Fluorescence polarization changes in electrically oriented solutions of dye-tagged polymers*

P. J. Ridler and B. R. Jennings
Electro-Optics Group, Physics Department, Brunel University, Uxbridge, Middlesex, UK
(Received 5 December 1977; revised 20 February 1978)

Dilute aqueous solutions of poly(methacrylic acid) ($M = 7.8 \times 10^5$) and sodium polystyrene sulphonate ($M = 1.3 \times 10^5$) when tagged with the dye acridine orange, have been subjected to pulsed electric fields. Changes have been recorded for the polarized components of the fluorescence with each polymer system, during the application of the electric fields. From these fluorescent changes, which were recorded for wavelengths above 515 nm when radiation of 488 nm was incident, the nature of binding of the dye to these polymers has been estimated. For each system, the planar dye molecules appear to bind parallel to the polymer backbone.

INTRODUCTION

Fluorescence is a two-fold optical property which involves the absorption of incident light followed by emission at a lower frequency. This double process has its origins in electronic transitions which have fixed directions with respect to the geometry of the fluorescent molecule. Their directions can in principle be located in space by measuring the intensity and polarization state of the light emitted when plane polarized light is incident on the system. Polymers are seldom fluorescent in the visible spectral region. In such cases, it is convenient to incorporate dye molecules whose orientation can be established.

More relevant to this study is the fact that the dye molecules can act as markers for any changes in the orientational order of polymer molecules. For solid polymers, this has been admirably described in a paper by Nobbs *et al.*[1]. Dilute solution studies of oriented dye-tagged polymers have not been so well rewarded. Claesson and Odami[2] attempted adventurous experiments in which they searched for shear induced polarization changes of fluorescein-tagged cellulose derivatives. They detected no fluorescence changes. Weill and Sturm[3] developed equations for fluorescent changes of solutions of rigid rod-like macromolecules when oriented in electric fields. Their related experiments on DNA solutions indicated such changes, but with rather poor sensitivity. Recently, we have designed and assembled a highly sensitive apparatus[4] with which transient changes in the polarized components of fluorescence from dilute solutions of dye-tagged native DNA were obtained with ease as the solutions were subjected to short duration pulsed electric fields. Such high sensitivity has encouraged us to investigate more complicated, flexible polymers in dilute solution.

Poly(methacrylic acid) (PMA) and sodium polystyrene sulphonate (SPS) have provided model systems for the study of polyelectrolytes in aqueous solutions. The nature of the association of cationic dyes with these two polymers has been extensively studied[5–12]. Aqueous solutions of PMS are particularly interesting as the molecules adopt a compact conformation at low pH and an extended, charged form at approximately neutral pH[13]. Under such conditions, acridine orange, which is the dye used in the present study, binds strongly due to coulombic attraction[7]. With aqueous SPS solutions, the polymer molecules are also negatively charged and interact strongly with cationic dyes[8,10,11]. From all these studies, no information is available on the direction of the dye molecules relative to the polymer geometry.

The object of the present study was two-fold. Firstly, to manifest the first transient electrically induced changes in the polarized components of fluorescence of synthetic polymers in dilute solution. Secondly, to reveal any specific orientational order of the dye molecules relative to the polymer backbone in PMA and SPS solutions.

EXPERIMENTAL

Atactic PMA was obtained as a colourless gel from Dr S. R. Oldland of Imperial Chemical Industries (Plastics) Ltd. A molecular weight of 7.8×10^5 was evaluated for this material from viscosity measurements on 0.002 M HCl solutions at 30°C in conjunction with the Mark–Houwinck type relationship $[\eta] = 66 \times 10^{-3} M^{0.5}$ cm^3/g given by Katchalsky and Eisenberg[14]. White crystallites of SPS were kindly donated by Dr M. A. Toynton of Zerolit Ltd. Viscosity measurements were performed on 0.005 M NaCl aqueous solutions at 25°C. Here again, a viscosity–molecular weight equation $[\eta] = 2.3 \times 10^{-3} M^{0.93}$ was used[15] to evaluate the molecular weight of 1.35×10^5. The acridine orange was a commercially purified sample purchased from G. T. Gurr Ltd. For the electro-optic experiments PMA and SPS were dissolved by slow agitation for 24 h and 2 h, respectively, to give solutions of pH 6.0 and 6.2 respectively, in filtered deionized water which had a specific conductivity of less than $2 \times 10^{-6} \Omega^{-1} \mathrm{cm}^{-1}$. Aqueous solutions of AO were added to these solutions to give final polymer concentrations of 4.5×10^{-5} g/cm^{-3} containing 1 dye per 150 monomers for the PMA solution and 1 dye per 300 monomers for the SPS solution. All experiments were performed at 21 ± 1°C.

---

[*] Presented at the Polymer Physics Group (Institute of Physics) Biennial Conference, Shrivenham, September 1977.

0032–3861/78/1906–0627$02.00

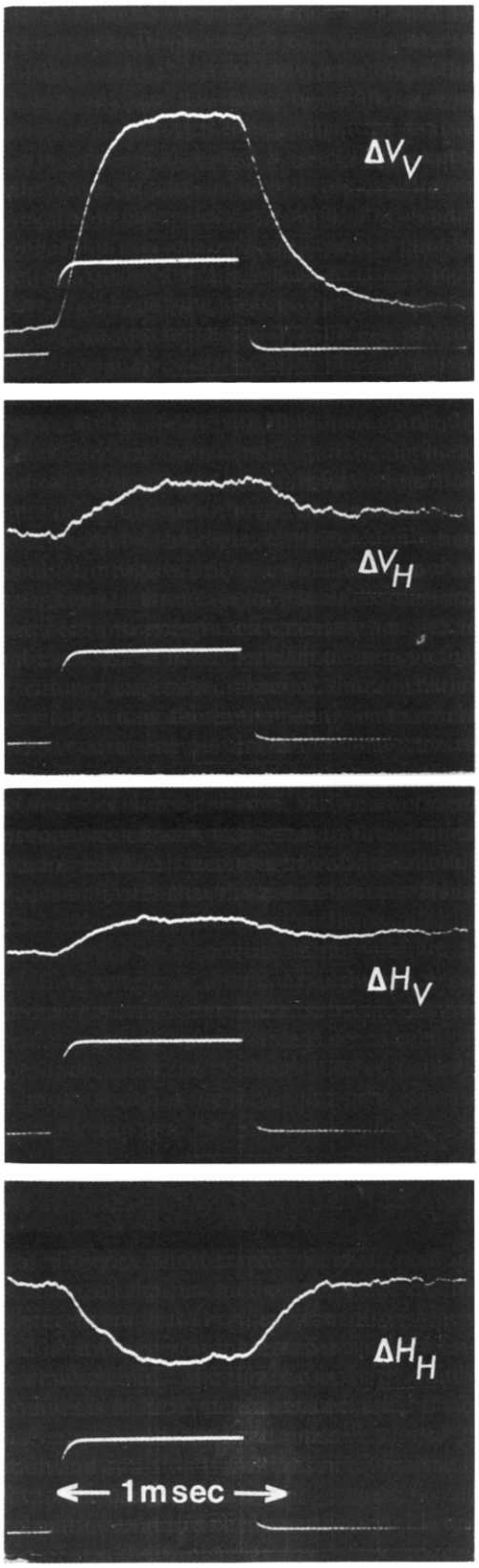

Figure 1 Transient changes in the polarized components of fluorescence for the poly(methacrylic acid) – acridine orange complex. In each frame, the lower trace is the applied field of 5.6 kV/cm and the upper trace the optical response. Time runs from left to right and the zero field intensities were made the same for each component. Relative magnitudes are $(\Delta V_V/V_V) = +0.31$; $(\Delta V_H/V_H) = +0.07$; $(\Delta H_V/H_V) = +0.06$; $(\Delta H_H/H_H) = -0.12$. Excitation wavelength was 488 nm and detection was for wavelengths exceeding 515 nm

For the fluorescent experiments, the solution was held in a glass cell of square cross-section which was fitted with steel electrodes arranged so that the electric field vector was in the vertical direction. Pulsed direct current fields of up to 800 μsec duration and 6 kV/cm amplitude were applied to the solutions. An argon ion laser emitted a beam of 488 nm wavelength which was then polarized either horizontally or vertically as required using a pair of Fresnel rhombs and a Glan–Taylor polarizing prism. This beam was then attenuated as required before being incident on the solution and exciting the fluorescence. Two detection limbs were mounted coaxially with the cell and received the light emitted from the solution under test at right angles to the incident beam. Each of these limbs was equipped with a pair of collimating apertures, a Glan–Thomson analysing prism, a colour glass cut off filter and a high sensitivity, low noise photomultiplier. Using two such limbs, we could record simultaneously both the horizontally and vertically polarized components of the emitted radiation for a given polarization state of the incident laser beam. With this arrangement, a total of four polarized components of the fluorescence could be recorded. These are designated V_V, V_H, H_V and H_H, where the capital letter indicates the vertical (*V*) or horizontal (*H*) polarization state of the incident light beam and the subscript refers to the vibration direction of the analysed beam. The changes in the amplitudes of any of these components upon application of the electric field are designated by the prefix Δ. These changes appear as fast transient effects when rectangular pulsed fields are applied. Upon establishment of the pulse, the electric field interacts with any permanent or induced electric dipoles of the polymer. The resulting orientation and deformation of the polymer coil is opposed by Brownian influences, and thus requires a finite time. The bound dye follows this process and manifests it through the observed fluorescence components. Orientational order is then established in the electric field and a molecular equilibrium condition is reached accompanied by steady values in the fluorescence components. Termination of the field pulse initiates a return to molecular disorder with the associated decay of each of the fluorescent component amplitudes to their pre-field values. The amplitudes and rates of these transient effects thus contain information on the molecular order and dynamics in the applied field.

RESULTS AND DISCUSSION

Figures 1 and *2* show typical oscilloscope photographs of the transient changes in the 4 polarized fluorescence components for each polymer system. From these Figures, it is seen that the establishment and decay processes involve rate processes of only fractions of a millisecond. The lifetime of the excited state of bound acridines is of the order of tens of nsec[16]. Hence, the changes of *Figures 1* and *2* indicate anisotropic ordering of the absorption and emission transitions of the dye molecules with respect to the dipolar axes of the polymers. The alternative explanation that the phenomena had their origins in the rotation of free dye molecules was eliminated by complete absence of the effects for dye solutions with no polymer present.

Qualitatively, the fluorescence component changes in *Figures 1* and *2* are similar both in sign and relative magnitude for each polymer system. The binding geometry of the acridine orange is likely to be the same for each polymer. In principle, it is possible to evaluate explicitly the directions of the absorption and emission transitions of the dye mole-

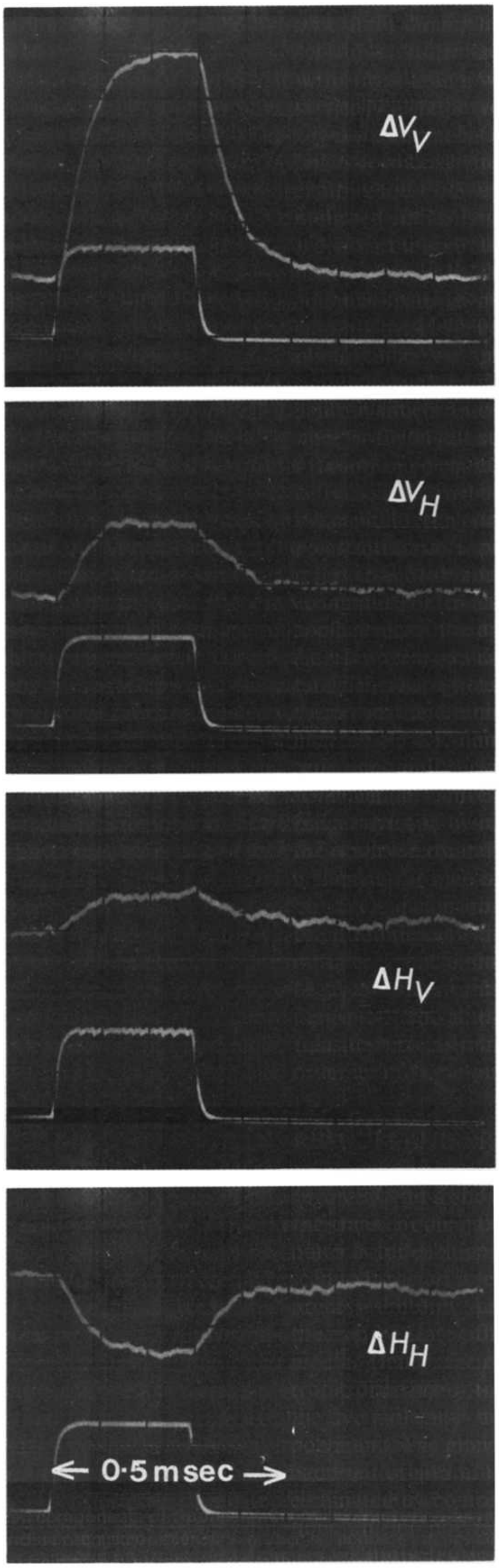

Figure 2 Transient changes in the polarized components of fluorescence for the sodium poly(styrene sulphonate) – acridine orange complex. Details as in *Figure 1* caption except that the applied field was 6.0 kV/cm and, for this polymer system, $(\Delta V_V/V_V) = +0.34$; $(\Delta V_H/V_H) = +0.10$; $(\Delta H_V/H_V) = +0.05$; $(\Delta H_H/H_H) = -0.11$

cules relative to the resultant dipole moment of the polymer. To date, relevant theory exists only for uncharged, rigid rod molecules which exhibit a quadratic dependence of the fluorescence component changes on the field strength at low field strength. This is equivalent to Kerr law behaviour[17] in electric birefringence. One also needs to know the direction of the resultant electric dipole moment of the molecule for a complete spatial evaluation. These polymers are not rigid rods as is shown by the exponent of the Mark–Houwinck expressions not being 1.8. Neither are they uncharged. Furthermore, in agreement with the electric birefringence studies of Nakayama and Yoshioka[18], *Figure 3* demonstrates the lack of quadratic dependence of the electro-optical effects on field strength for SPS solutions. Nevertheless, the ordering of the absorption and emission transition moments and hence the dye molecule orientations on the polymer may be estimated in the following way.

In conducting media such as water, the majority of polyelectrolytes orient with their major dimension along the applied electric field lines[17–22]. The mechanism for this is the anisotropic polarizability associated with the polarization of the cloud of counterions surrounding the polymer. This is enhanced by the rather extended configuration adopted by polyelectrolytes at the low concentration used in these experiments. With ever increasing field strength the polymer will tend to extend and orientate with the chain backbone drawing more nearly parallel to the applied field vector. With our experimental array, the polymers would tend partly to orient with their long axes increasingly parallel to the vertical direction. With vertically polarized incident light, the changes ΔV_V and ΔV_H are both seen to be positive (*Figures 1* and *2*). Hence, the *total intensity* of the fluorescence, which is proportional to the sum $[(V_V + \Delta V_V) + 2(V_H + \Delta V_H)]$ increases upon application of a vertical field. This indicates that the absorption process is more efficient

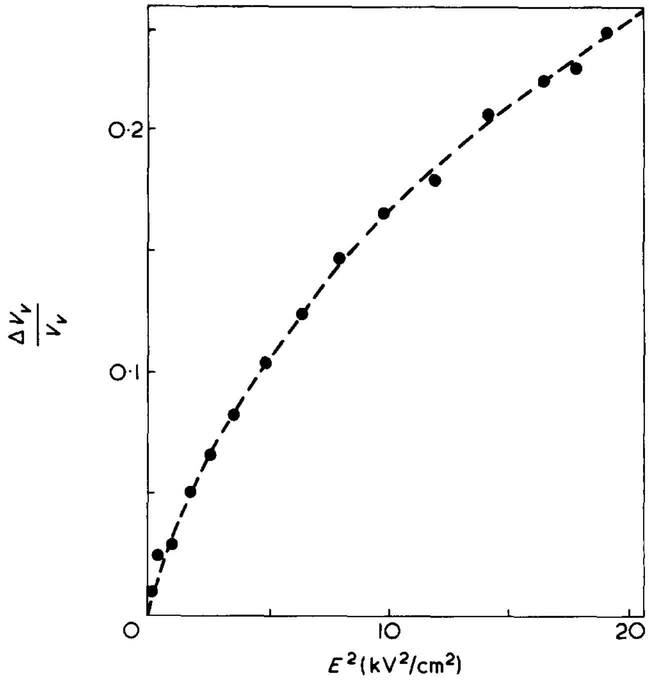

Figure 3 Typical field strength dependence of the relative change in a polarized component of the fluorescence. Data for the $(\Delta V_V/V_V)$ component of sodium poly(styrene sulphonate) complexed with acridine orange. Quadratic dependence on field strength is not in evidence

in the field and that the absorption transitions of the bound fluorescent dyes must align predominantly towards the direction of the electric vector of the laser beam. In our experimental arrangement, this is the vertical direction which is also the predominant direction of the long polymer axis in the field. The foregoing reasoning can be confirmed by a similar consideration of the component changes obtained using horizontally polarized incident light.

The predominant emission transition moment direction may be deduced from the *relative* amplitudes of the component changes. From the transients for each polymer system one notes that, whatever the polarization state of the incident beam, the corresponding vertically polarized emitted component is greater (i.e. more positive) than its horizontally polarized counterpart. In fact, $\Delta V_V > 2\Delta V_H$. The emission process is thus more efficient in the vertical direction. Hence the related transitions are predominantly associated with the vertical direction when the field is on. Like the absorption moment, this direction relates to that of the polymer backbone. With AO, the absorption and emission transitions are known to lie in the plane of the dye molecule[23] and to be parallel to the long axis of this three-membered ring planar molecule for visible light excitation[24]. Hence, the dye molecules must be predominantly ordered so that their long axes are more nearly parallel to the extended chains of the polymers.

From *Figures 1* and *2*, the rotary relaxation times (τ) following the field pulse termination may be evaluated from the decay rate of the fluorescence components. An approximate experimental average for a given polymer solution can be obtained[17] by considering the time taken for the fluorescence change to fall to e^{-1} of its maximum value. Such a value of τ will be influenced by the weight contributions of all molecules in a polydisperse solution. From *Figures 1* and *2*, relaxation times of 250 (±50) and 70 (±25) μsec are obtained for the PMA and SPS, respectively.

The majority of transient electro-optical experiments for the determination of τ are conducted on solutions of rigid molecules for which equations exist whereby conversion to particle dimensions is straightforward. Insertion of experimental relaxation times into Broersma's equation for rods[25], after estimating the equivalent molecular axial ratios from viscosity data[26], leads to molecular weights for both PMA and and SPS which are some thousand times too small. Therefore the molecular conformations are far from rod-like. This is confirmed by a consideration of the viscosity data. One would expect the exponent in the Mark–Houwinck equation $[\eta] = KM^{\alpha}$ to have the value $\alpha = 2$ for rigid rod-like molecules and $\alpha = 0.5$ for freely flexible coils[27]. For the systems studied here, $\alpha = 0.5$ and 0.93 for the PMA and SPS solutions, respectively.

Theories for the electrically induced orientational relaxation of flexible polymers are few. One of these, due to Zimm[28] gives an expression for the relaxation time, τ, of the low frequency cooperative motion of a complete polymer chain, namely:

$$\tau = f\frac{M[\eta]\eta_0}{RT} \qquad (1)$$

where f has the values 1.21 and 0.85 for free-draining and non-free-draining coils, respectively. Here, η_0 is the solvent viscosity and R and T have their usual meanings. Stockmayer and Baur[29] have discussed the application of this equation to electrically induced phenomena. They have shown that this particular mode of motion is predominant in electro-optical effects. The current data enable us to make an experimental appraisal of this equation.

The viscosity exponent for PMA indicates a truly flexible molecular conformation. Inserting the experimental value for τ into equation (1) leads to a molecular weight of 160 000. This is some five times smaller than the weight-average value obtained from the viscosity–molecular weight equation and may simply reflect sample polydispersity. Similar treatment of the relaxation time for SPS leads to a molecular weight of 20 000. This again is only 1/7 of the value obtained from the viscosity data alone. In this case, however, the viscosity exponent indicates some degree of molecular stiffness. The discrepancy may not be due therefore to polydispersity alone. An alternative explanation is that when conducting solutions are studied and the polarizability anisotropy has its origin predominantly in the polarization of the counterion cloud, equation (1) is not directly applicable.

In conclusion, we wish to point out that by observing the changes in the four polarized components of the fluorescence when solutions of flexible dye-tagged polymers are subjected to pulsed electric fields, one can estimate the geometry of dye binding. The data recording and analysis is extremely rapid and very dilute solutions can be studied. Further experiments on polymers of various stereoregular forms are envisaged.

ACKNOWLEDGEMENTS

The Science Research Council is thanked for a grant under which P. J. R. is employed. The facilities of this department are gratefully acknowledged.

REFERENCES

1 Nobbs, J. H., Bower, D. I., Ward, I. M. and Patterson, D. *Polymer* 1974, **15**, 287

2 Claesson, S. and Odani, H. *Discuss. Faraday Soc.* 1970, **49**, 268

3 Weill, G. and Sturm, J. *Biopolymers* 1975, **14**, 2537

4 Ridler, P. J. and Jennings, B. R. *J. Phys. (E)* 1977, **10**, 558

5 Barone, G., Caramazza, R. and Vitagliano, V. *Ric. Sci. Parte 2 Sez. A* 1962, **32(2)**, 554

6 Caramazza, R., Costantino, L. and Vitagliano, V. *Ric. Sci. Parte 2 Sez. A.* 1964, **34**, 67

7 Barone, G., Crescenzi, V., Quadrifoglio, F. and Vitagliano, V. *Ric. Sci.* 1966, **36**, 503

8 Crescenzi, V., Quadrifoglio, F. and Vitagliano, V. *J. Macromol. Sci. (A-1)* 1967, **5**, 917

9 Jordan, D. O., Kurucsev, T. and Martin, M. L. *Trans. Faraday Soc.* 1969, **65**, 612

10 Vitagliano, V. and Costantino, L. *J. Phys. Chem.* 1970, **74**, 197

11 Vitagliano, V., Costantino, L. and Zagari, A. *J. Phys. Chem.* 1973, **77**, 204

12 Vitagliano, V., Costantino, L. and Sartario, R. *J. Phys. Chem.* 1976, **80**, 959

13 Liquori, A. M., Barone, G., Crescenzi, V., Quadrifoglio, F. and Vitagliano, V. *J. Macromol. Chem.* 1966, **1**, 291

14 Katchalsky, A. and Eisenberg, H. *J. Polym. Sci.* 1951, **6**, 145

15 Takahashi, A., Kato, T. and Nagasawa, M. *J. Phys. Chem.* 1967, **71**, 2001

16 Duportail, G., Mauss, Y. and Chambron, J. *Biopolymers* 1977, **16**, 1397

17 Fredericq, E. and Houssier, C. 'Electric Dichroism and Electric Birefringence', Oxford University Press, London, 1973

18 Nakayama, H. and Yoshioka, K. *J. Polym. Sci (A)* 1965, **3**, 813

19 O'Konski, C. T. and Haltner, A. J. *J. Am. Chem. Soc.* 1957, **79**, 5634

20 Mandel, M. *Mol. Phys.* 1961, **4**, 489

21 Takashima, S. *Adv. Chem.Ser.* 1967, **63**, 232
22 Kikuchi, K. and Yoshioka, K. *Biopolymers* 1976, **15**, 583
23 Zanker, V. *Z. Phys. Chem.* 1954, **2**, 52
24 Ballard, R. E., McCaffery, A. J. and Mason, S. F. *Biopolymers* 1966, **4**, 97
25 Broersma, S. *J. Chem. Phys.* 1960, **32**, 1626
26 Scheraga, H. A. *J. Chem. Phys.* 1955, **23**, 1526
27 Tanford, C. 'Physical Chemistry of Macromolecules', Wiley, New York, 1961, Ch. 6
28 Zimm, B. H. *J. Chem. Phys.* 1956, **24**, 269
29 Stockmayer, W. H. and Baur, M. E. *J. Am. Chem. Soc.* 1964, **86**, 3485