). On this evidence, the application of a more complex mathematical model of the macromolecular dynamics could *not* be justified on statistical grounds. Figure 1 shows a typical fit to $R(t)$ for the PAA/1-VN system.

A major problem with this form of analysis is that it is impossible to derive a value for the intrinsic anisotropy, $r_0$, i.e. the anisotropy extant at "zero time" following excitation. This is a significant disadvantage since the anisotropy is subject, at early sampling times, to distortions induced by the finite width of the excitation pulse *and* the associated instrumental response, the presence of scattered excitation (which has a positively polarizing influence upon the observed data), timing instabilities of the excitation pulse (particularly in the "rising edge" of the fluorescence response), and the general lack of definition of $R(t)$ which occurs at short times as a result of poor fluorescence signal to noise ratios extant at times close to the onset of excitation. This situation introduces a degree of subjectivity to the data analysis: The experimenter is faced with a choice of "start channel" for analysis of the data. In the current analyses, we have initiated fitting at a value of $R$ broadly concurrent with that "expected" for $r_0$ from previous steady-state fluorescence polarization investigations of these polymer systems (but using other fluorescent labels[11]) and from studies of 1-VN and ACE labeled polymers dissolved in other media.[21-23]

Within the limits imposed by such a subjective form of analysis, the $R(t)$ data are consistent with adoption of a

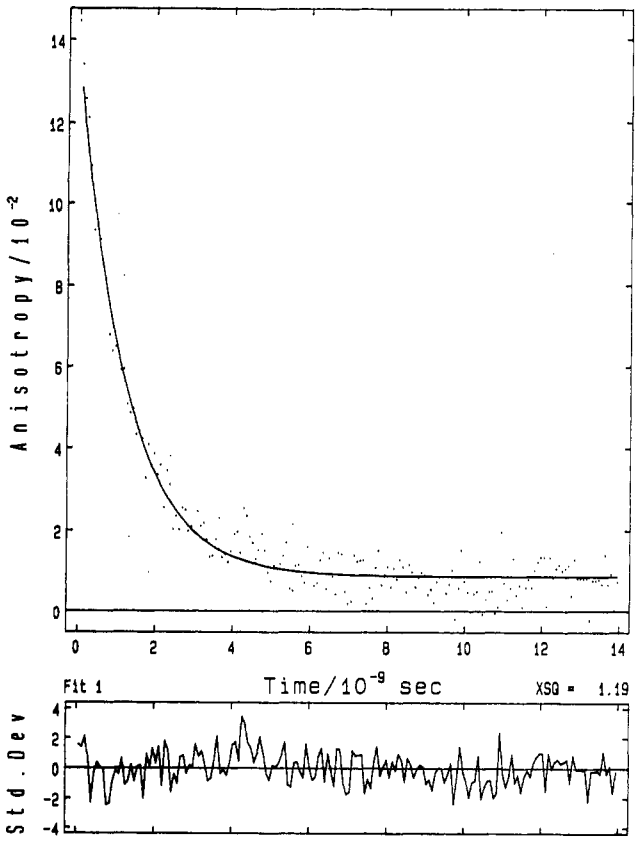


**Figure 1.** Direct analysis of $R(t)$ for PAA/1-VN ($10^{-3}$ wt %) in methanol at 298 K.

single exponential model for the decay of $R(t)$. Were this to represent a reasonable description of the decay of the true anisotropy function, $r(t)$, it would imply a functional form for the anisotropy decay of the type

$$r(t) = r_0 \exp(-t/\tau_r) \tag{2}$$

where $r_0$ is the "intrinsic anisotropy" of the chromophore and $\tau_r$ is the rotational correlation time. The correlation time resultant upon such analyses of $R(t)$ is independent of the initial channel of analysis (at *later* times) and the fits are supported in terms of the applied (see Experimental Section) statistical criteria. However, at earlier times of sampling, deviations from adequate fitting criteria are apparent.[23] These deviations might result from the susceptibility of $R(t)$ to the distortions evident at early times of data sampling, as discussed above. Alternatively, the inadequacy of a single exponential decay function in modeling $R(t)$ might result from the effect of fast relaxation processes of the macromolecule/label upon the overall behavior of the poly(acid) in solution.

Relatively little information is available in the literature for comparison with the data produced in the current study. Anufrieva et al.[11,24] have used steady-state fluorescence polarization methods to study the relaxation behavior of PMAA in methanol. The fluorescent labels employed were a (9-anthrylmethoxy)carbonyl species (incorporated as a "side group" of the polymer chain) and anthracene (incorporated as a main chain substituent covalently bound across the 9,10-positions). The resultant relaxation data are not directly comparable with those obtained in the current time-resolved studies: The motion of the (9-anthrylmethoxy)carbonyl label will reflect not only that of the polymer segments to which it is attached but also rotations about the (flexible) ester bonds linking it to the macromolecule. The anthracene unit, linked in a "bridging" fashion across its 9,10-positions, is disposed such that its absorption and emission vectors are aligned along the direction of the main-chain axis. This contrasts to the disposition of the chromophores used in the current work. The fluorescence for both the 1-VN and ACE labels is largely polarized in a perpendicular fashion with respect to the polymer backbone. Consequently, the naphthyl chromophores are expected to "sense" the polymer motion in a fashion comparable to that sampled in dielectric relaxation experiments by dipole components oriented orthogonally to the polymer chain.[25]

The Russian steady-state polarization studies[11,24] furnish relaxation times of 7.4 and 19 ns using the (9-anthrylmethoxy)carbonyl and anthracene main-chain labels, respectively. These, in turn, would correspond to rotational correlation times, $\tau_r$, of ca. 2.5 and 6.3 ns, respectively. Clearly, these values span that of 3.5 ns, resultant upon direct analysis of $R(t)$ of the current PMAA/1-VN and PMAA/ACE data. Reasonable agreement might therefore be considered to have been attained if it is considered that the longer sampling time (or higher $T/\eta$) polarization data accessed in the steady-state experiments on the (9-anthrylmethoxy)carbonyl-labeled system is taken to reflect the cooperative mechanisms which might "overtake" those of side groups under the high-frequency conditions represented by fluorescence determinations. Supportive evidence for cooperative involvement of flexible ester functionalities with that of the "host" macromolecule is afforded by early, steady-state studies of the relaxation of poly(methyl methacrylate) labeled with naphthyl methacrylate labels.[21] As we have observed above, the main-chain-linked anthracene label might well be expected to furnish different (but complementary) information on the motions of PMAA.

Anufrieva et al.[11] have, in addition, used steady-state polarization techniques to study the relaxation of PAA in methanol. A value of 2.3 ns is quoted for the relaxation time, "reduced" to correspond to a microviscosity of 0.38 cP.[11] Adjustment of this value to correspond to the viscosity of methanol at 298 K (0.55 cP) yields a relaxation time of 3.3 ns which, in turn, translates into a value of 1.1 ns for $\tau_r$. Given the deficiencies of the steady-state approach and the potential for mismatch of the data generated by (9-anthrylmethoxy)carbonyl data (as discussed above) with those of the 1-VN label, the agreement achieved is remarkable.

In terms of the "main thrust" of this paper, it is necessary to direct further discussion of the current data toward consideration of the alternative means of data retrieval, as addressed in sections 2–4 below.

**(2) Direct Vector Reconvolution (DVR).** In direct vector reconvolution, suitable model functions are chosen to represent the "true" fluorescence intensity profiles, $i_\parallel(t)$ and $i_\perp(t)$. These models are used in reconvolution analyses with the measured prompts $P_\parallel(t)$ and $P_\perp(t)$ to generate trial fits to $I_\parallel(t)$ and $I_\perp(t)$, respectively, which are then optimized.

In the simplest case of a spherical rotor characterized by a single reorientational correlation time, $\tau_r$, and a single fluorescence decay time, $\tau_f$, $i_\parallel(t)$ and $i_\perp(t)$ are given by

$$i_\parallel(t) = \exp(-t/\tau_f)[1 + 2r_0 \exp(-t/\tau_r)] \tag{3}$$

$$i_\perp(t) = \exp(-t/\tau_f)[1 - r_0 \exp(-t/\tau_r)] \tag{4}$$

Since these functional forms can be used as models in reconvolution analyses to fit $I_\parallel(t)$ and $I_\perp(t)$, $\tau_r$ can, in principle, be determined from either $I_\parallel(t)$ or $I_\perp(t)$. In practice, difficulties can be encountered[26,27] (even when the value of $\tau_f$ is "fixed" and set equal to that obtained in

**Table III**
**Data Representative of Attempted Data Retrieval by Reconvolution Analyses of $I_{\parallel}(t)$ and $I_{\perp}(t)$[a]**

| polymer | type of analysis | $\tau_r$/ns | $\tau_f$/ns | $\chi^2$ |
|---|---|---|---|---|
| PAA/1-VN | whole curve [$I_{\parallel}(t)$] | 2.0 | 19.7 | 4.2 |
| | whole curve [$I_{\perp}(t)$] | 4.1 | 20.2 | 2.0 |
| | peak [$I_{\parallel}(t)$] | 2.1 | 18.8 | 1.8 |
| | global | 2.0 | 19.8 | 4.2 |
| PMAA/1-VN | whole curve [$I_{\parallel}(t)$] | 2.2 | 20.4 | 3.0 |
| | peak [$I_{\parallel}(t)$] | 2.4 | 19.6 | 1.5 |
| | global | 3.3 | 20.9 | 3.5 |
| PMAA/ACE | peak [$I_{\parallel}(t)$] | 4.3 | 18.9 | 1.4 |
| | global | | | would not converge |

[a] Peak fits are those in which fitting commences at the channel of maximum population. Global[28] fits were attempted both upon whole curves and from the "peak". Global data listed refer to whole-curve fits.

**Table IV**
**Correlation Times Characteristic of Segmental Motion, Recovered Using Impulse Reconvolution (IR) and Autoreconvolution (AR)**

| polymer | $\tau_r$(IR)/ns | $\tau_r$(AR)/ns |
|---|---|---|
| PAA/1-VN | 1.3 (±0.1) | 1.1 (±0.1) |
| PMAA/1-VN | 3.7 (±0.3) | 4.0 (±0.3) |
| PMAA/ACE | 4.3 (±0.3) | 4.2 (±0.3) |

independent fluorescence lifetime determinations). Problems, in fitting, may arise even if a "global"[28] analysis of $I_{\parallel}(t)$ and $I_{\perp}(t)$ is adopted. These observations are amply demonstrated by the current data by reference to Table III.

First, it should be noted that global analyses of the data sets corresponding to $I_{\parallel}(t)$ and $I_{\perp}(t)$, with "deconvolution" of the instrumental response function, in which both $\tau_f$ and $\tau_r$ are sought in totally "free fitting" (i.e. without any constraint being applied to the value of $\tau_f$) provides poor resolution of the two "lifetime" components, $\tau_f$ and $\tau_r$, recovered: (i) In some cases, convergence of $\chi^2$ was not achieved. (ii) In few cases could reasonable agreement between the values of $\tau_r$ retrieved in this approach and those resultant upon any of the other procedures adopted in this work (cf. Tables II and IV) be achieved. (iii) Reasonable agreement between the values of $\tau_f$ retrieved by global analyses of $I_{\parallel}(t)$ and $I_{\perp}(t)$ with those (ca. 18.3 ns for PAA/1-VN, 19.6 ns for PMAA/1-VN, and 19.0 ns for PMAA/ACE) recovered either from direct determinations or from analysis of $S(t)$ was achieved (within limits of ±1 ns in most cases). (iv) Poor discrimination between the $\tau_r$ values attributed to the PAA and PMAA macromolecules is apparent in the data. (v) Inspection of eqs 3 and 4 shows that $I_{\parallel}(t)$ contains more anisotropy information than does $I_{\perp}(t)$. Consequently, it might be considered that combination of $I_{\perp}(t)$ with $I_{\parallel}(t)$ in a global analysis could hinder retrieval of information with confidence. However, independent fitting to $I_{\parallel}(t)$ [and $I_{\perp}(t)$] did not constitute a more reliable means of extracting $\tau_r$ from the empirical intensity data. (vi) Restriction of the "fitting range" to data channels corresponding to that of maximum population in either $I_{\parallel}(t)$ or $I_{\perp}(t)$ did not markedly affect the resultant values of $\tau_r$ or $\tau_f$.

The problems inherent in the DVR approach will be addressed in a separate communication with particular reference to the poly(methyl methacrylate) and poly(methyl acrylate) systems dispersed in dichloromethane as solvent.[26] In connection with the current work, it should be noted with respect to eqs 3 and 4 that modeling of the observed fluorescences $I_{\parallel}(t)$ and $I_{\perp}(t)$, in the instance whereby a single exponential decay of $r(t)$ is assumed, requires the adoption of double exponential models for both $i_{\parallel}(t)$ *and* $i_{\perp}(t)$. The "lifetime", $\tau_1$ and $\tau_2$, resultant upon such analyses are related to the anisotropy information in a manner such that $\tau_1 = \tau_f$ and $\tau_2^{-1} = \tau_r^{-1} + \tau_f^{-1}$ (cf. eqs 3 and 4). In consequence, determination of $\tau_r$ requires that $\tau_2$ is determined with high precision within dual exponential analyses of $I_{\parallel}(t)$ and $I_{\perp}(t)$. In order that a value of $\tau_r$ of 2 ns might be discriminated aqainst that of 4 ns, resolution of a $\tau_2$ value of 3.3 ns against that of 1.8 ns in a dual exponential analysis in which $\tau_f$ = 18 ns would be required. This is clearly an extremely stringent restriction which (demonstrably) cannot be met in analysis of the current data (given the results of other forms of data retrieval; cf. Table IV) despite the high quality of signal to noise ratio afforded using the synchrotron excitation and single photon detection methods applied.

Finally, it should be noted that even when $\tau_f$ is constrained to a "fixed" value corresponding to that of the fluorescence lifetime of the chromophore label, dual exponential analyses are *not* capable of resolving $\tau_r$, adequately, in these polymer systems.

**(3) Impulse Reconvolution[29,30] (IR).** In this, the most reliable method developed to date for the recovery of rotational orientation information from TRAMS data, the analysis procedure comprises two parts: The sum function $S(t)$ is analyzed using a suitable (i.e. one which will produce a fit of statistical adequacy) model function. If the best fit to $S(t)$ is represented by the impulse response function $\{I_s(t)\}$, it is obvious from the definition of the anisotropy

$$r(t) = d(t)/s(t) \tag{5}$$

that

$$\{I_r(t)\}\{I_s(t)\} = \{I_d(t)\} \tag{6}$$

Consequently, if a functional form is adopted to model $r(t)$, it may be combined with $\{I_s(t)\}$, according to eq 6, in a fitting procedure in which $\{I_r(t)\}$ is generated by optimization of the comparison with the empirical difference data, $D(t)$. The resultant "best fit", $\{I_r(t)\}$, allows a judgement to be made regarding the complexity of the anisotropy decay kinetics and furnishes, within the limits of the adopted model, rate parameters characteristic of the relaxation process.

The values of $\tau_r$ which result from impulse reconvolution analyses, in which the anisotropy is assumed to decay according to eq 2, are shown in Table IV. Reconvolution fitting to $D(t)$ was performed over all information from the channel corresponding to that of maximum population in $D(t)$. A typical fit is shown in Figure 2. [The impulse response function, $\{I_s(t)\}$, used in such fits was that obtained by multiexponential fitting to the experimentally derived sum function $S(t)$.] Analyses in which fitting was initiated at earlier channels in $D(t)$ resulted in poorer fit statistics. In particular, correlation between the residuals was apparent in the fitting region of the "rising edge" of $D(t)$. This may be a consequence of one or a combination of the following effects: (i) The anisotropy decay may not be strictly exponential. The polymer relaxation data may contain a contribution from a very fast reorientational process. (ii) Despite careful attention to monochromation of excitation and emission, the fluorescence data may be distorted, at early times, through the presence of a small amount of scattered excitation. In support of this proposition is the fact that the empirical anisotropy data, $R(t)$, sampled at early times following excitation, exhibit a smooth decay from values in excess of their theoretical maximum ($r_0$) for a random distribution of fluorescent

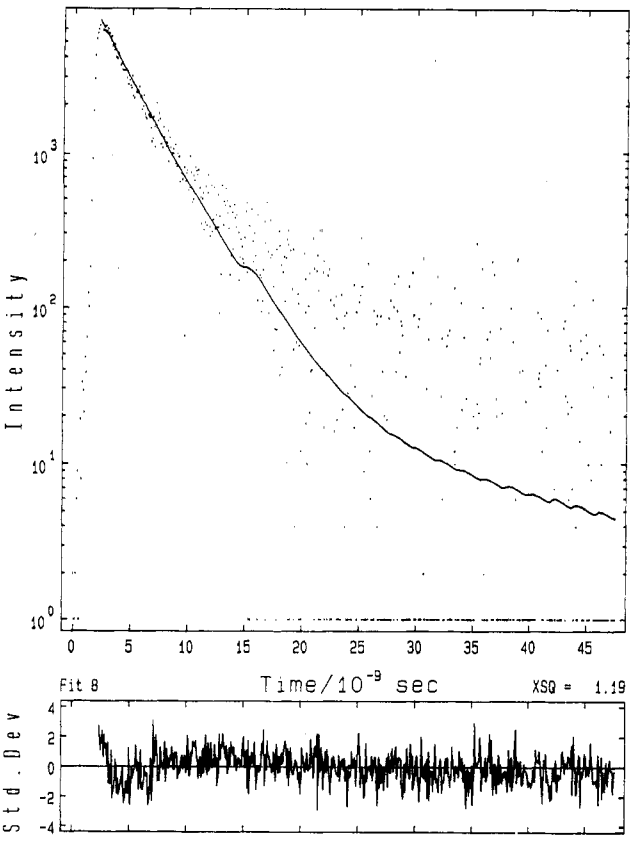


**Figure 2.** Difference function, $D(t)$, and impulse convolution fit for PMAA/ACE ($10^{-3}$ wt %) in methanol at 298 K.

species. (iii) The excitation pulse generated by the SRS might be subject to "short time" instabilities in *its* rising edge which would inhibit reliable fitting of fluorescence data in this region.

Given that the relaxations of the fluorescence labels are at least dominated to a large extent by a single component, the values of $\tau_r$ shown in Table IV can be taken as characteristic of the behavior of the macromolecules studied. We see that the estimates of $\tau_r$ afforded by IR analyses are, in all cases, slightly larger than those furnished by direct analyses of $R(t)$ (cf. Table II). Furthermore, the value of $\tau_r$ for the PMAA/1-VN system does not agree particularly well with that for PMAA/ACE. The reason for this is not immediately apparent. It might be that the imperfect polarization along the short axis of the naphthalene molecule would result in loss of anisotropy as a result of a degree of rotation of the 1-VN label about its bond of attachment to the polymer backbone. (This would only constitute a source of additional depolarization of the emitted radiation, provided this motion of the label did not become cooperative with that of the segmental motion of the polymer even at the high effective test frequencies accessed in fluorescence experiments.) Alternatively, it could be that the mode of attachment of the ACE label via the "bridge link" serves to impede the very segmental relaxation that we seek to study.

Whilst the reasons for the discrepancies between the $\tau_r$ values for PMAA/1-VN and PMAA/ACE cannot be identified at present, it may be that the difference is some artifact of the analysis procedure: It is to be noted that the values of $\tau_r$ for both labeled PMAA samples obtained by the method of autoreconvolution (described below) are self-consistent *and* agree reasonably well with that resultant upon IR analysis of the PMAA/ACE data.

**(4) Autoreconvolution[31] (AR).** In the method of autoreconvolution, functional forms appropriate to the decays of fluorescence [$s(t)$] and anisotropy are assumed. These, in turn, lead to assumed forms for $i_\parallel(t)$ and $i_\perp(t)$. [In the instance of first-order decays of fluorescence and emission anisotropy alike, $i_\parallel(t)$ and $i\perp(t)$ would be represented by eqs 3 and 4, respectively]. The method "recognizes" the fact that $i_\parallel(t)$ serves to augment $i_\perp(t)$ (implicit in eq 4) and thence functions in a manner similar to that of an excitation "prompt" feeding a fluorescence decay. $i_\parallel(t)$ may then be used to deconvolute $i_\perp(t)$ according to

$$I_\perp(t) = I_\parallel(t) \otimes m(t) + aI_\parallel(t) \tag{7}$$

where $m(t)$ is a model function of suitable complexity, as dictated by the functional forms of $i_\parallel(t)$ and $i_\perp(t)$ and

$$a = \frac{(1 - r_0)G}{(1 + 2r_0)} \tag{8}$$

Full details of the method are to be published.[32]

When $i_\parallel(t)$ and $i_\perp(t)$ are described by eqs 3 and 4, respectively, $m(t)$ is single exponential in form. The "G factor"[33] introduced in eq 8 corrects for differences in transmission and detection efficiencies in the determination of $I_\parallel(t)$ and $I_\perp(t)$. [In the current experiments, it has been shown that the optical/analyzer/photomultiplier combination, applied in the synchrotron-generated TRAMS, results (at all excitation/emission wavelengths accessed) in a G factor of unity, within experimental error. (G was estimated by comparison of the emission intensities analyzed in planes perpendicular and parallel, respectively, to the plane of *horizontally* polarized excitation.)]

The autoreconvolution approach not only allows TRAMS data to be resolved into relaxation parameters within the limits of a given model but also provides a rigorous test [through the link of the functional form appropriate to $m(t)$ to those descriptive of $i_\parallel(t)$ and $i_\perp(t)$] of the applicability of the relaxation model adopted. [The autoreconvolution approach is, in fact, the youngest of a family of deconvolution procedures having a similar concept within a convolution kinetics approach. Examples of previous applications in which one fluorescence function is used to deconvolute another include the "reference compound" approach to deconvolution of fluorescence data (e.g. see refs 34–36) or the detection and treatment of "transient effects" in excimer and/or energy-transfer kinetics (e.g. see refs 37–39). Martinho and Winnik[38] have shown that the functional form of eq 7 may be used in deconvolution of kinetic data in time-resolved studies of excimer formation {wherein the "excimer intensity" $I_E(t)$ replaces $I_\perp(t)$ and the "monomer intensity" $I_M(t)$ replaces $I_\parallel(t)$ in eq 7}. Their suggestion that eq 7 may be used to test the applicability of the model function $m(t)$ {itself dependent upon the mathematical functions assumed in description of $I_E(t)$ and $I_M(t)$} is an exact analogue of the application of autoreconvolution to the testing of the rigor of the modeling of the decay kinetics of anisotropy.]

$\tau_r$ data resultant upon application of the AR method are shown in Table IV. Typically, the entire fluorescence response curve, $I_\parallel(t)$, is used to deconvolute the corresponding $I_\perp(t)$, analyzing from the channel in $I_\perp(t)$ corresponding to that of maximum population in $I_\parallel(t)$. A representative example of such a fit is shown in Figure 3. [As in the case of the IR analyses described above, this procedure was adopted to avoid the effects of distortions in the rising edges of fluorescence response curves induced by excitation "leakage", fast relaxation processes, and/or "time-jitter" (vide supra, section 3).] The "goodness of fit" obtained in AR analyses (cf Figure 3b) justifies the adoption of a single exponential model for description of the anisotropy decay. Reasonable agreement between the

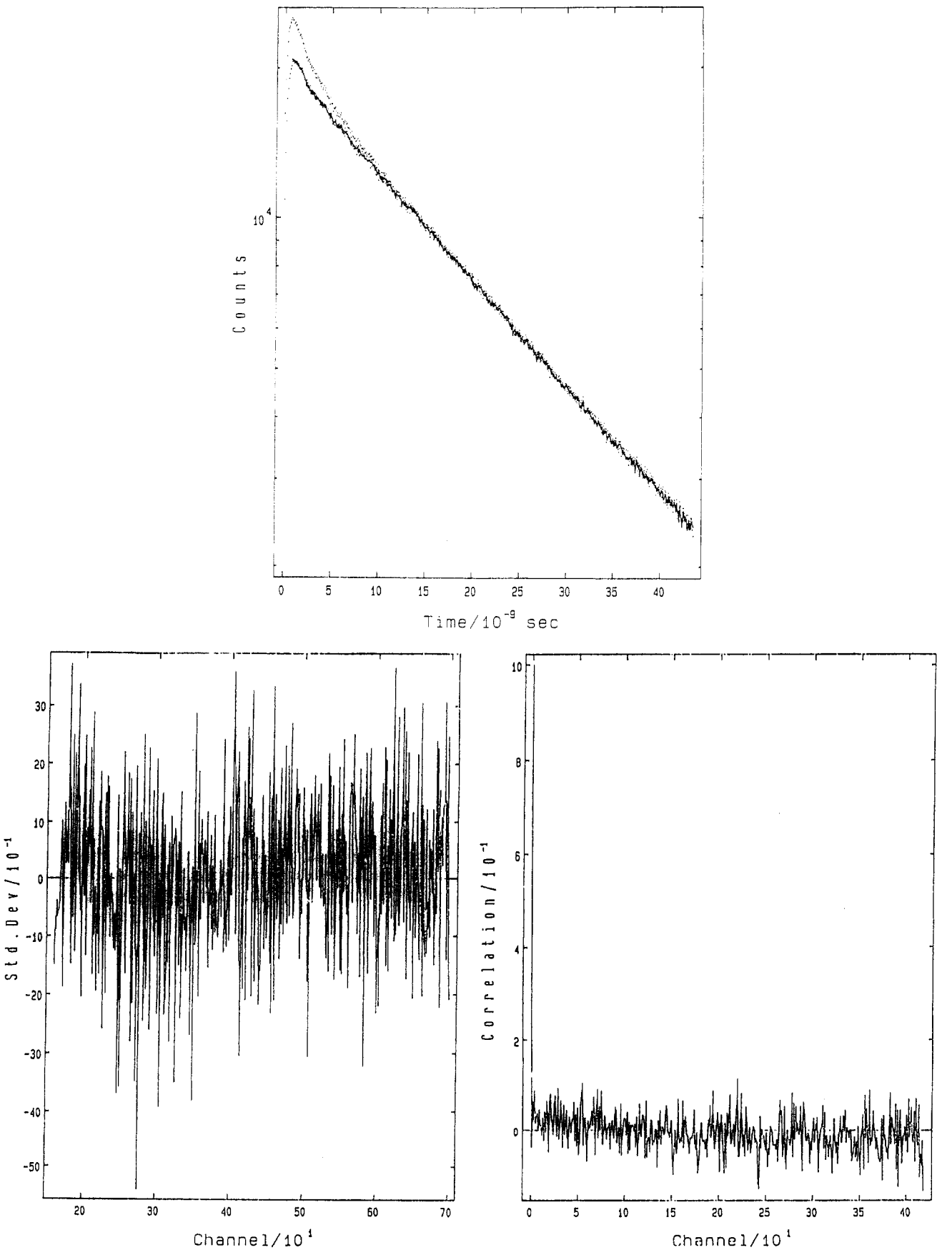


**Figure 3.** (a, Top) $I_{\parallel}(t)$ (upper curve) and $I_{\perp}(t)$ (lower curve) for PMAA/1-VN ($10^{-3}$ wt % in methanol at 298 K) and autoreconvolution fit to $I_{\perp}(t)$. (b, Bottom) Plots of residuals (left diagram) and autocorrelation of residuals (right diagram) for the AR fit to $I_{\perp}(t)$ shown in Figure 3a.

$\tau_r$ value obtained for PAA/1-VN and those from the DA and IR approaches is achieved. In addition (as commented upon above) good agreement is obtained between the relaxation data for the PMAA samples bearing the different labels (1-VN and ACE) and that obtained from IR analysis of the PMAA/ACE fluorescence data.

## Conclusions

(1) Fluorescence anisotropy data, as analyzed by a series of data retrieval procedures, do not substantiate adoption of a relaxation model which is more complex than that of a single exponential function, in description of the behavior of PAA or PMAA in dilute methanolic solutions.

(2) For the PAA/1-VN and PMAA/1-VN and PMAA/ACE systems in methanol, direct analysis of the "raw" anisotropy data provides a reasonably reliable means of retrieval of segmental correlation times: Fair agreement is achieved between the values of $\tau_r$ generated in this manner with those obtained using the more rigorous reconvolution methods of IR and AR.

(3) The segmental motion of PAA in dilute methanolic solutions is characterized by a correlation time of ca. 1.3 ns. That of PMAA is characterized by a $\tau_r$ value of ca. 4.2 ns. The difference in intramolecular segmental relaxation rates of PAA and PMAA reflects the steric hindrance imposed by the backbone methyl substituent of PMAA upon the backbone relaxation process.

(4) The DVR method was singularly unsuccessful in attempts to recover, with conviction, macromolecular relaxation data for these poly(acid) systems.

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**Registry No.** PAA (homopolymer), 9003-01-4; PMA (homopolymer), 25087-26-7.