value ($\bar{\gamma}_{\pm}'$, column 6, Table I) it would have if present alone at the same low concentration in the exchanger.

A qualitative discussion of the consequences of the fact that the electrical potential, $\bar{\psi}$, in the exchanger is not constant throughout, as was assumed implicitly in deriving eq 1, has been given by Tye.[23] In actuality, the electrical potential changes continuously from a large value in the neighborhood of the fixed charges to a minimum in the more remote regions in the interior of the molecular network of the exchanger. Consequently, on the average in a cation exchanger, for example, the cations are at a lower potential than the anions. Replacing $\bar{\psi}$ by the unequal potentials $\bar{\psi}_+$ and $\bar{\psi}_-$ in the derivation of the Donnan equation and comparing with the equation obtained assuming $\bar{\psi}$ to be constant gives the result

$$\bar{\gamma}_{\pm} = \bar{\gamma}_{\pm}' \exp[F(z_+\nu_+\bar{\psi}_+ + z_-\nu_-\bar{\psi}_-)/(\nu_+ + \nu_-)RT] \quad (4)$$

where $\bar{\gamma}_{\pm}'$ is an activity coefficient which is independent of the potentials caused by the fixed ions, F is the Faraday constant, ν is the moles of ion per mole of electrolyte, and z is the charge. The quantity $\bar{\gamma}_{\pm}'$ will resemble the activity coefficients of the solute in simple aqueous electrolyte solutions, and it may be referred to as the Debye–Hückel mean molal coefficient. Considering NaCl, eq 4 becomes

$$\bar{\gamma}_{\pm} = \bar{\gamma}_{\pm}' \exp[F(\bar{\psi}_+ - \bar{\psi}_-)/2RT] \quad (5)$$

The potential of the anions, $\bar{\psi}_-$, is larger than that of the cations, $\bar{\psi}_+$, and the exponential term will be less than unity so that $\bar{\gamma}_{\pm}$ will be less than $\bar{\gamma}_{\pm}'$ as observed. With highly cross-linked exchangers, the exponential term will be virtually constant for small external electrolyte concentrations, and $\bar{\gamma}_{\pm}'$ might be expected to increase as the internal electrolyte concentration decreases. Accordingly, $\bar{\gamma}_{\pm}$ should increase, and this appears to be the case as may be observed in Table I where the ratio $\bar{\gamma}_{\pm}/\bar{\gamma}_{\pm}'$ is nearly constant independent of concentration. The significantly lower values of $\bar{\gamma}_{\pm}$ for Na_2SO_4 appear to originate in the fact that $\bar{\psi}_-$ is larger and $\bar{\psi}_+$ remains unchanged.

The models assumed by Lazare, Sundheim, and Gregor,[24] by Shone,[25] and by Mackie and Meares,[26] who applied the theory of Katchalsky and Lifson, do not appear to apply to highly cross-linked exchangers as they predict either that $\bar{\gamma}_{\pm}$ becomes constant or decreases. Although the potential, $\bar{\psi}_-$, of co-ions (*i.e.*, Cl^- ions in a cation exchanger) in the vicinity of the like-charged structurally bound ions on the polymer network is large, because of efficient screening of the latter by the counterions, $\bar{\psi}_-$ is relatively constant throughout the remainder of the exchanger. With lightly cross-linked exchangers which become highly swollen in dilute electrolytes, the screening of the fixed charges will be less complete, and $\bar{\gamma}_{\pm}$ might be expected to decrease with diminishing external concentration. However, when $\bar{\psi}_+$ and $\bar{\psi}_-$ and hence $\bar{\psi}$ are constant within the ion exchanger, the Donnan theory will be valid.

(23) F. L. Tye, *J. Chem. Soc.*, 4784 (1961).

(24) L. B. Lazare, B. R. Sundheim, and H. P. Gregor, *J. Phys. Chem.*, **60**, 641 (1956).

(25) M. G. T. Shone, *Trans. Faraday Soc.*, **58**, 805 (1962).

(26) J. S. Mackie and P. Meares, *Proc. Roy. Soc.* (London), **A232**, 485 (1955).

Dispersion of the Kerr Constant of the Acridine Orange–Polyglutamic Acid Complex

John C. Powers, Jr.

Contribution from the IBM Research Laboratory, San Jose, California.
Received September 2, 1966

Abstract: The Kerr effect (linear electric birefringence) is generally measured in transparent regions of the spectrum and used to obtain information about the anisotropy of the polarizability. In the vicinity of an electronic absorption, the Kerr constant exhibits anomalous dispersion, and the form of this dispersion is related to the direction of the transition moment for the particular electronic transition. The origin of these effects is reviewed. Experimental work is presented on the dispersion of the Kerr constant for the acridine orange–polyglutamic acid complex in DMF. From the shape of the dispersion curve, the polarization of the absorption band for the free dye can be assigned. A structure of the dye–polymer complex compatible with these and other published data is that of a left-handed helix bound to the polymer helix so that the long axis of the dye molecule is parallel to the long axis of the polymer helix.

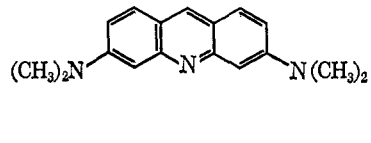
The study of the Kerr effect (linear electric birefringence) in both large and small molecules has been generally restricted to regions of the spectrum where the system under study does not absorb. In such transparent regions, the extensive researches of O'Konski[1] and Tinoco[2] have shown how dipole moments and polarizabilities can be obtained for macromolecules under conditions of saturation of birefringence. Information about the size and shape of

(1) (a) C. T. O'Konski, K. Yoshioka, and W. H. Orttung, *J. Phys. Chem.*, **63**, 1558 (1959); (b) C. T. O'Konski and A. J. Haltner, *J. Am. Chem. Soc.*, **78**, 3604 (1956).

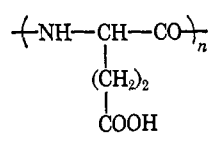
(2) (a) D. N. Holcomb and I. Tinoco, Jr., *J. Phys. Chem.*, **67**, 2691 (1963); (b) I. Tinoco, Jr., and C. A. Bush, *Biopolymers, Symp.*, **1**, 235 (1964).

large molecules is also available from measurements of the decay of the birefringence.[1] Le Fèvre and his co-workers have, on the other hand, concentrated on the study of small molecules and the determination of their polarizability ellipsoids.[3] Some conformational assignments[4] have been made by this group with the aid of measurements of the Kerr constant; however, such determinations are still difficult to perform, requiring some fairly detailed calculations.

Measurement of the dispersion of the Kerr constant in a region of absorption offers the possibility of obtaining information about the polarization of an absorption and about properties of the excited state.[5] Experimentally this poses problems because concentrated solutions or pure liquids can be satisfactorily employed in a transparent region, but only very dilute solutions can be used in absorbing regions. Although the Kerr constant is substantially enhanced in such regions, measurements on small molecules in the vicinity of strong electronic absorption bands are still quite difficult to perform with standard light sources. Only two studies of the dispersion of the Kerr constant in the vicinity of an absorption have been reported. In the infrared spectrum of nitrobenzene[6] an anomalous dispersion was shown by the Kerr constant for several bands. The existence[7] of a transverse dipole moment in hemoglobin was inferred from measurements at two or three wavelengths in the visible region. This paper will consider the measurement of the dispersion of the Kerr constant in the region of the visible absorption for the complex formed between acridine orange (AO) and polyglutamic acid (PGA) in the solvent dimethylformamide (DMF); measurements in water solution were not possible (see Experimental Section).



acridine orange



polyglutamic acid

The acridine orange–polyglutamic acid complex is a system which has a group possessing an intense absorption ($\epsilon \sim 10^4$) attached to a molecule that has a large dipole moment and Kerr constant. Stryer and Blout[8] demonstrated that optical activity could be induced in the visible absorption band of AO when the dye was bound to the helical conformation of the polymer. This is a general effect exhibited by several dyes and disappears if the polymer is in the random-coil conformation. Acridine orange is also known to exhibit induced optical activity when complexed with other macromolecules, *e.g.*, heparin[9] and DNA.[10] From a determination of the Kerr constant dispersion for the AO–PGA complex, the polarization of the absorption relative to the known molecular geometry of the polypeptide can be obtained.[11] The information is complementary to that available from measuring the circular dichroism of partially ordered solutions of the complex.[12]

Theory

The observable in the Kerr effect[3] is the phase change, δ, induced in an incident, linearly polarized light beam propagated at right angles to an applied electrostatic field (F). For low fields, the effect is governed by Kerr's equation

$$B = \frac{\delta}{2\pi l F^2} = \frac{(n_{\parallel} - n_{\perp})}{\lambda F^2} \tag{1}$$

where l is the length of the cell, $(n_{\parallel} - n_{\perp})$ is the induced difference in index of refraction of the medium parallel ($\parallel$) and perpendicular ($\perp$) to the field, and λ is the wavelength of light used; B is the Kerr constant, and the field (F) is expressed in statvolts. In terms of the classical Langevin–Born orientation theory[3] this becomes

$$B = \frac{\pi N}{27 n \lambda}(n^2 + 2)^2(\epsilon + 2)^2(\theta_1 + \theta_2) \tag{2}$$

where N is the number of molecules/unit volume, and n and ϵ are the refractive index and dielectric constant of the material. θ_1, the "anisotropy term," is given by

$$\theta_1 = \frac{1}{45kT}[(a_x - a_y)(\alpha_x - \alpha_y) + (a_y - a_z)(\alpha_y - \alpha_z) + (a_z - a_x)(\alpha_z - \alpha_x)] \tag{3}$$

where a_i is the component of the induced moment and α_i is the component of the polarizability both with regard to the principal axes of the molecule. θ_2, the "dipole term," is given by

$$\theta_2 = \frac{1}{45k^2T^2}[(\mu_x^2 - \mu_y^2)(\alpha_x - \alpha_y) + (\mu_y^2 - \mu_z^2)(\alpha_y - \alpha_z) + (\mu_z^2 - \mu_x^2)(\alpha_z - \alpha_x)] \tag{4}$$

where μ_i is the component of the molecular dipole moment.

For a dipolar molecule, θ_1 is much smaller than θ_2 and is generally neglected. If the dipole moment lies along one of the principal axes (say the z axis), then[13]

$$\theta_2 = \frac{\mu_z^2}{45k^2T^2}(2\alpha_z - \alpha_x - \alpha_y) \tag{5}$$

The magnitude and sign of the Kerr effect as a function of λ are thus dependent on the final term. From the quantum mechanical definition of the polarizability[14]

$$\alpha_i = \frac{2}{3h}\sum_i \left[\frac{\nu}{(\nu_i^2 - \nu^2)}\langle 0|\mu_i|i\rangle\langle i|\mu_i|0\rangle\right] \tag{6}$$

the Kerr constant as a function of frequency ν rises as ν approaches ν_i if $2\alpha_z > (\alpha_x + \alpha_y)$ (a parallel transition). For a perpendicular transition, the opposite effect is seen. Thus, for the simple case of orientation polarization, the dispersion of the Kerr constant should have the

(3) C. G. Le Fevre and R. J. W. Le Fèvre, *Rev. Pure Appl. Chem.*, **5**, 261 (1955), and succeeding papers.
(4) See, for example, (a) J. M. Eckert and R. J. W. Le Fèvre, *J. Chem. Soc.*, 1081 (1962); (b) C. Y. Chen and R. J. W. Le Fèvre, *ibid.*, 234 (1964); (c) J. M. Eckert and R. J. W. Le Fèvre, *ibid.*, 358 (1964).
(5) A. D. Buckingham, *Proc. Roy. Soc.* (London), **267**, 271 (1962).
(6) E. Charney and R. S. Halford, *J. Chem. Phys.*, **29**, 221 (1958).
(7) W. H. Orttung, *J. Am. Chem. Soc.*, **87**, 924 (1965).
(8) L. Stryer and E. R. Blout, *ibid.*, **83**, 1411 (1961).
(9) A. L. Stone, *Biopolymers*, **2**, 315 (1964).
(10) D. N. Neville, Jr., and D. F. Bradley, *Biochem. Biophys. Acta*, **50**, 397 (1961).
(11) J. C. Powers, Jr., *J. Am. Chem. Soc.*, **88**, 3679 (1966).
(12) R. E. Ballard, A. J. McCaffery, and S. F. Mason, *Biopolymers*, **4**, 97 (1966).
(13) H. Labhardt, *Tetrahedron Suppl.*, **2**, 223 (1963).
(14) H. Eyring, J. Walter, and G. E. Kimball, "Quantum Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1944, p 121.

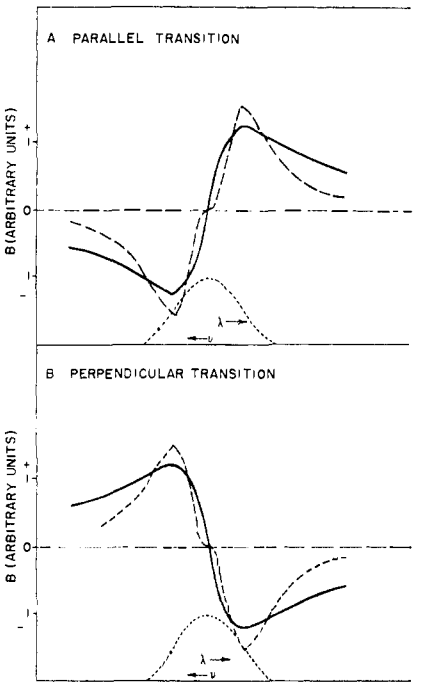


Figure 1. Theoretical dispersion curves of the Kerr constant B for a transition polarized parallel (plot A) or perpendicular (plot B). The solid lines arise from the electrooptic polarizability, the dashed lines from the hyperpolarizability; the absorption is indicated at the bottom. The diagrams are not meant to be quantitative (see ref 5).

form shown in Figure 1 for a transition centered at ν_i. Because of the width of the absorption band ($2\Delta\nu_i$), this dispersion varies as $(\nu_i - \nu)/[(\nu_i - \nu)^2 + (\Delta\nu_i)^2]$ for frequencies ν near ν_i.[5]

Buckingham and Pople[15] have considered the effect of high fields on the Kerr effect and have derived expressions for the deviations from a linear polarization law. The term "hyperpolarizability" was used to describe the distortion produced by a high field. Buckingham[5] has carried this analysis further by treating the frequency dependence of the Kerr constant due to orientation polarization (low field) and to the "distortion polarization" (hyperpolarizability). Although his derivation is primarily for small molecules, it is general enough to be valid for large molecules. The Kerr constant can be expressed as

$$B = \frac{2\pi N}{81\lambda n F^2}(n^2 + 2)^2(\epsilon + 2)^2\overline{\pi_{zz} - \pi_{xx}} \qquad (7)$$

where the last term, $\overline{\pi_{zz} - \pi_{xx}}$, is the mean difference in molecular polarizabilities in the x and z directions (the field is the z direction). The difference in polarizability is due to two temperature-dependent terms (in $1/kT$ and $1/k^2T^2$) which correspond to θ_1 and θ_2 found in the classical Langevin–Born orientation theory, another term dependent on $1/kT$ which arises from the first-order expansion of π in terms of F, and a temperature-independent term due to the hyperpolarizability. This last term becomes significant only in the vicinity of an absorption band. For the case of a molecule symmetric about the z axis in both ground (0) and excited states (i), the Kerr constant is given by

$$B = \frac{4\pi N}{15\lambda}\left[\frac{(n^2 + 2)^2(\epsilon + 2)^2}{81n}\right]\frac{(\mu_i - \mu_0)^2(\alpha_{\parallel}^i - \alpha_{\perp}^i)}{h^2(\nu_i - \nu)^2} \qquad (8)$$

where $\alpha_{\parallel}^i$ and $\alpha_{\perp}^i$ are the excited state electrooptic polarizabilities parallel and perpendicular to the molecular axis, and μ_i and μ_0 are the dipole moments in the excited and ground states, respectively. The dispersion curve here varies as $1/(\nu_i - \nu)$.[3] Since the absorption band has a finite line width $2\Delta\nu_i$, the dispersion lines are broadened, and the actual dispersion would behave as $\{(\nu_i - \nu)/[(\nu_i - \nu)^2 + (\Delta\nu_i)^2]\}^3$.

Owing to the cubic dependence, the dispersion curve arising from the hyperpolarizability is much narrower and sharper than that due to orientation and, as Buckingham points out, an inflection is present at $\nu_i = \nu$. The ideal dispersion curves for parallel and perpendicular transitions are given in Figure 1. Over a wide range of ν, the frequency dependence of n must also be considered. Equation 8 shows that for a linear molecule, if the direction of the transition can be assigned and the oscillator strength is known, the excited-state dipole moment can be estimated. The direction of the transition can easily be assigned from the shape of the dispersion curve.

An alternate treatment of this problem from the standpoint of quantum mechanics has recently been given by Lin, Lin, and Eyring.[16] The dispersion curves for the Kerr constant of a polar molecule are the same as those resulting from the classical treatment of Buckingham.

Experimental Section

A. Materials. DMF (dimethylformamide) (Merck) was dried and fractionated from barium oxide under reduced pressure, bp 80° (12 mm). The resistivity of the liquid was greater than 10^6 ohm cm, close to that obtained by Thomas and Rochow.[17]

The L-polyglutamic acid was a commercial sample (Pilot Chemical Co.) of molecular weight 77,000. It was used without further purification.

Acridine orange was also a commercial sample (National Aniline). The zinc-free dye was obtained by dissolving the dye in water, adding sodium hydroxide solution until a pH of 10 was reached, and then extracting the mass with benzene. After two recrystallizations from benzene, the material melted at 181°.[18]

The PGA–AO complex was formed by mixing stock solutions of the materials in DMF. Fresh solutions were made up for each run as the dye solutions deteriorate slowly. Spectra taken before and after a run were the same. A Cary Model 14 spectrophotometer was used for spectral determinations.

B. Apparatus. A conventional arrangement was used similar to that of Watanabe, *et al.*[19] The light source is a 1000-w Osram lamp whose output is passed through a 500-mm Bausch and Lomb monochromator; a slit width of 1 mm gave an output band width less than 20 A. Light of the desired wavelength was collimated by a 10-cm focal length lens and then passed through a Glan–Thompson prism (Karl Lambrecht Crystal Optics) whose axis is aligned at 45° to the direction of the applied field. Upon emerging from the polarizing prism, the beam is passed through a 4-cm Kerr cell, through another Glan–Thompson prism, and focused on a plate in front of an RCA 1-P-21 photomultiplier tube. The entire assembly was mounted securely on a triangular rod optical bench manufactured by Spindler and Hoven, Göttingen, Germany. The prisms were equipped with a vernier scale, and the position of the optic axis was determined by use of a previously calibrated Glan prism. The Kerr cell itself was originally designed by Shah[20] and is com-

(15) A. D. Buckingham and J. A. Pople, *Proc. Phys. Soc.* (London), **A68**, 905 (1958).

(16) S. H. Lin, C. Y. Lin, and H. Eyring, *J. Phys. Chem.*, **70**, 1756 (1966).

(17) A. B. Thomas and E. G. Rochow, *J. Am. Chem. Soc.*, **79**, 1843 (1957).

(18) K. L. Moudgill *J. Chem. Soc.*, **121**, 1506 (1922).

(19) H. Watanabe, K. Yoshioka, and A. Wada, *Biopolymers*, **2**, 91 (1964).

posed of a 4-cm Beckman cell surrounded by a polystyrene casing for thermostating. A hollow aluminum screw allows passage of the light beam. The cell plates made of platinum-plated steel are 3 mm thick and 4 cm long and are attached to a machined nylon block with a spacing of 4 mm between surfaces. Connections to the high-voltage line are made with thin wires spot welded to the cell plates. The actual dimensions of the cell cavity itself are 4 mm by 4 mm by 4 cm. Rectangular high-voltage pulses of duration 0.1 to 10 msec could be applied across the cell plates by a Sorenson No. 230-6P high-voltage power supply which is gated by an RCA No. 811A electronic tube, which, in turn, is controlled by a 25-v square-wave pulse applied to the grid and generated by a Tektronix pulsing unit (a Type 160 power supply, a Type 162 wave-form generator, and a Type 163 pulse generator). The voltage developed across the cell plates is monitored by a calibrated probe and displayed simultaneously with a phototube output signal on a Tektronix Type 545A oscilloscope. The maximum voltage that could be developed across DMF with this setup is 700 v, corresponding to a field of 1750 v/cm across the sample.

The observable in this experiment is the phase shift, δ, resulting from the application of a field at right angles to the direction of propagation of a light beam polarized at an angle of 45° to the direction of the applied field. The emergent light from the cell is thus elliptically polarized. The intensity of light passed by an analyzer in such a situation is given by the equation[21]

$$I = I_0[\cos^2(\alpha - \beta) - \sin 2\alpha \sin 2\beta \sin^2 \delta/2]$$

where I is the resultant intensity of light, I_0 is the incident intensity of light, α and β are the angles between the field direction and the optic axes of the polarizing and analyzing prisms, respectively, and δ is the induced phase change in the light. For the case of crossed polarizing prisms and the incident beam polarized on an angle of 45°, this relation becomes

$$I = I_0 \sin^2 \delta/2$$

I_0 can be determined from Malus' law by measuring intensities when the analyzer is rotated from the crossed position. To do this, one must assume that a linear relation exists between the light incident on the phototube and the voltage developed by the tube. With the low intensities employed in this work, this assumption was borne out.

The apparatus was calibrated by measuring the Kerr constant of nitrobenzene at 35° and 5000 A. The value of 4.0×10^{-5} cm/statvolt^2 is in satisfactory agreement with previously obtained values.

C. Kerr-Constant Measurements. All determinations of Kerr constants were made at 35.52 ± 0.03°. A voltage pulse of 1 msec duration was employed. A variable capacitance of 0.01 to 0.001 μf was used to reduce noise in the output line. Heating effects were minimal as the current flow was less than 10 μa. A (comparatively) large resistance (10^5 ohms) was placed across the cell in order to produce a pulse of constant amplitude. The magnitudes of the applied field and the phototube response were obtained from photographs of their oscilloscope traces, and δ was determined from the relations given above. A plot of δ *vs.* F^2 was used to determine the Kerr constant. The much higher conductivity of water solutions precluded the establishment of a static field higher than 100 v; consequently, it was not possible to make birefringence measurements with sufficient accuracy to determine the dispersion curve in water.

Results

The Spectrum of the AO–PGA Complex in DMF. The spectrum of acridine orange in hydroxylic solvents has been thoroughly investigated from the standpoint of dependence on both pH and concentration.[22] In DMF the spectrum of zinc-free acridine orange has a maximum of 4250 A corresponding to that of the free molecule in water at pH's greater than 11 (4350 A). Upon addition of acetic acid, this peak shifts to about 4950 A, again near to that observed in water at pH 6

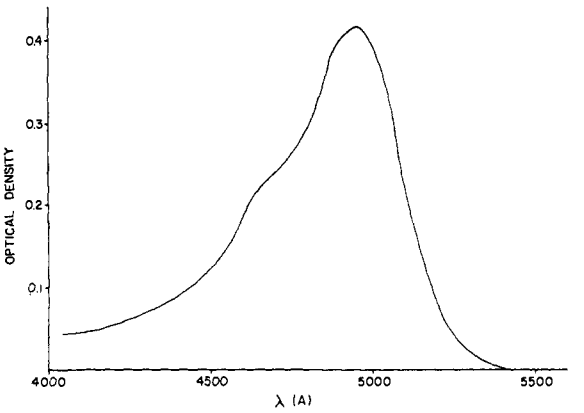
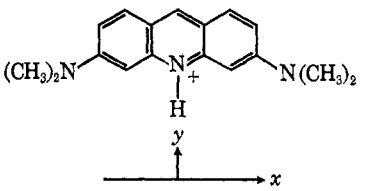


Figure 2. Absorption spectrum of the AO–PGA complex (AO = 7.6×10^{-6} *M*, PGA = 2.33×10^{-2} *M*).

(4900 A). The spectrum of the acridine orange–polyglutamic acid complex in DMF shows a maximum at 4950 A and a shoulder at 4650–4700 A (see Figure 2). This spectrum is identical with that obtained in water solution with the same concentrations of dye and polymer at pH 4.5. The shoulder occurs in the region which has been assigned to absorption by dye aggregates (presumably the dimer).[22,23] Although a band appears in the same region in the spectrum of the free dye molecule, the shoulder observed here is more pronounced and indicates the presence of dye–dye interactions. The dye associates very easily and a careful study by Bradley and Wolf[23] has indicated that this aggregation is strong enough to direct binding of the dye onto adjacent sites. The spectrum of the acridine orange–zinc chloride complex in DMF shows two peaks of equal intensity at 4950 and 4650 A, indicating a much greater extent of aggregation. Spectra of this zinc salt complex in the presence of polyglutamic acid were, however, identical with those obtained with zinc-free dye, indicating that binding has taken place. The relative intensities of the two peaks are, of course, a function of the dye/polymer ratio.

The direction of the transition moments in acridine orange has been experimentally determined in PVA films,[24] and calculations by Mason, *et al.*,[12] have confirmed this assignment. The absorption at 5000 A is long-axis (x) polarized (see below). The direction of the transition moment in the aggregate has not been assigned since very little is definitely known about the structure.



Kerr-Constant Measurements. The Kerr constants of the dye–polymer complex were measured over a wavelength region of 5700–4300 A for several different concentrations of dye and polymer. In all cases, the number of binding sites was greatly in excess of the number of dye molecules. The observed birefringence

(20) M. J. Shah, D. C. Thompson, and C. M. Hart, *J. Phys. Chem.*, **67**, 1170 (1963).
(21) R. B. Ditchburn, "Light," Interscience Publishers, Inc., New York, N. Y., 1959, pp 385–386.
(22) V. Zanker, *Z. Physik Chem.* (Leipzig), **199**, 225 (1952).
(23) D. F. Bradley and M. K. Wolf, *Proc. Natl. Acad. Sci. U. S.*, **45**, 944 (1959).
(24) H. Jakobi and H. Kuhn, *Z. Elektrochem.*, **66**, 46 (1962).

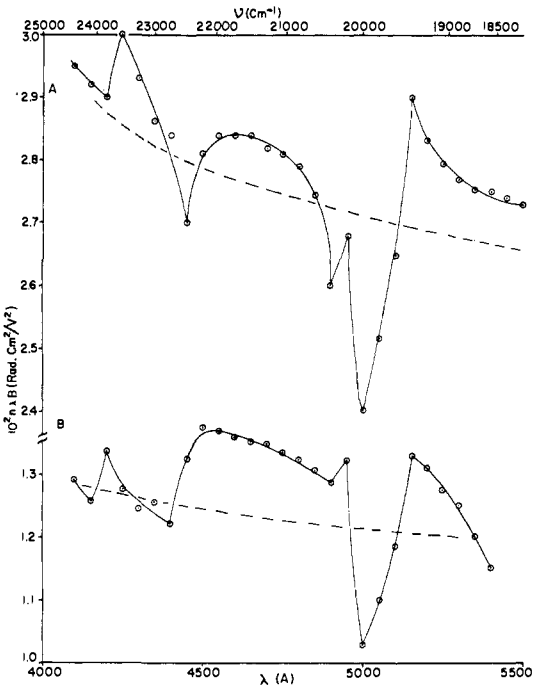


Figure 3. Dispersion of $n\lambda B$ for the AO-PGA complex (solid line) and PGA (dotted line): (A) PGA = 2.33×10^{-2} *M*, AO = 7.6×10^{-6} *M*; (B) PGA = 7.75×10^{-3} *M*, AO = 7.6×10^{-6} *M*.

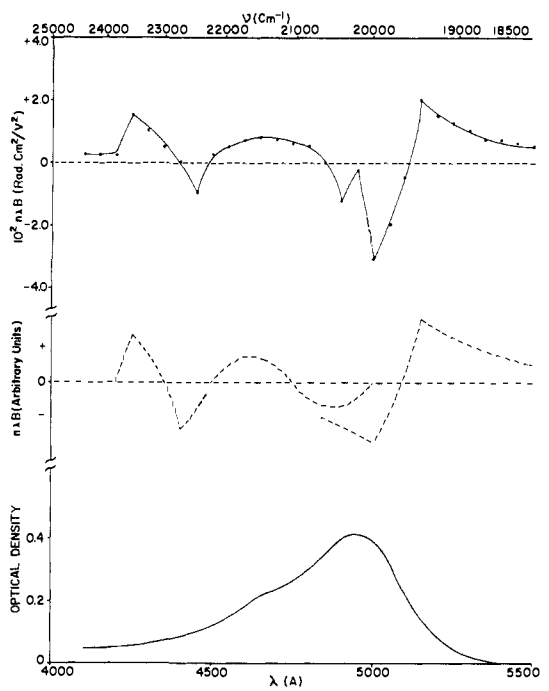


Figure 4. Comparison of spectrum (bottom curve) and dispersion of $n\lambda B$ (top curve) for PGA = 2.33×10^{-2} *M*, AO = 7.75×10^{-3} *M* corrected for PGA birefringence (see Figure 3). Center plot shows hypothetical resolved dispersions.

is due both to the bulk anisotropy of the polymer and to the orientation polarization and the (presumed) distortion polarization of the bound dye. Since the Kerr constant of polyglutamic acid in DMF is positive and, under the conditions employed, larger than any arising from the dye, the sign of the latter is easily obtained at any wavelength from the relation

$$B_{\text{total}}(\lambda) = B_{\text{polymer}}(\lambda) + B_{\text{dye}}(\lambda)$$

The dispersion of the Kerr constant for PGA was measured at intervals of 300 A over the spectral range of interest at concentrations of 7.75×10^{-3}, 1.55×10^{-2}, and 2.33×10^{-2} *M*. The specific Kerr constants at each wavelength are invariant, indicating the absence of polymer aggregation.

A summary of the measurements on the AO–PGA complex is given in Table I. Points were taken every

Table I. Summary of Results of Kerr-Effect Measurements on the AO–PGA Complex

Run	$10^6 \times$ [AO][a]	$10^2 \times$ [PGA][b]	Monomer/dye	$10^4 \times \Delta B_{5000\,A}$	$\Delta B_{5000}/M_{AO}$
1	3.8	1.55	4100:1	0.5	13
2	8.7	2.96	3400:1	0.8	9.2
3	7.6	2.33	3100:1	0.55	7.2
4	10.2	2.33	2330:1	0.7	6.9
5	4.5	0.775	1700:1	0.3	6.7
6	7.6	0.775	1000:1	0.4	5.3

[a] Moles/liter. [b] Moles of monomer/liter.

100 A over the entire wavelength region and every 50 A in the region of absorption; detectable signals in this region could not be obtained at dye concentrations much higher than 10^{-5} *M*. While the dispersion curves all have qualitatively similar features, the exact dependence of the Kerr constant on wavelength is a function of the monomer/dye ratio. This is indicated in the last column of the table where the strength of the dispersion in the vicinity of 5000 A is given in terms of the difference between the maximum and minimum values of the Kerr constant (*i.e.*, $\Delta B_{5000A} = (B_{max} - B_{min})_{5000A}$) and as a specific value/unit amount of dye ($\Delta B_{5000A}/M_{AO}$). The dispersion in this area has a maximum at 5150 A and a minimum at 5000 A.

It is evident that the polymer-to-dye ratio sensibly influences the dispersion curves, presumably by altering the amount of dye–dye interaction (*vide infra*). In Figure 3 are shown the data obtained in runs 3 and 6 plotted as $n\lambda B$ *vs.* λ. Treating the experimental results in this manner removes the simple wavelength dependence of the birefringence due to the polymer and polymer dye complex and makes it easier to compare the features of the two dispersion curves. The two curves have similar characteristics, both having a positive dispersion centered around 5100 A and two negative dispersions in the 4000–4800-A region.

Discussion

The absorptions responsible for the dispersions noted above can be assigned from the spectral information cited previously and are indicated schematically in Figure 4 for run 3. The top curve in this figure represents the birefringence of the complex only (the bulk polymer contribution has been subtracted). The strong positive dispersion occurs near the center of the absorption of free protonated acridine orange, and a weaker negative dispersion is centered in the region of dimer absorption. Although the center of the strong dispersion is shifted somewhat to the red relative to the absorption at 4950 A, this same effect is found in both the optical rotatory dispersion[8] and circular dichroism[9] spectra in water. This is not due to electrical di-

chroism in the present case since none could be detected at 5000 A. This small shift has been attributed[8] to head-to-tail coupling of the long-axis transition moments of dye molecules.[25] Although this effect would be expected to show up in the absorption spectrum, there does not seem to be an appreciable shift. Since polyglutamic acid is in the helical conformation in DMF and has a positive Kerr constant, it must be oriented so that the long axis of the molecule is parallel to the field. The above results indicate that the dye molecule must be bound with its long axis approximately parallel to the polymer axis in order to generate a positive dispersion. This assignment follows from the known long-axis polarization of the long-wavelength absorption of the free dye.

The negative dispersion in the 4500–4800-A region occurs at the site of an absorption assigned to the acridine orange dimer.[22] The existence of this aggregation (independent of all other observations) is strongly indicated by the data in the above table. A lowering of the monomer/dye ratio lowers the magnitude of the long-wavelength dispersion (free-dye dispersion). The dye can be bound on the surface of the polymer so that a helical aggregate is formed (the carboxyl groups form a helix of opposite screw sense to that of the polymer helix). The transition moment of a dimer or trimer (*i.e.*, only nearest neighbor interactions being considered) would then be tilted with respect to the axis of the polymer and split into perpendicular and parallel components. The existence of multiple absorptions is suggested by the fact that the optical rotatory dispersion[8] is normal,[26] yet the circular dichroism[12] is complex. The rotatory dispersion must be due to a transition polarized perpendicular to the polymer axis,[26] and the observed Kerr constant dispersion in this wavelength region indicates a perpendicular polarization in agreement with Mason's assignment.[12] Similar remarks can be made concerning the other negative dispersion which falls in the neighborhood of an absorption assigned to the trimer[22] of acridine orange. This absorption would also be expected to have a perpendicular polarization.

The original work of Stryer and Blout[8] permits the assignment in principle of the gross structure of the bound dye since the induced optical activity is shown only by dyes which can aggregate. It should be noted that the signs of the Cotton effects are the same as those observed for the dispersion of the Kerr constant and occur at the same place in the spectrum. The acridine orange is probably bound as a left-handed superhelix,[8,12] and, as Mason has suggested,[12] the assumption of three binding points/dye molecule (each amino group is bound to a carboxyl group) which lie on successive turns of the polymer helix would generate such a helix since there are 3.3 monomers/turn. This conformation would lead to a nearest neighbor interaction primarily located along the long axis of the molecule, yet the evidence is that dyes of this type prefer a stacking type of aggregation with the planes of the molecules approximately parallel.[27] There is a close similarity in the spectra of acridine orange bound to PGA and acridine orange that is aggregated by concentration of the dye. It would seem reasonable that the dye aggregates in a similar manner in both systems. Thus, two alternatives can be suggested to the extended chain dye helix. Either the dye is bound to a single site and a "card pack" aggregate is formed to permit stacking, or separate dye helices are formed and the aggregate absorption involves nearest neighbors on neighboring helices. In both cases, the geometry of the binding sites on the helical rod would generate the required left-hand helix. The card-pack aggregate would seem to be more favorable since the distance between neighboring molecules would be smaller. Although the card-pack structure has been used as a model for calculations,[28] the assumed structure placed the planes of the dye molecules across the long axis of the polymer. The present work shows this definitely not to be the case; the dispersion measurements unequivocally show that the acridine orange is bound with its long axis predominantly parallel to the long axis of the polymer.

Acknowledgment. The author is pleased to acknowledge the expert technical assistance of Mr. John H. Smith in making the measurements.

(25) M. Kasha, *Rev. Mod. Phys.*, **31**, 162 (1959).
(26) I. Tinoco, Jr., *J. Am. Chem. Soc.*, **86**, 297 (1964).
(27) D. M. Blow and A. Rich, *ibid.*, **82**, 3752 (1960).
(28) (a) I. Tinoco, Jr., R. M. Woody, and D. F. Bradley, *J. Chem. Phys.*, **38**, 1317 (1963); (b) D. F. Bradley, I. Tinoco, Jr., and R. M. Woody, *Biopolymers*, **1**, 239 (1963).