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Electric Dichroism and Polymer Conformation. I. Theory of Optical Properties of Anisotropic Media, and Method of Measurement[1]

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ABSTRACT: The use of Mueller matrix algebra to analyze the optical properties of anisotropic media is discussed. It is shown that the treatment of collinear transmission in a general anisotropic medium can be simplified, since the contribution from the average absorption can be factored out of the Mueller matrix. The various anisotropic optical properties contribute to the rotation of the plane of the incident linearly polarized light, and these properties can be measured. The major contributions to the net rotation (for weak interaction of the anisotropic medium with the light beam) are the linear dichroism, the linear birefringence, and the change in optical rotatory dispersion upon application of an external field. Since these all depend differently on the angle, θ, between the major axis of the output elliptically polarized light and the direction of the external (electric) field, they can be separated and measured; for light propagating perpendicular to the axis of a uniaxial medium, the linear dichroism is much larger than the other two effects in the region of an absorption band. A sum rule is applicable to the total linear dichroism spectrum. On the basis of these considerations, a technique is developed for measuring electric dichroism, in which the Cary Model 60 spectropolarimeter is used (together with a parallel-plate cell) to obtain the dependence of the rotation on θ, on the strength of a static external electric field, and on the wavelength of the incident light. This method is much more sensitive than a conventional direct measurement of the separate absorbances of two mutually perpendicularly polarized light beams. Representative data on several α-helical poly(amino acids) indicate that the rotation shows the expected dependence on θ and on field strength.

Linear dichroism (the anisotropic absorption of linearly polarized light) arises primarily from the interaction between the electric dipole transition moment $\boldsymbol{\mu}_{0i}$ of a molecule and the electric field E of the incident electromagnetic radiation. No linear dichroism (LD) is observed in an isotropic (or random) distribution of molecules because the average value of the $\boldsymbol{\mu}_{0i}$'s is such that absorption is the same in all directions, regardless of the polarization of the incident radiation; in such a case, one acquires information only about $|\boldsymbol{\mu}_{0i}|$. On the other hand, if the molecules are distributed anisotropically, *i.e.*, if they have been forced to align preferentially in some direction, linear dichroism will be observed (with detection being possible even for less than 1% of complete orientation), and the vector properties (both magnitude and direction) of $\boldsymbol{\mu}_{0i}$ can be determined.

The direction of $\boldsymbol{\mu}_{0i}$ is an important quantity since it provides additional information, beyond that obtained from $|\boldsymbol{\mu}_{0i}|$, about the electronic structure, conformation, and interactions in molecules. For example, from LD studies of crystals, Albrecht and Simpson assigned the theoretically derived transitions to specific absorption bands in benzene,[4] and Peterson and Simpson determined the direction of polarization of the NV_1 transition in an amide[5] (a quantity required for theoretical calculations of the optical properties of polypeptides). By measuring the LD of steroids incorporated in stretched polyethylene films, Yogev, *et al.*, studied the polarization of transitions in these compounds.[6] LD studies of biopolymers have yielded information not only about electronic states and conformation, but also about flexibility and aggregation; in these investigations, orientation has been produced by streaming in a hydrodynamic field,[7] by stretching of films,[8] and by application of an external electric field[9,10] which interacts with the permanent dipole moment $\boldsymbol{\mu}$ of the molecule. From these examples, it is clear that a wide variety of methods can be used to establish an anisotropic distribution for the study of LD.

Once the problem of producing a known distribution of orientations of the molecules is overcome, it is necessary to detect the resulting LD. While this has been difficult in the past because of the low degree of orientation induced in the

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system, progress is now possible (especially for polymers of high dipole moment and high axial ratios) because of recent instrumental developments.[8-13] More specifically, in the method developed here (and described briefly in a preliminary communication[9]), the rotation of plane polarized light arising from the LD is measured with an increase by a factor of about 33 in the LD signal over that obtained from conventional direct measurement of separate absorbances of two mutually perpendicularly polarized light beams. Although we have developed the technique to measure the LD induced by an electric field (ED), the basic method has been used successfully in strain-oriented systems.[14] In addition, the same type of instrument has been used to measure linear birefringence;[14,15] in principle, it can also measure changes in optical rotatory dispersion (ΔORD) induced by orienting the optically active molecules.

Any optical measurement on an anisotropic medium requires careful consideration of the variety of optical properties that the medium displays. Isotropic media can exhibit absorbance, circular dichroism (CD; $\epsilon_L - \epsilon_R$), and optical rotatory dispersion, *i.e.*, circular birefringence (ORD or CB; $n_L - n_R$), where ϵ and n are the molar extinction coefficient and refractive index, respectively, of left and right circularly polarized light. On the other hand, anisotropic media can exhibit not only CD and ORD, but also LD ($\epsilon_u - \epsilon_v$), LB ($n_u - n_v$), ΔCD, and ΔORD, where u and v indicate two mutually perpendicular types of linearly polarized light. Hence, the analysis of the net effect of the interaction of electromagnetic radiation with anisotropic media can be quite complicated. Ramachandran and Ramaseshan[16] have utilized the Poincaré sphere representation to analyze the optical properties of anisotropic media. Jones[17,18] and Mueller[18,19] have independently developed simple matrix methods for treating anisotropic media.

In section I of this paper, we outline the Mueller matrix treatment of the effect of an anisotropic medium on elliptically polarized light. Special emphasis is placed on *weak* interactions of the anisotropic medium with the light beam (such as encountered in our LD technique), which are treated as an example of the applicability of the Mueller method. In particular, the Mueller matrix is recast in a form in which it is simplified by factoring out the effect of the average absorption (for collinear transmission), and, further, a valid approximate form of the matrix is obtained for *weak* interactions of the anisotropic medium with the light beam. The purpose of this presentation is to provide a simple physical description and sound theoretical basis for the LD method developed here (in section I), and to make generally available a convenient method for the analysis of anisotropic media and for the development of new techniques and apparatus for their study. In section II, we describe and evaluate the ED apparatus developed here. In papers II–IV we will apply this method to the study of the electronic and conformational properties of several polymers.

I. Theory of Optical Properties of Anisotropic Media

(A) The Medium as a Linear Operator. If $\mathbf{s}_I$ and $\mathbf{s}_O$ are vectors representing the magnitude and polarization of the incident and output light beams, with propagation vectors $\mathbf{k}_I$ and $\mathbf{k}_O$, respectively, then the medium may be represented by a linear operator **F** which changes the intensity and polarization of the incident beam, *i.e.*

$$\mathbf{s}_O = \mathbf{F}\mathbf{s}_I \qquad (1)$$

The cross sections of the incident and output beams must be uniformly polarized, and the wavelength must be the same in the $\mathbf{k}_I$ and $\mathbf{k}_O$ directions, for eq 1 to apply.[18] The operator F can take on many forms depending on the direction of the incident and output beams, and the relation of F to molecular properties is complicated. However, for the case of interest here, where $\mathbf{k}_I$ and $\mathbf{k}_O$ are collinear, the form of F is simpler.[20-22]

(B) Stokes Parameters of Polarized Light. In the Mueller matrix treatment,[18] the vectors $\mathbf{s}_I$ and $\mathbf{s}_O$ are 4 × 1 column matrices with Stokes parameters as elements, *viz.*[18,21]

$$\mathbf{s} = \begin{pmatrix} s_0 \\ s_1 \\ s_2 \\ s_3 \end{pmatrix} = \begin{pmatrix} E_x^2 + E_y^2 \\ 2E_xE_y \cos\delta \\ 2E_xE_y \sin\delta \\ E_x^2 - E_y^2 \end{pmatrix} \qquad (2)$$

for completely polarized light propagating along the $+z$ axis. In eq 2, E_x and E_y are the rms amplitudes for the polarized light components in the x and y directions, respectively, and δ is the phase difference between the x and y components of the light. From eq 2, it follows that

$$s_0^2 = s_1^2 + s_2^2 + s_3^2 \qquad (3)$$

Except in cases where the relative magnitudes of $(s_0)_I$ and $(s_0)_O$ are of interest, one may factor out a constant from **s** and, therefore, set $s_0 = 1$ for *both* the incident and output beams.

The dependence of **s** on the polarization of the light is given, according to Gō,[21] by

$$\tan 2\alpha = s_1/s_3 \qquad (4)$$

and

$$\tan 2\beta = s_2/(s_1^2 + s_3^2)^{1/2} \qquad (5)$$

where α is the orientation of the major axis of the elliptically polarized light with respect to the x axis (taking rotation from the $+x$ axis to the $+y$ axis as positive—the right-hand rule) and β is the ellipticity. Examples of Stokes vectors for different kinds of polarized light are given in Figure 1.

(C) Mueller Matrix for Collinear Transmission. In the Mueller matrix treatment, **F** is a 4 × 4 matrix (the Mueller matrix) characterizing the macroscopic optical properties of the anisotropic medium.[18] It contains 16 elements, but only 7 of them are independent if the light is not depolarized. Gō[21] has related **F** to molecular properties, for the case where $\mathbf{k}_I$ and $\mathbf{k}_O$ are collinear, by expressing **F** in terms of a 4 × 4 matrix **H** whose elements are the microscopic optical properties of the medium. The relation between **F** and **H** is analogous to that between transmittance and molar extinction coefficient for isotropic media and, according to Gō,[21] is

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$$\mathbf{F}(l) = \exp(-2\eta l\mathbf{H}) \tag{6}$$

where η is the number density of molecules, l is the path length in the medium, and

$$\mathbf{H} = \begin{pmatrix} c & b_1 & b_2 & b_3 \\ b_1 & c & a_3 & -a_2 \\ b_2 & -a_3 & c & a_1 \\ b_3 & a_2 & -a_1 & c \end{pmatrix} \tag{7}$$

The relations between Gō's parameters and the microscopic optical properties of the anisotropic medium can be shown to be

$$\begin{aligned} \gamma c &= \text{total average absorbance} \\ \gamma b_3 &= 1.151(\epsilon_x - \epsilon_y)lc && \text{(LD)} \\ \gamma a_3 &= (2\pi/\lambda)(n_x - n_y)l && \text{(LB)} \\ \gamma b_2 &= -1.151\,(\epsilon_\mathrm{L} - \epsilon_\mathrm{R})lc && \text{(CD)} \\ \gamma a_2 &= -(2\pi/\lambda)(n_\mathrm{L} - n_\mathrm{R})l && \text{(CB or ORD)} \\ \gamma b_1 &= 1.151(\epsilon_{+45} - \epsilon_{-45})lc && \text{(LD}') \\ \gamma a_1 &= (2\pi/\lambda)(n_{+45} - n_{-45})l && \text{(LB}') \end{aligned} \tag{8}$$

where $\gamma = 2\eta l$, 1.151 is (ln 10)/2, c is the molar concentration, n is the refractive index, λ is the wavelength *in vacuo*, and +45, −45, x, and y refer to the values of u and v, indicated as the linearly polarized light examples 1–4, respectively, of Figure 1. LD′ and LB′ (defined by the last two of eq 8) differ from LD and LB, respectively, by the values of u and v. In general, $\epsilon_u - \epsilon_v$ (with $|u - v| = 90°$) will vary as u and v vary, *i.e.*, with the choice of the location of the laboratory-fixed axes (x, y, z) with respect to the optic axes of the medium.

Using a quantum mechanical treatment of the collinear transmission of photons by an anisotropic distribution of molecules, and selecting a laboratory-fixed set of axes (x, y, z) with the $+z$ axis in the **k** direction, Gō[21] related γa_i, γb_i, and γc to molecular properties such as $\boldsymbol{\mu}_{0i}$. For the most general distribution of molecules, all elements of **H** may be nonzero. For a uniaxial crystalline medium (an example of which is a system of molecules oriented by an external electric field **E**), a_1 and b_1 are zero if the x and y axes are chosen parallel and perpendicular, respectively, to the external field **E**. On the other hand, if the x and y axes are chosen at +45 and −45°, respectively, to **E**, then a_3 and b_3 are zero. Thus, if the symmetry of the anisotropic distribution is that of a uniaxial crystal, either (a_1, b_1) or (a_3, b_3) may be eliminated by rotating the laboratory axes relative to the molecular distribution.

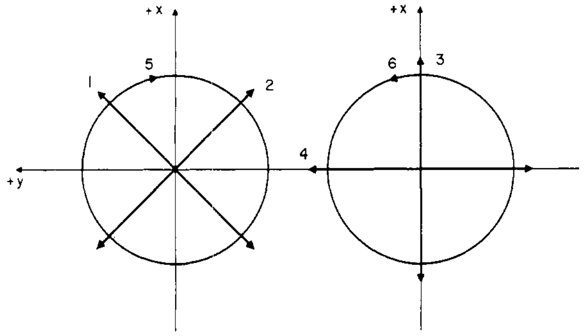

Figure 1. Examples of Stokes vectors for different kinds of polarized light,[21] with the $+z$ axis and the propagation vector, **k**, directed up out of the paper: (A) linearly polarized light ($\beta = 0°$), (1) $\alpha = +45°$, $\mathbf{s} = (1, 1, 0, 0)^+$; (2) $\alpha = -45°$, $\mathbf{s} = (1, -1, 0, 0)^+$; (3) $\alpha = 0°$, $\mathbf{s} = (1, 0, 0, 1)^+$; (4) $\alpha = 90°$, $\mathbf{s} = (1, 0, 0, -1)^+$; (B) circularly polarized light, (5) right handed, $\beta = +45°$, $\mathbf{s} = (1, 0, 1, 0)^+$; (6) left handed, $\beta = -45°$, $\mathbf{s} = (1, 0, -1, 0)^+$, where the plus sign indicates the transpose.

(D) Simplification of F by Factoring out the Average Absorbance. The matrix **F**, expressed in terms of the microscopic properties (a_i's, b_i's, c), can be simplified further. The first step is to factor out γc, the average absorbance, which is generally large. Hence, since the c's lie only on the diagonal of **H**, eq 6 becomes

$$\mathbf{F}(l) = e^{-\gamma c} e^{-\gamma \mathbf{H}'} \tag{9}$$

where

$$\mathbf{H}' = \begin{pmatrix} 0 & b_1 & b_2 & b_3 \\ b_1 & 0 & a_3 & -a_2 \\ b_2 & -a_3 & 0 & a_1 \\ b_3 & a_2 & -a_1 & 0 \end{pmatrix} \tag{10}$$

In most experimental studies, the rotation, ellipticity, and similar properties of polarized light generally can be measured with sufficient accuracy in the range of 10–2000 mdeg. Since γa_i and γb_i are in radians, a power series expansion of $e^{-\gamma \mathbf{H}'}$ will converge after a few terms if the elements of $\gamma \mathbf{H}'$ have magnitudes not greater than a few degrees. For a uniaxial medium whose optic axis is oriented in the xz plane, in which case $a_1 = b_1 = 0$, we obtain expression 11, where

$$\mathbf{F}(l) = e^{-\gamma c}\left[\mathbf{I} - \gamma \begin{pmatrix} 0 & 0 & b_2 & b_3 \\ 0 & 0 & a_3 & -a_2 \\ b_2 & -a_3 & 0 & 0 \\ b_3 & a_2 & 0 & 0 \end{pmatrix} + \frac{\gamma^2}{2} \begin{pmatrix} b_2^2 + b_3^2 & a_2b_3 - b_2a_3 & 0 & 0 \\ a_3b_2 - a_2b_3 & -a_2^2 - a_3^2 & 0 & 0 \\ 0 & 0 & b_2^2 - a_3^2 & a_2a_3 + b_2b_3 \\ 0 & 0 & a_2a_3 + b_2b_3 & b_3^2 - a_2^2 \end{pmatrix} - \ldots\right] \tag{11}$$

I is the identity matrix.

It is simpler, and theoretically correct, to consider the medium to be composed of a sequential collection of sets of seven infinitesimally thin lamellae (each of which exhibits one of the seven elementary anisotropic properties indicated in eq 8), taking as many sets of seven as are required to produce the observed optical effect.[17] Then when polarized light is incident on the lamellae, we have the much simpler problem of dealing with one microscopic anisotropic property at a time. In the case of weakly anisotropic media for which quadratic and higher terms in the expansion in powers of **H**′ are zero, the incident light behaves as though the individual elementary properties *in the whole medium* behave independently of each other. When quadratic and higher terms become important, the anisotropic properties interact with each other and with themselves in their net effect on the incident beam. In this case, we must consider what the preceding portion of the medium has done to the incident beam before proceeding to determine how the next portion of the medium changes the polarization. The cross terms, arising from quadratic and higher terms, are especially interesting and complicating. For example, when considering a medium in which only LD and ORD are present, the observed rotation of polarized light involves not only γb_3 and γa_2 but also the cross term $\gamma a_2 \gamma b_3$. If the cross terms were not taken into account (*i.e.*, if the

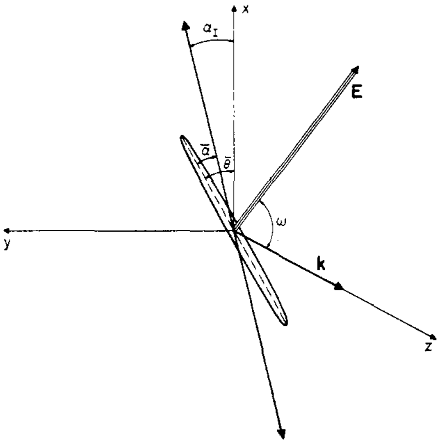

Figure 2. Relative orientations of major polarization axes of incident and output light beams (at angles α_I and $\bar{\theta}$, respectively, with respect to the x axis). The external electric field **E** is in the xz plane at an angle ω with respect to **k**; the ED experiment is arranged so that $\omega = 90°$, for which **E** lies along the x axis. See text for definitions of other quantities.

lamellae were considered to be independent), then (for a medium in which the optic axis is, say, along the x axis) a nonzero LD (*i.e.*, γb_3) would induce a rotation that is proportional to $\sin 2\alpha_I$ (according to eq 18), and this rotation would be zero when $\alpha_I = 0$. However, by taking the cross term ($\gamma a_2 \gamma b_3$) into account, we find that the ORD lamella (γa_2) rotates the plane of polarization of the incident beam slightly so that α_I is some small value δ (rather than zero) when the light then enters the LD lamella (γb_3), so that $\sin 2\alpha_I$ is no longer zero; *i.e.*, γb_3 produces a rotation of the plane of polarization even though α_I was zero for the original incident beam. Thus, using the lamellar representation, one can easily understand qualitatively how complicated media, which contain all possible anisotropic optical properties, interact with the incident polarized beam (even if some quadratic and higher terms are involved).

In eq 11, **F** is in its simplest form for treating a general medium exhibiting LD, LB, CD, CB, and absorbance properties. Generally, if the γa_i's and γb_i's are large, quadratic and higher terms become more important, and it becomes more difficult to separate the various observed optical effects from each other; the same will apply to any other theoretical treatment. One can readily solve for **F** for special cases in which only one particular property exists or perhaps in which one property is of large magnitude and a second one is small. However, in the interesting systems, one usually finds that the medium exhibits many elementary optical properties of comparable magnitude. Furthermore, present instruments can measure with accuracy the effects which are produced even when the γa_i's and γb_i's are small. If one of the properties happens to be large, its magnitude can be decreased readily by reducing the concentration, decreasing the path length, or decreasing the degree of orientation. Therefore, the situation of weak interaction of the anisotropic medium with the light (small γa_i and γb_i) is attainable and measurable with sufficient accuracy for applicability of the theory using only a few terms in the expansion in eq 11.

From the above discussion, we see that the Mueller matrix method provides a simple way to determine completely the factors which will contribute to the experimentally observable quantities of an existing or proposed instrument. Furthermore, *the Mueller matrix is useful for evaluating the effects of* instrumental imperfections. The analytical procedure to determine the experimental quantity of interest involves writing the Mueller matrices for the optical components of the instrument (*e.g.*, polarizers, Faraday modulators, and Pockel cells),[23,24] multiplying the incident Stokes vector by each matrix (including the sample medium) in the order in which the beam traverses through the system, and solving for the observable property using the incident and resulting Stokes vectors, *i.e.*, **F** of eq 1 is a product of the **F**'s for the sequence of optical components.

(E) Rotation of Linearly Polarized Light by an Anisotropic Medium. When linearly polarized light is incident on a uniaxial medium, the plane is rotated because of LD, ORD, and LB. In electric dichroism (ED), the uniaxial character of the medium is produced by the external field, with the optic axis in the direction of the field.

As described in section II, the Cary Model 60 spectropolarimeter will be used to measure the rotation induced by the medium. While one would, in principle, have to write a Mueller matrix for each optical unit in this instrument, as outlined in section ID, this is not necessary, since the Cary Model 60 spectropolarimeter measures only the rotation, *i.e.*, even if the output light is elliptically polarized because the medium is birefringent or circularly dichroic, the instrument was designed to measure only rotation. Thus, we need solve only the simpler problem of determining the rotation produced by the medium.

The medium is oriented so that the optic axis (direction of **E**) is perpendicular to **k**, the propagation vector of the linearly polarized light beam, *i.e.*, ω, the angle between the optic axis and **k**, is 90°, as shown in Figure 2. The plane containing the electric vector of the incident linearly polarized light is oriented at an angle α_I with respect to the x axis. The major axis of the elliptically polarized output light beam is oriented at an angle $\bar{\theta}$ with respect to the x axis, *i.e.*, the major axis is rotated in the medium by an amount $\bar{\alpha}$, where

$$\bar{\alpha} = \bar{\theta} - \alpha_I \tag{12}$$

According to eq 2–5 (with $\beta = 0$), the incident Stokes vector is

$$\mathbf{s}_I = \begin{pmatrix} s_{I0} \\ s_{I1} \\ s_{I2} \\ s_{I3} \end{pmatrix} = \begin{pmatrix} 1 \\ \sin 2\alpha_I \\ 0 \\ \cos 2\alpha_I \end{pmatrix} \tag{13}$$

The output Stokes vector is given by eq 1, and the direction of its major axis is, according to eq 4 (where α is now $\bar{\theta}$)

$$\tan 2\bar{\theta} = s_{O1}/s_{O3} \tag{14}$$

where $\mathbf{s}_O$ is obtained by substituting eq 11 and 13 in eq 1, *i.e.*

$$s_{O1} = (\gamma^2/2)(a_3b_2 - a_2b_3) + [1 - (\gamma^2/2)(a_2^2 + a_3^2)] \sin 2\alpha_I + \gamma a_2 \cos 2\alpha_I \tag{15}$$

$$s_{O3} = -\gamma b_3 + [1 + (\gamma^2/2)(b_3^2 - a_2^2)]\cos 2\alpha_I - \gamma a_2 \sin 2\alpha_I \tag{16}$$

including quadratic terms in **H**′. The neglect of the higher order terms introduces an error of no more than ½% of the first-order terms for γa_i and $\gamma b_i \sim 10°$.

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[23] Walker[24] gives matrices for polarizers and retardation devices, but his Stokes vector $(i, q, u, v)^+$ corresponds to $(s_0, s_3, s_1, s_2)^+$. Thus, Walker's matrix elements must be reordered before use with our **F** matrix.

[24] M. J. Walker, *Amer. J. Phys.*, **22**, 170 (1954).

We now express s_{01} and s_{03} of eq 15 and 16 in terms of $\bar{\alpha}$, $\bar{\theta}$, and properties of the medium. Since $|\bar{\alpha}|$ is usually $<2°$ in the experiment, we make the valid approximations that $\cos 2\bar{\alpha} = 1$ and $\sin 2\bar{\alpha} = 2\bar{\alpha}$. Since the Mueller matrix method follows the right-hand rule convention, the resulting definitions of $\bar{\alpha}$ and $\bar{\theta}$ must be converted to those of the left-hand rule, the more commonly used convention of optical rotation, by introducing α' and θ by means of the relations

$$\begin{aligned} \alpha' &= -\bar{\alpha} \\ \theta &= -\bar{\theta} \end{aligned} \qquad (17)$$

Thus, using eq 12 and 14–17, we obtain

$$\alpha' = \frac{1}{2}\,\frac{(\gamma^2/2)(a_2b_3 - a_3b_2)\cos 2\theta + \gamma b_3 \sin 2\theta - (\gamma^2/4)(a_3{}^2 + b_3{}^2)\sin 4\theta - \gamma a_2}{1 - (\gamma^2/2)[a_2{}^2 + a_3{}^2\cos^2 2\theta - b_3{}^2 \sin^2 2\theta]} \qquad (18)$$

for $|\alpha'| < 2°$, where α' is in radians. Equation 18 can be simplified further since the γa_i's and γb_i's are much less than unity. First of all, the quadratic terms in the denominator are all very small compared to unity and can be neglected. Secondly, except for the term in $(\gamma a_3)^2$, the quadratic terms in the numerator can be neglected compared to the first-order terms. Obviously, if a medium displayed only LB, *i.e.*, γa_3, the $(\gamma a_3)^2$ term would determine the rotation entirely. In some cases, a situation may arise in which $\gamma a_3 \gg \gamma b_3$ or γa_2, thus also requiring inclusion of the $(\gamma a_3)^2$ term (see section IG). Hence, eq 18 becomes

$$\alpha' = (1/2)[\gamma b_3 \sin 2\theta - (\gamma^2/4)a_3{}^2 \sin 4\theta - \gamma a_2] \qquad (19)$$

In the absence of the external field, the only term of eq 19 that would contribute to α' is $-\gamma a_2$, the ORD. Hence, the difference in α' in the presence and absence of the external field is

$$\alpha_E \equiv \alpha'_{E\neq 0} - \alpha'_{E=0} = (1/2)[\gamma b_3 \sin 2\theta - (\gamma^2/4)a_3{}^2 \sin 4\theta - \Delta(\gamma a_2)] \qquad (20)$$

Substituting for γb_3, γa_3, and γa_2 from eq 8, eq 20 becomes

$$\alpha_E\ (\text{deg}) = 180\{[(\ln 10)/4\pi](\epsilon_{\parallel} - \epsilon_{\perp})lc \sin 2\theta - (\pi/2)[(n_{\parallel} - n_{\perp})(l/\lambda)]^2 \sin 4\theta + \Delta(n_{\rm L} - n_{\rm R})(l/\lambda)\} \qquad (21)$$

for $|\alpha_E| \lesssim 2°$. The coefficient of the sin 2θ term is the LD, that of the sin 4θ term the LB, and the angle-independent term is the difference in ORD in the presence and absence of the field, and ($\parallel$, $\perp$) have been substituted for (x, y) of eq 8 because **E** is perpendicular to **k**. Each anisotropic property has a unique dependence on θ, which allows the LD, LB, and ΔORD to be separated. In the region of an absorption band, LD is usually the major component because LB arises from the quadratic term and ΔORD is a minor optical effect (for **E** perpendicular to **k**).[25] LB has been successfully studied[14,15] by this method with **E** perpendicular to **k**, but not ΔORD.

For the special case in which $\theta = 45°$, and ΔORD is negligible, eq 21 reduces to

$$(\alpha_E)_{45°}\ (\text{deg}) = 33(\epsilon_{\parallel} - \epsilon_{\perp})lc \qquad (22)$$

The foregoing theory can be generalized for any value of ω. In our experiments, we restrict ω to 90°. However, in general (*e.g.*, in the study of crystal optics), ω can take on any value. For $\omega \neq 0$ or 90° and in the limits of the theory ($|\alpha'| < 2°$), LB will cause negligible splitting of the beam into two rays, the E and O rays. For our case, in which $\omega = 90°$, if there is any LB, the E and O rays are exactly superimposed on each other. Tinoco[26] and Hoffman and Ullman[27] have used an apparatus with $\omega = 0°$ to measure ΔORD and ΔCD in poly(γ-benzyl L-glutamate) (PBLG) oriented by an external electric field **E** (see section IF).

(F) Anisotropic CD and ORD. The first-order term $\Delta(n_{\rm L} - n_{\rm R})(l/\lambda)$, *i.e.*, anisotropic ORD, appears in eq 21. Therefore, we consider in this section the question as to whether anisotropic ORD (and, in general, also anisotropic CD) is significantly large compared to the other terms of eq 21. Another interesting question is whether ΔORD itself can be used[22] to obtain information about $\boldsymbol{\mu}_{0i}$. We will show here that ΔORD and ΔCD are very small, and therefore that LD is a better quantity to study than ΔORD or ΔCD to learn about $\boldsymbol{\mu}_{0i}$.

As seen in eq 21, ΔORD is independent of θ, and it can be shown by Gō's method[20,21] that it depends on ω. In his analysis, Gō[20,21] used tensors with complex elements a_{ij}, a_{ijk}, . . . to describe the interaction of light with a molecule. The a_{ij}'s contain quantities like $(p_j)_{0n}(p_i)_{n0}$, where $(p_j)_{0n}$ is the linear momentum transition moment along the molecular axis j between states 0 and n. The a_{ij} terms produce isotropic absorption, LD, and LB. The a_{ijk} elements include quantities such as $(p_jr_k)_{0n}(p_i)_{n0}$, from which anisotropic ORD and CD arise (see eq 6 of ref 20). For a medium oriented by an external field **E**, it can be shown that

$$(a_2 - ib_2)_{\omega=0°} = [1 - (1/2)f(x)]A + (1/4)f(x)(a_{123} - a_{213}) \qquad (23)$$

$$(a_2 - ib_2)_{\omega=90°} = [1 + (1/4)f(x)]A - (1/8)f(x)(a_{123} - a_{213}) \qquad (24)$$

where $f(x)$ is the degree of orientation induced by **E** [see paper II[28] for the form of $f(x)$], and $A = {}^1/_6(a_{123} + a_{231} + a_{312} - a_{321} - a_{213} - a_{132})$, which is the isotropic optical activity, with subscript 3 indicating the direction of the permanent dipole moment. Equations 23 and 24 refer to the ORD (a_2) and CD (b_2) for **E** parallel to **k** ($\omega = 0°$) and **E** perpendicular to **k** ($\omega = 90°$), respectively, at a given field strength **E**. Since $f(x) = 1$ for complete orientation, eq 23 and 24 reduce to

$$(a_2 - ib_2)_{\omega=0°} = A/2 + (1/4)(a_{123} - a_{213}) \qquad (25)$$

$$(a_2 - ib_2)_{\omega=90°} = 5A/4 - (1/8)(a_{123} - a_{213}) \qquad (26)$$

in this case. Hence, we see that anisotropic (CD, ORD) is of the same order of magnitude as A, the isotropic (CD, ORD). By subtracting A from eq 23 and 24, we obtain ΔCD and ΔORD, which are seen to be proportional to $f(x)$, as are LD[28] and LB.[28]

Since (ΔCD, ΔORD) and (LD, LB) are all proportional to $f(x)$ then, at any given $f(x)$, the relative magnitudes of a_{ij} and a_{ijk} provide an estimate of the relative sizes of γb_3 and $\Delta\gamma a_2$ in eq 20, *i.e.*, of LD and ΔORD. The elements a_{ij} and a_{ijk} differ by two factors:[20] (1) a_{ijk} has an additional factor of $1/\lambda$ which is 4×10^4 cm^{-1} at 250 mμ and (2) a_{ijk} contains **r** in $(p_jr_k)_{0n}$, the magnitude of r being <5 Å in the region in which a localized wave function has an appreciable value.

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(25) See section IF.

(26) I. Tinoco, Jr., *J. Amer. Chem. Soc.*, **81**, 1540 (1959).

(27) S. J. Hoffman and R. Ullman, *J. Polym. Sci., Part C*, No. 31, 205 (1970).

(28) T. C. Troxell and H. A. Scheraga, *Macromolecules*, **4**, 528 (1971).

Combining these two factors, we would expect a_{ij}/a_{ijk} to be $\sim$500. An examination of experimental data[26,27] on anisotropic ORD and CD of PBLG verifies this estimate. Thus, one would expect the ΔORD to be insignificant compared to the LD term in eq 21, for $\omega = 90°$.

As a result of this estimate, it is usually wiser to measure either LD or LB if information about $\boldsymbol{\mu}_{0i}$ is desired. Furthermore, extreme caution must be used to measure anisotropic ORD with $\omega = 0°$ because slight imperfections in the apparatus (such as inhomogeneities in E or incomplete collimation of the beam) will introduce a significant LB component, especially if the instrument is sensitive to changes in ellipticity in the beam; in other words, since LB is large compared to ΔORD, the former can falsify the measurements of the latter. In any case, if one wants to measure ΔORD, one should test that α_E (for $\omega = 0°$, it can be shown by Gō's method[20,21] that LD and LB both vanish) is independent of θ to prove that the observed effect is free of instrumental artifacts.

(G) Kronig–Kramers Transforms. In order to examine the relative magnitudes of γb_3 and γa_3, which appear in eq 20, we consider the Kronig–Kramers optical transforms which relate not only these quantities but all dispersive (a_i) and absorptive (b_i) phenomena. The Kronig–Kramers relations for a_3 and b_3 are[29,30]

$$a_3(\nu) = \frac{2}{\pi}\nu \oint_0^\infty \frac{b_3}{\nu'^2 - \nu^2}\,d\nu' \qquad (27)$$

$$b_3(\nu) = -\frac{2}{\pi}\nu^2 \oint_0^\infty \frac{a_3}{\nu'(\nu'^2 - \nu^2)}\,d\nu' \qquad (28)$$

where ν is the frequency of the light, and the integration is carried out over ν'. With these transforms, one can calculate, say, $a_3(\nu)$ from $b_3(\nu')$, and compare the result with the experimental values of $a_3(\nu)$. Differences would be due to contributions from bands in inaccessible regions of the $b_3(\nu')$ spectrum.

To obtain some idea of the relative magnitudes of a_3 and b_3, we use a simple gaussian band to represent $b_3(\nu')$; then eq 27 leads to

$$|a_3|_{\max} \sim 0.6|b_3|_{\max} \qquad (29)$$

where the maxima in a_3 and b_3, respectively, occur at slightly different wavelengths λ. Equation 29 indicates that $|a_3|_{\max}$ and $|b_3|_{\max}$ are comparable in size.

For $(\gamma a_3, \gamma b_3) < 10°$, we saw from eq 19 that the contribution to the induced rotation, α', from LD is

$$\alpha' = (1/2)\gamma b_3 \sin 2\theta \qquad (30)$$

Further, it can be shown by the Mueller matrix method that the contribution to the induced *ellipticity*, β, from LB is

$$\beta = -(1/2)\gamma a_3 \sin 2\theta \qquad (31)$$

From eq 29–31 we see that the maximum values of α' and β are comparable in magnitude; the same is true of all quantities a_i and b_i which are related by a Kronig–Kramers transform. Since dispersive (a_i) phenomena cover a larger spectral region than their absorptive (b_i) counterparts, it is easier to separate individual transitions which contribute to b_i than to a_i. But, on the other hand, a_i can be observed in a nonabsorbing region of the spectrum where there is no interference from absorption bands.

While $|a_3|_{\max}$ and $|b_3|_{\max}$ are comparable in size, the contribution of b_3 to α' is very much larger than that of a_3 within a particular absorption band, since $a_3{}^2$ appears in eq 19, and both a_3 and b_3 are small. However, for a very weak LD band in the neighborhood of a strong LD band, the LB (a_3) of the strong band, in the region of $\lambda_{\max}$ of the weak band, corresponds (by a Kronig–Kramers transform) to a relatively large LD (b_3) from the strong band, which is superimposed on the LD of the weak band in the measurement of α_E; therefore, in order to obtain the LD of the weak band, one must separate out the LB contribution from the nearby strong band. Thus, the LB term must be retained in eq 19 in this case, as well as when the medium exhibits only LB. Of course, if θ is set equal to 45°, the LB term vanishes.

(H) Form Dichroism and Form Birefringence. Up to this point, we have considered LD and LB to arise from intrinsic molecular properties ($\boldsymbol{\mu}_{0i}$), independent of the shape of the molecule. In oriented systems containing nonspherical macromolecules, one observes shape-dependent or *form* LD and LB, which arise from scattering as the result of the form of the solute and a difference between the indices of refraction of the solute and solvent. Form dichroism and form birefringence are also mutually related by Kronig–Kramers transforms.

Form dichroism $(\tau_\parallel - \tau_\perp)$ is the analog, for an oriented system, of the turbidity (τ) of an isotropic system. In a turbid medium, the intensity of the forward propagating beam is partially reduced because part of the light has been scattered by the solute particles. If the particles are long thin rods, the ratio $(\tau_\parallel - \tau_\perp)/\tau$ is positive and has the asymptotic limit of $^{15}/_{22}$ for completely oriented infinitesimally thin rods.[15,31–34] Form dichroism (like turbidity in general[35]) can be detected easily since it leads to a nonzero apparent absorbance (with a monotonic dependence on wavelength), which can be observed even in spectral regions that are devoid of electronic transitions. However, the turbidity must be fairly large for form dichroism to be observable.

Thus, when γa_3 and γb_3 are related to the microscopic properties of a medium, the contributions from form dichroism and form briefringence must be taken into account (using a technique described elsewhere[35]), in addition to the intrinsic chromophoric LD and LB. In the results to be presented in this series of papers, this correction was not required, since no form dichroism or significant turbidity was detected.

(I) Linear Dichroism Sum Rule. Since the interpretation of ED measurements will require an analysis of the electronic transitions of the chromophores of the molecule, it is useful to inquire into the possible existence of a sum rule for LD, analogous to the Kuhn–Thomas sum rule for isotropic absorption[36] and the rotational strength sum rule for optical activity.[37] While we could not find an LD sum rule in the current literature, Born essentially states one in a discussion of the dispersion of anisotropic polarizability,[38] and we derive it here (making use of Kauzmann's derivation[36] of the Kuhn–Thomas sum rule) for completeness in the treatment of LD theory.

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(29) L. J. Oosterhoff, in "Modern Quantum Chemistry," Part III, O. Sinanoglu, Ed., Academic Press, New York, N. Y., 1963, p 6, eq 4 and 5. These equations were checked by starting from the general Kronig–Kramers integral transform.[30]

(30) J. R. MacDonald and M. K. Brachman, *Rev. Mod. Phys.*, **28**, 393 (1956).

(31) S. Higashi, M. Kasai, F. Oosawa, and A. Wada, *J. Mol. Biol.*, 7, 421 (1963).

(32) J. E. Mayfield and I. J. Bendet, *Biopolymers*, **9**, 669 (1970).

(33) E. W. Taylor and W. Cramer, *Biophys. J.*, 3, 127 (1963).

(34) J. Y. Cassin and E. W. Taylor, *ibid.*, **5**, 531 (1965).

(35) S. J. Leach and H. A. Scheraga, *J. Amer. Chem. Soc.*, **82**, 4790 (1960).

(36) W. Kauzmann, "Quantum Chemistry," Academic Press, New York, N. Y., 1957, p 651.

(37) Reference 36, p 711.

(38) M. Born, "Optik," Springer-Verlag, Berlin, 1933, p 526.

The Kuhn–Thomas sum rule[36] is

$$\sum_{\substack{\text{all}\\ j\neq 0}} [(f_x)_{j0} + (f_y)_{j0} + (f_z)_{j0}] = N \qquad (32)$$

where (x, y, z) are arbitrary orthogonal molecular axes, $(f_x)_{j0}$ is the x component of the oscillator strength for the transition $0 \rightarrow j$ [similarly for $(f_y)_{j0}$ and $(f_z)_{j0}$], N is the number of electrons in the molecule, and j represents all discrete and continuum states excluding the initial state 0. Equation 32 holds because the total oscillator strength is N. From Kauzmann's[36] eq A-32, A-44, and A-45, we obtain

$$\sum_{\substack{\text{all}\\ j\neq 0}} (f_x)_{j0} = \sum_{\substack{\text{all}\\ j\neq 0}} (f_y)_{j0} = \sum_{\substack{\text{all}\\ j\neq 0}} (f_z)_{j0} = N/3 \qquad (33)$$

i.e., the x, y, and z components of the oscillator strength are equal. Hence, the LD sum rule follows from eq 33, *viz.*

$$\sum_{\substack{\text{all}\\ j\neq 0}} [(f_x)_{j0} - (f_y)_{j0}] = 0 \qquad (34)$$

where (x, y) is any pair of molecular axes. Further, since[39]

$$f_x \propto \int \epsilon_x \mathrm{d}\nu \qquad (35)$$

eq 34 may be written as

$$\sum_{\substack{\text{all}\\ j\neq 0}} [(\int \epsilon_x \mathrm{d}\nu)_{j0} - (\int \epsilon_y \mathrm{d}\nu)_{j0}] = 0 \qquad (36)$$

from which an alternative form of the LD sum rule follows, *viz.*

$$\int_{\substack{\text{all}\\ \text{transitions}}} (\epsilon_u - \epsilon_v)\mathrm{d}\nu = 0 \qquad (37)$$

where (u, v) are the principal axes of the linear dichroic medium, usually taken as ($\|$, $\perp$) as in eq 21.

The LD sum rule can be used in the same way as the other two sum rules are. For example, since the integral in eq 37 is zero, LD and LB, respectively, from different transitions, tend to cancel. In LB (in which individual bands have a large dispersion), the sum rule would imply that the contributions from remote bands would cancel, leaving the LB contributions from nearby transitions at the observed wavelength as the major ones; this is in contrast to the isotropic index of refraction, where the oscillator strengths sum to N instead of zero.

As another example of the applicability of the sum rule of eq 37, consider the problem of coupled oscillators, and distribute a set of identical chromophores regularly on a helix. In the absence of coupling, the transition $0 \rightarrow i$ of any chromophore would give rise to the linear dichroism LD_i of a monomer on the helix. Now introduce a strong coupling so that the transition $0 \rightarrow j$ in these chromophores forms an exciton band by identical oscillator interaction; this results in the splitting of the $0 \rightarrow j$ transition into two bands polarized parallel and perpendicular, respectively, to the helix axis. According to the sum rule, the integral of the LD from the $0 \rightarrow j$ transition plus that from all other transitions must be zero, *independent* of the strength of coupling. Hence, if no transitions, other than the $0 \rightarrow j$, are affected by the coupling, then the sum of the intensities of the (exciton) split band must equal that of the unsplit (zero coupling) band. However, when the constraint of the LD sum rule is combined with those from the other two sum rules,[40] we find that there is a severe restriction as to how the intensity is distributed between the split bands. Furthermore, because the value of the LD integral $\int(\epsilon_u - \epsilon_v)\mathrm{d}\nu$ *over the* $0 \rightarrow j$ *transition* is independent of the strength of coupling,[5] we can determine the LD of the monomer band by evaluating the LD integral over the two exciton bands arising from this transition; as shown in paper II,[28] this will enable us to determine the direction of the $0 \rightarrow j$ transition in the monomer with respect to the helix axis.

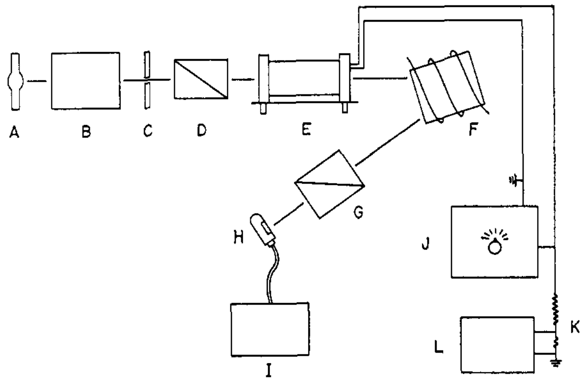

Figure 3. Schematic diagram of ED apparatus: (A) light source, (B) double monochromator, (C) slit, (D) motor-driven polarizer, (E) ED cell and holder; (F) Faraday modulator, (G) fixed analyzer, (H) photomultiplier tube, (I) electronic signal processor, (J) static high-voltage generator, (K) fixed potential divider, and (L) potentiometer.

II. Method of Measurement of Electric Dichroism

(A) Apparatus. The ED apparatus consists of two components, a Cary Model 60 spectropolarimeter and an ED modification, as shown in Figure 3. The Cary instrument supplies a well-collimated, monochromatic, linearly polarized light beam. After the ED cell introduces rotation (and ellipticity), the Faraday modulator of the Cary instrument adds a 60-Hz sinusoidal variation to the major axis of the polarized light. The electronics lock on to and amplify the 60-Hz component of the light falling on the photomultiplier, using it to drive the polarizer until the combined polarizer and ED cell are crossed with the analyzer, while simultaneously recording the orientation of the polarizer.

The ED modification consists of a cell, cell holder, high-voltage generator, fixed-potential divider, and potentiometer. The cell is a cylindrical Kel-F body with removable polished parallel stainless steel electrodes, 1.3 cm wide and 1–3 cm long, separated by a gap of 0.8 cm and arranged so that **k** is perpendicular to **E**. Stainless steel screws hold the electrodes in place and serve as contacts to the high-voltage terminals in the cell holder. Strain-free quartz end windows are butted, within 0.1 mm, against the ends of the electrodes to minimize end effects in the electric field, and are held in place by phenolic resin end caps with intervening Teflon spacers and O rings to provide a strain-free seal. The cell is removable from the holder to facilitate filling with a hypodermic needle and syringe and cleaning by disassembling.

Use is made of the three-point locating platform of the Cary instrument in mounting the cell holder. Vertical phenolic resin pieces, attached to the platform, provide a rigid mount for a rotatable phenolic resin cylinder which houses the cell and contains the high-voltage terminals. The angle θ can be varied by rotating the cylinder.

The high voltage is generated by a Spellman (Model 2040) variable dc power supply. A fixed potential divider, consisting of a series of 60 precision 332-kohm resistors encased in polyethylene tubing, reduces the voltage by a factor of 10^4 so that it can be measured by a simple potentiometer with an accuracy of better than 1%. The usable electric field range is 9–90 statvolts/cm.

With this apparatus α can be measured as a function of θ, E, and λ.

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[39] Reference 36, p 581.

[40] D. F. Bradley, I. Tinoco, Jr., and R. W. Woody, *Biopolymers*, **1**, 239 (1963).

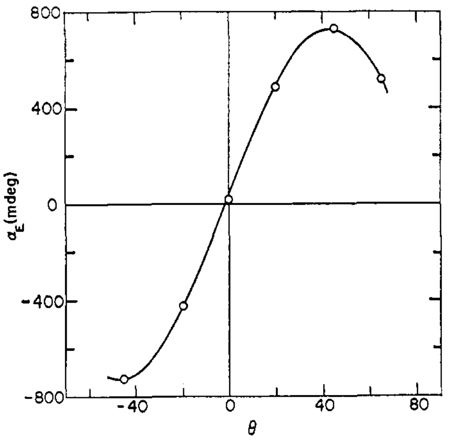

Figure 4. ED of *p*-Cl-PBLA in dioxane (residue concentration = 1.1×10^{-4} *M*) at 225 mμ in a cell of 1-cm path length at a field of 52.7 statvolts/cm. The points are the experimental values of α_E as a function of θ and the curve is that of sin 2θ (normalized to pass through the maximum and minimum values, and centered at $\theta = -2°$).

(B) Performance. The sensitivity of the method is simply that of the Cary Model 60 spectropolarimeter. According to the manufacturer's specifications, the maximum rms noise is 0.7 and 3.3 mdeg at 300 and 200 mμ, respectively, with no cell in the beam, for a 3-sec pen period and a 1-mμ half-band width. Actual instrument performance is variable but usually significantly better than the specifications. We find the limiting sensitivity (*i.e.*, the sensitivity as the optical density approaches zero) of our instrument to be about 1 mdeg around 250 mμ, which is equivalent to $(\epsilon_{\parallel} - \epsilon_{\perp})lc$, *i.e.*, Δabs, of 3×10^{-5}. This may be compared with a value of about 1×10^{-3} when Δabs is obtained by a conventional direct measurement of the separate absorbances of two mutually perpendicularly polarized light beams. The increased sensitivity attainable with a spectropolarimeter arises primarily from the factor of 33 (in eq 22) which multiplies the value of Δabs in the direct measurement of α_E.

Figures 4–6 show representative data for poly(*p*-chloro-β-benzyl L-aspartate) (*p*-Cl-PBLA) and *p*-Cl-PBLG in dioxane. In Figure 4 the dependence of α_E on θ at 225 mμ (an absorbing region) is given. The curve is the function sin 2θ, adjusted to pass through the maximum and minimum points. The best fit to the experimental points was obtained by shifting the zero of the θ scale to $-2°$; this is necessitated, presumably, by some determinate error in the measurement of θ. In a nonabsorbing region (286 mμ), the average value ($\sim$9 mdeg) of $|\alpha_E|$ was much less than the values shown in Figure 4. This dependence on λ, and the sin 2θ dependence at 225 mμ, indicates, according to eq 21, that the overwhelmingly main contribution to α_E is from the LD term. Since the curve fits the experimental points so well, there does not appear to be any significant inhomogeneity in the applied electric field (especially considering that the cross section of the cell which is illuminated varies with θ). In all of our work, the adherence of α_E to a sin 2θ dependence is taken as a demonstration that the origin of α_E is in LD induced by the electric field, *i.e.*, ED.

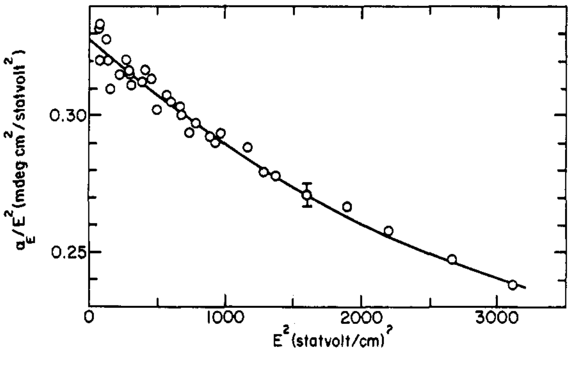

Figure 5. Dependence of α_E/E^2 on E^2 for the same solution, cell, and wavelength as in Figure 4, at $\theta = 45°$. The points are experimental and the curve is the least-squares fit to eq 38, together with the standard deviation.

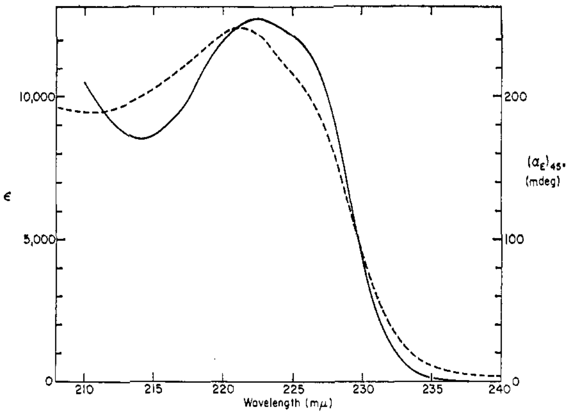

Figure 6. Extinction coefficient in absence of electric field (dashed curve), and ED spectrum at 69 statvolts/cm, path length = 1 cm, $\theta = 45°$ (solid curve), of *p*-Cl-PBLG in dioxane (residue concentration = 4.2×10^{-5} *M*). The values of $(\alpha_E)_{45°}$ have been corrected, by subtracting 5 mdeg at all wavelengths, to take account of the apparatus artifact; this correction is 2% of the maximum value of $(\alpha_E)_{45°}$.

The dependence of α_E on **E**, for the same solution, cell, and wavelength used in Figure 4, at $\theta = 34°$, is shown in Figure 5, plotted as α/E^2 *vs.* E^2. The points in Figure 5 were fit, by least squares, to the equation

$$\alpha_E = A_0E^2 + A_1E^4 + A_2E^6 \tag{38}$$

with a standard deviation in α_E/E^2 of about 4.0×10^{-3} (1.3% at $\alpha_E/E^2 = 0.3$). The constants in eq 38 are $A_0 = 0.328$, $A_1/A_0 = -1.30 \times 10^{-4}$, and $A_2/A_1 = -1.07 \times 10^{-4}$. The relative values of these constants are as predicted[28,41] from the ED theory for the interaction of permanent dipoles with the electric field. It should be noted that the primary dependence of α_E on E resides in the E^2 term.

The absorption curve in the absence of an electric field and the dependence of α_E on λ in the presence of a field (at $\theta = 45°$), of *p*-Cl-PBLG in dioxane, are shown superimposed on each other in Figure 6. For a strongly allowed transition, the wavelength dependence of ϵ (at $E = 0$) and α_E would be expected to be the same. The curves in Figure 6 are similar, with differences arising from overlapping bands and from variation in the direction of transition moments within weak bands.

An assessment of end effects (*i.e.*, inhomogeneity in **E** at the ends of the cell) was obtained by varying the path length of the cell. Increasing the path length from 1 to 3 cm had little effect on α_E/l, the ratio of this quantity for 3- and 1-cm path lengths being 1.000 ± 0.035 in four experiments on three different polymers in dioxane. The homogeneity of the electric field was also checked by introducing a circular slit between E and F of Figure 3. For example, in a particular case, the slit reduced the observed illuminated cross-sectional area of the cell by about 75%, but A_0 of eq 38 increased by only 2.4%.

The behavior of α_E with variation in θ, **E**, λ, l, and slit width indicates that the ED apparatus functions satisfactorily. Nevertheless, it was desired to have a check on the magnitude of the observed ED to demonstrate that no significant errors are introduced by the cell or by the voltage-measuring apparatus. Since no polymers are available which can serve as a check on the magnitude of the observed ED, resort was had to an alternative test. Instead of measuring ED, the EB of chlorobenzene was measured (Kerr

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(41) The theory of ED is presented in paper II.[28]

effect), since the Kerr constant of this substance is known. The idea here is that, if one obtains the correct Kerr constant using the same cell and voltage-measuring equipment (but with a different optical system), then one has a check on the proper operation of the cell and electrical system, not only for this EB measurement but also for the ED measurements on dichroic substances. Hence, another optical system was used for the measurement of the Kerr constant of chlorobenzene, with a quarter wave plate to convert the ellipticity induced by the EB to a rotation. The orientation of the resultant plane of polarization was measured photoelectrically by detecting the analyzer positions of equal intensity on each side of the extinction position. The observed Kerr constant of chlorobenzene was 1287×10^{-9} cm statvolt^{-2} at 578 mμ compared to the literature value[42] of 1271×10^{-9}, corrected from 589 to 578 mμ. In addition to this excellent agreement, the EB followed a sin 2θ dependence very closely, as expected from eq 31.

(C) Discussion. As might be expected with a highly sensitive apparatus, rotational artifacts were observed when, for example, EB (γa_3) was large and $\gamma a_3 \gg \gamma b_3$. For example, in a spectral region in which LD, CD, and CB are zero, the LB departed from a sin 4θ dependence on θ. Numerous experiments were carried out to try to locate and possibly eliminate the instrumental imperfection which couples to the EB to produce the artifact. It was possible to show that reflection from the electrodes was not responsible for the artifact. The artifact appears to be relatively independent of the cell path length, cell alignment, and instrumental slit width. It differs somewhat between two different Cary Model 60 spectropolarimeters, but not sharply enough to be able to attribute it *definitely* to the spectropolarimeter. Its magnitude varied over a period of several months, and also with strains applied to the cell windows. However, it could be demonstrated that the artifact itself was not due to strains in the cell windows. No such artifact was observed by Ingwall and Flory,[14] who also used a Cary Model 60 spectropolarimeter (to measure strain-induced LB).

One possible source of the artifact may be a slight ellipticity in the *incident* beam, from strains in the optics of the Cary Model 60 spectropolarimeter or in our ED cell windows, which is converted into a rotation by the EB in the cell. A Mueller matrix analysis of an optical element of the instrument, which introduces an ellipticity β before the linearly polarized light is incident on the polymer *solution*, indicates that the following term should be added to eq 21

$$\alpha_E(\text{deg}) = (90/\pi)\gamma a_3 \tan 2\beta \cos 2\theta \qquad (39)$$

For $\beta = 0.5°$ and $\gamma a_3 = 10°$, α_E has a maximum value (at $\theta = 0°$) of 86 mdeg, while the rotation from the natural EB (eq 21) is 218 mdeg (at $\theta = 67.5°$). An ellipticity of 0.5° corresponds to a beam in which $I_\perp$ is $\sim 0.008\%$ of $I_\parallel$ and is barely detectable by visual observation of crossed nicol prisms.

A second possible source of the artifact could arise from strain birefringence in an optical element of the spectropolarimeter *following* the cell. Such an element could be the Faraday modulator which may also be the major source of the relatively large base-line dispersion ($\Delta\alpha \sim 70$ mdeg from 500 to 210 mμ) of the Cary instrument. For relatively small strains, significant contribution to α_E can be produced when the EB in the cell is large, as in the first possibility.

As a third possibility, second-order inhomogeneities in E may give rise to the artifact.[43]

Although we cannot definitely assign the source of the artifact, we suspect that the Faraday modulator may give rise to the difficulty. This is a typical example of problems which can be encountered in such highly sensitive apparatus, and indicates the importance of testing and analyzing such instruments in considerable detail.

Because of this artifact, our ED method cannot be used to measure EB or ΔORD (eq 21) because these are small in the spectral region where the artifact is of comparable magnitude. However, similar apparatus[14,15] has been used to measure LB. On the other hand, in the region of an absorption band which is not weak, where ED is measured, such artifacts are negligible. For example, for an individual band (*e.g.*, one for which $\gamma b_{3_{\max}} = 1°$ and, according to eq 29, $\gamma a_{3_{\max}} = 0.6°$), the resulting value of $(\alpha_E)_{\max}$ would be 500 mdeg (arising from the LD), while that from the artifact (eq 39, with $\beta = 0.5°$) would be ~ 5 mdeg and hence negligible; this is confirmed by the sin 2θ dependence of the ED in Figure 4. On the other hand, for a very weak band in the neighborhood of strong bands, the situation may be different. In PBLG, there is a very weak band ($\epsilon_{\max} \sim 220$) at 260 mμ, with neighboring strong far-ultraviolet bands (*e.g.*, $\epsilon_{185} \sim 50{,}000$). Assuming that the transition moments are in the same direction for each of these bands, it can be seen that large EB tails, originating in the far-ultraviolet bands, will overlap the 260-mμ band in ED. In this situation, γa_3 from the strong band is $\gg \gamma b_3$ from the weak band at 260 mμ, and the artifact may contribute significantly. Since the EB tails of strong bands give rise to the artifact in the region of the weak band, the spurious rotations will vary slowly and monotonically with λ. Thus, it is often possible to separate the ED of a weak band from the artifact in an α_E spectrum, even though the two are superimposed. Of course, as indicated above, this problem does not arise in the region of stronger bands. Notwithstanding the artifact, the method developed here is a powerful one to study ED.

Other varieties of sensitive LD apparatus have been developed in the laboratories of Brahms[8] and Holzwarth.[12] The Brahms method permits studies in the vacuum ultraviolet, although its noise is somewhat higher than ours (Δabs $\sim 3 \times 10^{-4}$) and their method required the development of a complete instrument. Mandel and Holzwarth[12] have modified a Cary Model 6001 attachment to the spectropolarimeter to make LD measurements on oriented films. The sensitivity of their instrument is similar to ours, and their apparatus is relatively unaffected by ORD, CD, or LB (precluding LB studies). Their Cary Model 6001 modification has inadequate space in the sample compartment to introduce orientation devices such as the ED cell or a Couette device so that they are unable to study anisotropic *solutions*.

Allen and Van Holde[10] have recently developed a pulsed-field LD instrument. However, the short duration of the field pulse decreases the sensitivity with which the signal can be observed. The Cary Model 60 spectropolarimeter and the other apparatus cited could not be adapted to pulsed-field experiments because the instrument response time is of the order of seconds. Thus, we measure only steady-state ED and cannot measure rotational diffusion times or ED in conducting solutions.

III. Conclusion

The Cary Model 60 spectropolarimeter is easily converted into a highly sensitive steady-state ED apparatus for nonconducting solutions. In fact, the only component needed for LD measurements is a device to orient the sample. The Mueller matrix algebra shows that spectropolarimeters similar in design to the Cary instrument are capable of measuring ΔORD (for $\omega = 0°$) and LB. The latter two properties normally produce small rotations compared to LD. Furthermore, the Mueller matrix algebra is seen to be easily adaptable for the analysis of the optical properties of complicated anisotropic media and thereby to have utility in developing instruments for studying these systems. The simplicity of this analysis was achieved by separating out the contribution from the average absorption.

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