Optical Spectroscopy of Oriented Molecules

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I. Introduction

This review covers the interpretation of experiments in the field of polarization modulation spectroscopy. Only transmission spectroscopy will be discussed, so such subjects as fluorescence, reflection spectroscopy, Rayleigh and Raman scattering, and other types of spectroscopy will not be considered. Other recent reviews in this general area are given in ref 1–8, though none of these covers the same ground as the present review.

Over the past 50 years or so the information sought in spectroscopic investigations has gradually become more sophisticated. At first, spectral bands were characterized only by their wavelengths, i.e., by the energy differences between the excited and the ground states. Later, it was shown that, with the assumption of Born–Oppenheimer separability, the integrated absorption over a vibronic band is directly proportional to the square of the electronic transition moment with fixed nuclei.[9] As a result experimental and theoretical results for intensities could be compared to obtain increased information on selection rules, assignments, molecular structure, etc. For molecules with high symmetry the orientation of electric transition moments in the molecular framework can be deduced by symmetry arguments. The interest in finding the direction of transition moments in molecules of low symmetry led to the study of polarized absorption in crystals and other oriented systems. Out of this has grown the current status of the field in which absorption, circular dichroic, and linear dichroic spectra are being obtained for a large variety of systems. Applications have developed simultaneously in the areas of inorganic, organic, and biological chemistry.

One could paraphrase this quest for sharpened spectroscopic information in the following way. An electronic transition is characterized by a series of transition matrix elements, one for each relevant quantum mechanical operator. The frequency of absorption establishes the energy matrix element for the excited state; the measurement of intensity usually provides a value for the square of the electric dipole transition matrix element. Polarized absorption spectroscopy permits the evaluation of the three components of the electric transition moment in the molecular framework. Circular dichroism studies, which became common 20 years ago, provide information about the components of the orbital magnetic moment operator. Finally, in the contemporary state of development, studies are made on oriented systems using both linearly and circularly polarized light. Such studies provide the possibility for the measurement of transition matrix elements of operators of the form $r_\alpha p_\beta$, where r represents a Cartesian coordinate, p, the linear momentum, and $\alpha,\beta = x,y,z$. As is well-known, magnetic moments and quadrupole moments can be generated as linear combinations of these operators, so they are included in this extended set. The motivation for the experimental determination of these quantities is that all of these matrix elements are easily calculated when suitable wave functions are available. These matrix ele-

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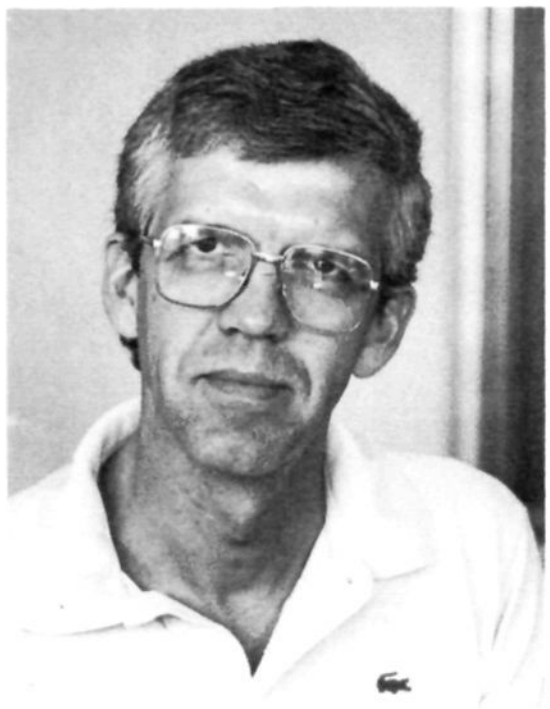

Hans P. Jensen was born June 11, 1943, in Copenhagen, Denmark. He received his M.S. degree from the University of Copenhagen in June 1968 and his Ph.D. degree from Chalmers University of Technology, Sweden, in 1981. He has served as both assistant professor (1969–1972) and associate professor (1972 to present) at the Technical University of Denmark. His scientific work falls within the structure of coordination compounds and ligands, mainly studied by circular dichroism spectroscopy, theoretical considerations in the area of phase-modulated differential absorption spectra of anisotropic compounds, and history of chemistry.

ments are functions of molecular conformation as well as electronic structure, and their experimental determination can be used either to determine conformation or to provide a powerful basis for testing the adequacy of our theoretical understanding of the electronic structure of molecules. At present the agreement between experiment and theory for the more subtle matrix elements of circular polarization spectroscopy is usually qualitative at best. Rather than being discouraging, this should be regarded as an incentive for increased experimentation in this field, since the data obtained provide guidelines on the path to a better understanding of the electronic excited states of molecules.

As is usual, these new measurements have fluorished only because of technological developments that have made them possible and convenient. A number of years ago, H. Cary stated that the accurate measurement of small difference spectra requires the comparison of signals in space or in time.[10] Methods such as half-shade devices, compensators, and double-beam arrangements separate signals in space so that simultaneous comparisons can be made. Newer methods using choppers and other modulation devices, coupled with ac circuitry, permit the comparison of intensities over a short period of time with a single beam. The invention of such devices as the Faraday modulator or the polarization modulator described later in this review, coupled with devices such as the lock-in amplifier, has opened the path to the determination of all the optical constants of an oriented system with great accuracy. (We refer the reader to a little-known work of J. Hofrichter, who determined the absorption, linear dichroism, birefringence, circular dichroism, and optical rotatory dispersion of a number of biological molecules in a flow instrument.[11]) Since the incident beam and the transmitted beam may both be represented by Stokes vectors of four components, the optical behavior is determined by a matrix of 16 coefficients, of which 7 are independent if there is no depolarization by scattering or other processes. This is called the Mueller matrix. It will be shown later how all of these (with one uninteresting exception) may be measured directly with simple optical and electronic apparatus involving a modulator and two polarizers.

Techniques are being developed[12] that use more than one modulator, operating at more than one frequency. These are used for the study of circularly and linearly polarized scattering, where 10 of the 16 matrix elements are independent.[13] We also mention here briefly the work of Fuller and his associates, who have developed techniques and instruments for measuring several of the Mueller matrix elements simultaneously.[13a,b] Their methods will be discussed in section II.J.

It is reasonably safe to conclude that the problem of the measurement of the elements of the Mueller matrix of a system have been essentially solved, though artifacts which arise from the combination of unavoidable anisotropies of the measuring system with the anisotropies under investigation can cause severe dificulties. Such problems can be overcome in favorable cases and will be discussed later. The principal difficulty lies in unscrambling the optical effects contained in the measured optical constants (Mueller matrix elements). For "pure" cases, i.e., cases that show either linear or circular anisotropies, but not both, this does not constitute a problem. But, as will be seen, the relationships are very complicated for the general case. Only by understanding the interplay of effects at the phenomenological level as well as the molecular level can one hope to design experiments and interpretive procedures that realize the full potential of the method.

This review is an attempt at describing the phenomenological theory necessary for the interpretation of modulation spectroscopy for the general case. We also discuss the approximate formalism used to relate these results to the optical tensors of molecular systems. We

have not tried to present a survey of results in the field. We have, however, included several examples in the section on applications. These examples have been selected to indicate the diversity of applications and methods of molecular orientation.

The subject matter that follows is divided into three main sections. In section II the transfer function for a general optical sample (i.e., a system possessing both linear and circular anisotropy) is developed in the Jones and Stokes formalism. This leads to a phenomenological definition of circular dichroism, linear birefringence, etc. for the general case. It is then shown how phase-modulation techniques permit the measurement of all useful Mueller matrix elements (up to 15 out of 16 with the apparatus of Figure 2). The main problem is to convert knowledge of these matrix elements into information on the seven basic optical quantities, and this is discussed at some length. Section III deals with the molecular interpretation of the optical quantities. Since this is mainly done by using lattice gas equations, the first half of the discussion analyzes the applicability of these formulas to real systems, while the second half relates the phenomenological parameters to quantum mechanical spectroscopic matrix elements that are dependent on orientation, conformation, and electronic structure. Section IV presents a number of applications in summary form. Work is being vigorously pursued at present in the fields of inorganic, organic, and biophysical chemistry, and the examples are selected to demonstrate the diversity of applications. Finally, since sections III and IV describe results and their interpretation, whereas section II deals with problems and technicalities, the nonspecialist may wish to defer the reading of this section by assuming that the basic set of optical properties are experimentally available. The sorting out of these experimental properties is, however, the fundamental challenge of the field.

II. Phenomenology: Definition of the Problem

This review will discuss the measurement and interpretation of the polarized spectroscopy of anisotropic systems such as crystals or oriented macromolecules or films. To avoid unnecessary complications the sample will be considered to be confined between two plane surfaces perpendicular to a collimated incident light beam. Only the properties of the transmitted light will be discussed, so the phenomena under consideration are absorption and refraction, dichroism, and birefringence. Reflections from the surface of the sample or from the cell that contains it are also ignored, though in practice corrections for reflection must often be made. It is also assumed that the scattering of light by the sample is sufficiently small that it does not affect the intensity or polarization of the transmitted beam appreciably. Thus we exclude the effects of differential light scattering, which are at present being intensively investigated in a number of laboratories.[14]

The discussion will also be restricted to linear optical systems. A system is linear if signals transmitted by it are linearly superposable. If input signals $f_1(t)$ and $f_2(t)$ produce output signals $g_1(t)$ and $g_2(t)$, respectively, then an input signal of $c_1f_1(t) + c_2f_2(t)$ will produce an output of $c_1g_1(t) + c_2g_2(t)$, where c_1 and c_2 are arbitrary constants. The importance of this is that we can regard a real input signal, which is a very complex function of time, as a Fourier superposition of monochromatic waves and consider only the response of the system to monochromatic light. Thus all the formulas of this section will refer to a monochromatic beam of light that can be regarded as a Fourier component of a real light signal. If it is necessary, the input beam can be Fourier analyzed and the output beam synthesized by the principle of superposition. This is rarely necessary in standard (continuous wave) spectroscopy. In the phenomenological analysis, where use is made of optical calculus, we will be dealing with the transfer function[15] for the simple process

incoming light → light passing through sample → emerging light

which can be represented by the operator equation

$$\mathbf{v}_F = \boldsymbol{O}_p\mathbf{v}_1 \tag{1}$$

$\mathbf{v}_F$ and $\mathbf{v}_1$ comprise the set of parameters that describe the state of the incoming and final (outgoing) light beam, i.e., intensity, polarization, and phase. $\boldsymbol{O}_p$ is an operator representing the effect produced by the sample. There are two well-known vector representations of light and one matrix representation. Since our analysis will concentrate mainly on the vector representations, the operator $\boldsymbol{O}_p$ has the property of transforming one vector into another and thus can be represented by a matrix.

We know from the theory of the propagation of electromagnetic waves in anisotropic media that the transmission of light within the medium may be regarded as the superposed propagation of two waves, each with its own polarization, velocity, extinction, and direction. In the case of simple transparent crystals like calcite, the propagating waves are orthogonal linearly polarized waves with different refractive indices. The direction of polarization of the eigenrays is determined by the symmetry of the crystal and the direction of propagation. With crystals that are absorbing and also optically active, the two propagating waves are elliptically polarized and in general are not orthogonal. Two elliptically polarized light waves are orthogonal when the principal axes of their respective ellipses are perpendicular, they have opposite sense (right and left handed), and they have the same axial ratio. For some crystals there is a special direction of propagation for which there is only one eigenray.[15a]

The observation of transmitted light reveals two extreme cases. In the limit of thick specimens, long optical paths, coupled with strong birefringence, can lead to the emergence of two separated beams. In this case two operators, one for each beam, are required to describe the experiment. The polarization states of the two beams are the eigenpolarizations of the crystal. Their polarization states depend on the direction of the incoming beam, but not on its polarization. When the beams are separated, the system is acting as a refractive polarizer. The operators for the optical system are described mathematically as projection operators. The other extreme is when the sample is sufficiently thin that the emergent beam can be regarded as the coherent superposition of the doubly refracted beams. Spectroscopists almost invariably work under conditions that approach the thin-specimen limit, and the remainder of the discussion will be confined to this case.

TABLE I. Jones Vectors for Several Polarizations

linear	$\begin{pmatrix} c \\ s \end{pmatrix}$	$c = \cos\alpha$, $s = \sin\alpha$, α = polarization angle from vertical
right (+) or left (−) circular	$1/2\begin{pmatrix} 1 \\ \pm i \end{pmatrix}$	
right (+) or left (−) elliptical, long axis vertical	$\begin{pmatrix} a \\ \pm ib \end{pmatrix}$	$\pm b/a = \tan\beta$, β = ellipticity
general elliptical, long axis at angle α to vertical	$R(\alpha)\begin{pmatrix} a \\ \pm ib \end{pmatrix}$	$R = \begin{pmatrix} \cos\alpha & -\sin\alpha \\ \sin\alpha & \cos\alpha \end{pmatrix}$

We would like to emphasize here an important point. The behavior of light in an absorbing, optically active, anisotropic medium is extremely complex. The waves are a superposition of the two eigenpolarizations with different refractive indices and absorption coefficients usually going in different directions. The extraordinary wave is not even transverse. It is important to bear in mind that the operator phenomenology does not attempt to describe the nature of light in the sample. The two beams that are compared are the light before it enters the sample and the light after it leaves the sample. Thus the intensity and state of polarization is being compared in the same medium, usually air. Of course it is the anisotropic medium that produces the changes in the light, and the principal interest is to determine phenomenological coefficients of the sample and eventually formulate a molecular interpretation.

There are several mathematical ways of representing light. The most common are the Jones vector, the Stokes vector, and the density matrix. Our analysis will be based mainly on the vector representations, but the density matrix will arise at times in the theoretical discussion. We assume that the reader is familiar with the Jones and Stokes representations of light. They are described in many sources.[15-20] The Jones vectors may be thought of as being analogous to any of the Maxwell field vectors **E**, **B**, **H**, **D**, and **A**. Like the transverse components of these vectors, the Jones vector is two-dimensional and is written in complex form in order to introduce phase differences between the components. The components of the Stokes vector are intensities. The first component is the total intensity, and the other three components describe the polarization of the light in terms of the extent to which it is linearly polarized at 0° and 45° relative to a chosen axis and the degree to which it is circularly polarized. For completely polarized light, one can transform freely from the Jones to the Stokes representation. The two representations are limited but in different ways. The Stokes vector can describe unpolarized or partially depolarized light but contains no phase information. The Jones vector is associated with a phase factor and can be used in describing interference phenomena, but it cannot be used for partially polarized light. With the methods discussed in this paper, neither unpolarized light nor absolute phase effects will be of importance, so that it is possible to base the discussion on either vector representation. In practical experiments one is always ultimately measuring intensities rather than wave amplitudes, and this makes it convenient to express results in terms of the Stokes components. On the other hand, it is easier to develop fundamental relationships with the Jones vectors, partly because of their similarity to the field vectors, but mainly because they are two-dimensional and satisfy a simple spinor algebra. Consequently formulas will be developed with the Jones representation and then will be transformed to the Stokes representation for practical applications. Representative Jones vectors are shown in Table I.

A. Jones or Field Vectors

Jones vectors have the general form

$$\mathbf{m} = \begin{pmatrix} m_1 \\ m_2 \end{pmatrix}$$

and are usually normalized with the condition

$$\mathbf{m}^*\cdot\mathbf{m} = \mathbf{m}^\dagger\mathbf{m} = |m_1|^2 + |m_2|^2 = 1$$

where a normalized vector indicates unit intensity. The above formula indicates two types of notation. The form at the left is a vector scalar product, and the asterisk means complex conjugation. The next form is matrix notation, and the superscript dagger indicates the conjugate transpose so that $\mathbf{m}^\dagger$ is a row matrix. The vector for the incoming radiation will always be represented by a unit vector. After the radiation passes through the sample, there are changes in intensity, polarization, and phase. The latter is ambiguous since phase is a function of time and position and varies whether there is a sample or not. We define the change in phase relative to the case where there is no sample: χ' = the phase in the presence of the sample minus the phase in the absence of a sample. χ' is given by the Fresnel formula

$$\chi' = [2\pi(n-1)Z]/\lambda_0 \qquad (2)$$

where n is the refractive index, Z is the path length through the sample, and λ_0 is the vacuum wavelength. It is convenient also to represent absorption processes by the diminution of the magnitude of the polarization vector. Thus for a homogeneous, isotropic medium, we represent the outgoing light wave as

$$\mathbf{m}_F = \mathbf{m}_1 \exp[(-i\chi' - A_e)/2] \qquad (3)$$

where A_e is the absorbance in base e. The relative intensity of the outgoing beam is given by

$$\mathbf{m}_F^\dagger\mathbf{m}_F = e^{-A_e}\mathbf{m}_1^\dagger\mathbf{m}_1 = e^{-A_e} \qquad (4)$$

since $\mathbf{m}_1$ is normalized. The change in absolute phase has disappeared from the intensity formula. It is useful to define a general phase shift with a real and an imaginary part $\chi = (\chi' - iA_e)/2$. This is similar to using real and imaginary parts of the refractive index and is preferable since the retardance and absorbance are closer to the actual experimental quantities that are measured. For generality we will represent the properties of an isotropic sample without optical activity by the matrix $e^{i\chi}\sigma_0$, where σ_0 is the unit matrix in two dimensions.

B. Matrix Representations of Optical Systems

We now wish to set up a correspondence between the sample under consideration, or any transmitting optical

TABLE II. Notations and Definitions

1. Basic Retardances and Absorbances

effect	phenomenological symbol	definition[a]	relation to expt	Jones[19]	Hofrichter and Schellman[47]	Gō[30]
mean absorbance	A_e	$\ln 10(A_x + A_y)/2$	$(\ln 10)\epsilon cl$	2κ	$2\kappa''$	γc
mean refractive index	n		$2\pi n(l/\lambda_0)$	η	κ'	
$(x - y)$ linear dichroism	LD	$\ln 10(A_x - A_y)/2$	$(\ln 10)\Delta\epsilon c(l/2)$	$-2p_0$	$2\delta_0''$	γb_3
$(x - y)$ linear birefringence	LB	$[2\pi(n_x - n_y)l]/\lambda_0$	$2\pi\Delta n(l/\lambda_0)$	$-2g_0$	$2\delta_0'$	γa_3
45° linear dichroism	LD′	$\ln 10(A_{45} - A_{135})/2$	$(\ln 10)\Delta\epsilon' c(l/2)$	$-2p_{45}$	$2\delta''_{45}$	γb_1
45° linear birefringence	LB′	$[2\pi(n_{45} - n_{135})l]/\lambda_0$	$2\pi\Delta n'(l/\lambda_0)$	$-2g_{45}$	$2\delta'_{45}$	γa_1
circular dichroism	CD	$\ln 10(A_- - A_+)/2$	$(\ln 10)\Delta\epsilon_{\pm} c(l/2)$	2δ	$2\phi''$	$-\gamma b_2$
circular birefringence	CB	$[2\pi(n_- - n_+)l]/\lambda_0$	$\pi(\alpha/90)$	-2ω	$2\phi'$	$-\gamma a_2$

2. Generalized Retardances

$$L' \equiv \mathrm{LB}' - i\mathrm{LD}'$$
$$C \equiv \mathrm{CB} - i\mathrm{CD}$$
$$L \equiv \mathrm{LB} - i\mathrm{LD}$$
$$T \equiv \mathrm{TB} - i\mathrm{TD}$$

3. Mixed Retardances

$$\mathrm{T} = (L', -C, L)$$
$$\mathrm{T}\cdot\mathrm{T}^* = |L|^2 + |C|^2 + |L|^2 = \mathrm{LB}'^2 + \mathrm{LD}'^2 + \mathrm{CB}^2 + \mathrm{CD}^2 + \mathrm{LB}^2 + \mathrm{LD}^2$$
$$\mathrm{T}\cdot\mathrm{T} \equiv T^2 = a - ib$$
$$|T^2| = TT^* = |T|^2 = (a^2 + b^2)^{1/2}$$
$$a = \mathrm{TB}^2 - \mathrm{TD}^2 = \mathrm{LB}'^2 + \mathrm{CB}^2 + \mathrm{LB}^2 - \mathrm{LD}'^2 - \mathrm{CD}^2 - \mathrm{LD}^2$$
$$b = 2\mathrm{TB}\cdot\mathrm{TD} = 2(\mathrm{LB}'\cdot\mathrm{LD}' + \mathrm{CB}\cdot\mathrm{CD} + \mathrm{LB}\cdot\mathrm{LD})$$
$$\mathrm{TB} = \pm{}^1\!/_2(|T|^2 + a)^{1/2}$$
$$\mathrm{TD} = \pm{}^1\!/_2(|T|^2 - a)^{1/2}$$

[a] A stands for standard absorbance, n for refractive index, l for path length through the medium, c for molar concentration, and λ_0 for the vacuum wavelength of light. Subscripts specify the polarization of light as x, y, 45° to the x axis, 135° to the x axis, circular right (+), or left (−); α is the rotation in degrees in the polarimeter convention.

TABLE III. Jones Matrices

$$\text{45° linear retardance} \begin{pmatrix} \cos L'/2 & -i\sin L'/2 \\ -i\sin L'/2 & \cos L'/2 \end{pmatrix} = \begin{pmatrix} \cos \mathrm{LB}'/2 & -i\sin \mathrm{LB}'/2 \\ -i\sin \mathrm{LB}'/2 & \cos \mathrm{LB}'/2 \end{pmatrix} \begin{pmatrix} \cosh \mathrm{LD}'/2 & -\sinh \mathrm{LD}'/2 \\ -\sinh \mathrm{LD}'/2 & \cosh \mathrm{LD}'/2 \end{pmatrix}$$

$$\text{optical activity} \begin{pmatrix} \cos C/2 & \sin C/2 \\ -\sin C/2 & \cos C/2 \end{pmatrix} = \begin{pmatrix} \cos \mathrm{CB}/2 & \sin \mathrm{CB}/2 \\ -\sin \mathrm{CB}/2 & \cos \mathrm{CB}/2 \end{pmatrix} \begin{pmatrix} \cosh \mathrm{CD}/2 & i\sinh \mathrm{CD}/2 \\ -i\sinh \mathrm{CD}/2 & \cosh \mathrm{CD}/2 \end{pmatrix}$$

$$x,y \text{ linear retardance} \begin{pmatrix} e^{-iL/2} & \\ & e^{iL/2} \end{pmatrix} = \begin{pmatrix} e^{-i\mathrm{LB}/2} & \\ & e^{i\mathrm{LB}/2} \end{pmatrix} \begin{pmatrix} e^{-\mathrm{LD}/2} & \\ & e^{\mathrm{LD}/2} \end{pmatrix}$$

in terms of magnitudes and angles

$$\mathrm{LD} = \mathrm{LD}_\chi \cos 2\chi \qquad \mathrm{LD}' = \mathrm{LD}_\chi \sin 2\chi$$
$$\mathrm{LB} = \mathrm{LB}_\theta \cos 2\theta \qquad \mathrm{LB}' = \mathrm{LB}_\theta \sin 2\theta$$

device, and a set of 2 × 2 matrices. Since the input and output waves are represented by two-dimensional complex vectors, the relation between them can always be represented by the most general matrix transformation in two dimensions, i.e., a two-by-two matrix containing four independent complex numbers, or eight parameters in all. As was established by Jones,[20] these eight parameters correspond to the eight physical measurements one can make on such a system with a given light path: mean absorption, mean refraction, optical rotation, circular dichroism, and two parameters each for linear birefringence and linear dichroism. The mean absorbance and mean refractive index are the means of the two eigenvectors, not the isotropic absorbance and refractive index. The latter four parameters can be defined either in terms of the magnitude of the birefringence and dichroism and the orientation of their principal axes or in terms of the linear birefringence and dichroism determined with respect to two axes at an angle of 45° to one another. The latter is the most useful in optical calculus since the parameters come in linearly. It is easy to set up a matrix representation of the eight physical effects, taken one at a time, and this has been done in many places.[1,4,12,15-20] We wish to keep our theoretical discussion as close as possible to experiment, and for this purpose we have introduced the eight quantities A_e, n, CB, CD, LB, LD, LB′, LD′. These are defined in Table II, which also gives formulas for the matrices for the eight physical effects. They are standard quantities except that all absorptions and dichroisms are in base e and must be divided by ln 10 for the usual absorbances. Also, the circular birefringence is twice the optical rotation. It is useful to go one step further and define a general retardation for each of the birefringence–dichroism pairs; thus

$$L = \mathrm{LB} - i\mathrm{LD}$$
$$L' = \mathrm{LB}' - i\mathrm{LD}' \qquad (5)$$
$$C = \mathrm{CB} - i\mathrm{CD}$$

Several experimentalists have expressed dissatisfaction with this set of definitions since they do not represent the actual measured quantities, which are usually differences in absorbance, refractive indices, or optical rotation. A glance ahead to Table VI or eq 62 certainly convinces one of their appropriateness to the Mueller calculus. It seems to us to be worthwhile to present this complicated theory in a harmonious form with variables all of the same dimensionality. Conversion to the various experimental quantities is then easily done via the formulas of Table II.

All the quantities in eq 5 have the units of radians, and the real and imaginary parts of each of the general retardances are Kronig–Kramers transforms of one

another.[8] As can be seen in Table III, defining general retardances as sums of their dispersive and absorptive parts is equivalent to setting up operators that are products of the dispersive and absorptive operators. Note also that the pairs of operators on each line of eq 5 commute with one another, but do not commute with the operators on the other lines.

When the symmetry of the sample is high enough (uniaxial crystals, flow- or field-oriented molecules, monoclinic crystals in certain orientations, etc.), the principal axes for linear retardation and for linear dichroism are symmetry determined and coincide, and it is possible to choose a coordinate system such that the linear birefringence and dichroism can be represented by a single matrix, usually the L matrix of Table III. In this case the X axis is identified with the slow or fast axis of the sample. In the general case, suppose that the linear dichroism has a maximum value of LD_χ (or a minimum value, i.e., change of sign) at an angle of χ and a maximum value of LB_θ at an angle θ. Then the standard parameters of L and L' are related to LD_χ, LB_θ, χ, and θ by the formulas

$$LD = LD_\chi \cos 2\chi \qquad LD' = LD_\chi \sin 2\chi$$
$$LB = LB_\theta \cos 2\theta \qquad LB' = LB_\theta \sin 2\theta$$

There is a one-to-one correspondence between the mathematical properties of the matrices of Table III and the physical measurements that can be made. The fact that the components of C, L, and L' commute with their partners indicates that the measurements also commute. A system that has optical rotation and circular dichroism may be simulated by passing light through a circularly dichroic sample and then an optically active one, by reversing this order, or by mixing the effects in one sample. The net result is that CD measurements are not affected by CB and vice versa. Also, LD measurements are not distorted by the presence of LB, but they are affected by LB'! As long as the matrix algebra is applied to simple systems, i.e., materials or devices that do not produce simultaneous, noncommuting effects, the calculation of polarization properties is quite straightforward and well established. The theory of instruments that measure circular birefringence and dichroism and linear birefringence and dichroism is easily described in a simple and effective manner using matrix methods.

Conversely, if a system requires more than one of the matrices C, L, or L', the purity of the measurement is destroyed and the analysis of the optical effects can be rather complicated. For example, certain combinations of linear birefringence and dichroism in a sample will appear as CD signals in a CD spectrometer. In fact any two of the three optical effects of eq 5 can combine to simulate signals for the third. Our principal concern for the rest of this section will be the problem of handling mixed, or noncommuting, optical properties theoretically and the way in which this mixing affects the measurements of polarization spectroscopy.

C. Infinitesimal Layers and Infinitesimal Transformations, the General Jones Matrix

One of the most cogent ways of conceiving of the optical properties of a system is as the cumulation of the effects of progressive infinitesimal layers. The Beer–Lambert law is derived in this way, and the derivation may be extended to linear and circular dichroism. Alternatively, one may formulate the transmission properties of an optical sample in the manner of Fresnel as the coherent superposition of incident and scattered radiation. In this way absorbance, refraction, optical rotation, etc. may be conceived of as the superposition or interference of secondary waves scattered from layers of the sample with the primary or incident wave. Absorptive properties arise from destructive interference. These methods, especially in the form of a generalization by Ewald and Oseen have been applied to refracting and absorbing systems[21,22] and to optically active ones.[23,24] To the authors' knowledge no one has succeeded in developing formulas in this rigorous way for mixed optical properties. Fortunately we are only interested in obtaining phenomenological formulas for restricted systems of the kind described above, and we thus avoid the formidable problem of the physical description of the transmission of light in optically active, anisotropic, absorbing media.

TABLE IV. Infinitesimal Jones Matrices

absorption refraction	$\begin{pmatrix} 1-i\chi & 0 \\ 0 & 1-i\chi \end{pmatrix}$	=	$\sigma_0 - i\chi\sigma_0$
L'	$\begin{pmatrix} 1 & -iL'/2 \\ -iL'/2 & 1 \end{pmatrix}$	=	$\sigma_0 - (iL'/2)\sigma_1$
C	$\begin{pmatrix} 1 & C/2 \\ -C/2 & 1 \end{pmatrix}$	=	$\sigma_0 + (iC/2)\sigma_2$
L	$\begin{pmatrix} 1-iL/2 & 0 \\ 0 & 1+iL/2 \end{pmatrix}$	=	$\sigma_0 - (iL/2)\sigma_3$

$$\sigma_0 = \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix} \quad \sigma_1 = \begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix} \quad \sigma_2 = \begin{pmatrix} 0 & -i \\ i & 0 \end{pmatrix} \quad \sigma_3 = \begin{pmatrix} 1 & 0 \\ 1 & -1 \end{pmatrix}$$

As shown by Jones many years ago, it is very useful to conceive of the effect of an optical sample as the cumulation of the operators for infinitesimal layers. In the work below we will be comparing the optical behavior of a sample of thickness Z with that of a sample of thickness $Z + dZ$ and we thus inquire about the added effect of the infinitesimal layer of thickness dZ. This layer may contain all eight of the properties discussed above. When the thickness and therefore the optical effects become infinitesimal, the four basic matrices take the form shown in Table IV. This result is obtained by expanding the trigonometric functions and discarding quadratic and higher terms. We note that there is a one-to-one correspondence between the four infinitesimal matrices and the Pauli matrices, including the identity σ_0. Each of the four infinitesimal matrices is equal to the identity plus an infinitesimal transformation. These transformations constitute a Lie group of the kind that has important implications in quantum mechanics.[25,26] To the first order in infinitesimals the four optical matrices commute with one another. An infinitesimal sample with all four of the basic optical properties may be represented as the product of the four infinitesimal matrices, in any order, which we evaluate as

$$(\sigma_0 - i\chi\sigma_0)(\sigma_0 - i(L'/2)\sigma_1)(\sigma_0 + i(C/2)\sigma_2) \times (\sigma_0 - i(L/2)\sigma_3) \cong \sigma_0 - i[\chi\sigma_0 + (L'/2)\sigma_1 - (C/2)\sigma_2 + (L/2)\sigma_3] + \ldots \cong \sigma_0 - i\boldsymbol{R} \quad (6)$$

where $\boldsymbol{R}$ is the retardance matrix for the infinitesimal layer

$$\boldsymbol{R} = {}^1/_2 \begin{pmatrix} \chi + L & L' + iC \\ L' - iC & \chi - L \end{pmatrix} \quad (7)$$

It should be noted from Table II that the four quantities χ, C, L, and L' contain the thickness of the specimen via the Fresnel or Lambert formulas. To simplify the analysis, the thickness of an infinitesimal layer will be written as $dZ = Z/N$, where N is the number of layers, and the length factor will be removed from $\boldsymbol{R}$ by defining

$$\boldsymbol{R} = \boldsymbol{R}'(Z/N) \tag{8}$$

where $\boldsymbol{R}'$ is now a finite matrix with components defined per unit length. With $(\sigma_0 - i\boldsymbol{R}'Z/N)$ as the operator for one of the infinitesimal layers, the operator for the N layers of the sample is given by[51]

$$\boldsymbol{J} = \lim_{N\to\infty}(\sigma_0 - i\boldsymbol{R}'(Z/N))^N = e^{-i\mathbf{R}'Z} = e^{-i\mathbf{R}} \tag{9}$$

In the last step Z has been reincorporated into $\boldsymbol{R}$ to form a finite matrix based on the real thickness of the sample. This is done so that the elements of $\boldsymbol{R}$ correspond with the absorbances and retardances measured experimentally.

It should be clear from the foregoing that the Jones calculus in polarization space is very closely related to the algebra of spin $^1/_2$ particles. We will now make use of this analogy. The required spin formulas will be found in most intermediate texts on quantum mechanics. The book of Merzbacher is especially appropriate for our application.[27] The matrix for a rotation of a spin system by an angle θ about an axis defined by unit vector $\mathbf{n}$ is given by

$$U = \exp[-i(\theta/2)\mathbf{n}\cdot\sigma] \tag{10}$$

where σ is the "vector" whose components are the three Pauli matrices σ_1, σ_2, and σ_3. If we define a generalized retardance vector $\mathbf{T} = (L', -C, L)$ (see Table II) and normalize it by dividing by

$$T = (L'^2 + C^2 + L^2)^{1/2} \tag{11}$$

then, using eq 7, we can express $\boldsymbol{R}$ as $^1/_2(\chi\sigma_0 + \mathbf{T}\cdot\sigma)$ and the general Jones matrix can be written as

$$\boldsymbol{J} = \exp[-i\boldsymbol{R}] = e^{-i\chi/2}\exp[-i(\sigma\cdot\mathbf{T})/2] = e^{-i\chi/2}\exp[-i(T/2)(\mathbf{T}/T)\cdot\sigma] \tag{12}$$

The notation $\mathbf{T}\cdot\sigma$ to represent the last three terms of eq 6, or the anisotropic part of $\boldsymbol{R}$, will be used in the subsequent analysis. Here $\mathbf{T}/T$ is a normalized complex vector that plays the role of the unit vector $\mathbf{n}$, and T, a complex number, plays the role of θ. Jones obtained his general matrix for the combination of all linear transmission effects by recognizing that $\boldsymbol{J}$ and $\boldsymbol{R}$ of eq 7 have the same eigenvectors, transforming to the principal axes of $\boldsymbol{R}$, expanding the exponential operator in the principal axis basis, and then transforming back to the original basis. The result is given in eq 13 below. We obtain this result in one step using the spin equation[28]

$$\exp[i(\theta/2)\mathbf{n}\cdot\sigma] = \sigma_0\cos(\theta/2) - i\mathbf{n}\cdot\sigma\sin(\theta/2)$$

which translates as

$$\mathbf{J} = e^{-i(\chi/2)}\{\sigma_0\cos(T/2) - (i/T)(\mathbf{T}\cdot\sigma)\sin(T/2)\} \tag{13}$$

This equation is fundamental to the treatment of noncommuting optical effects.

We are trying to develop a notation with as much physical content as possible. The quantity T introduced above (T stands for total since it depends on all six retardance effects) is a complex number, which must be distinguished from the absolute magnitude $(\mathbf{T}^*\cdot\mathbf{T})^{1/2}$. It may be shown, as was first done by Jones, that the eigenvalues of the general optical sample are given as $\exp[-i(\chi/2) \pm i(T/2)]$. Thus the quantity that describes the real birefringence and dichroism of the sample, i.e., the difference in phase shift and absorbance of the ordinary and extraordinary rays, is T. T may be written as the sum of real and imaginary parts

$$T = \mathrm{TB} - i\mathrm{TD} \tag{14}$$

In the general case TB and TD are the physical birefringence and dichroism of the doubly refracted waves which are normally elliptical. On the other hand, LD, CB, etc. are constructs that are useful, but artificial, since in the general case the birefringence and dichroism are neither linear nor circular.

TB and TD may be expressed in terms of the six standard linear and circular anisotropies. We write

$$\mathbf{T}\cdot\mathbf{T} = a - ib = T^2 = \mathrm{TB}^2 - \mathrm{TD}^2 - 2i\mathrm{TD}\cdot\mathrm{TB} \tag{15}$$

Using de Moivre's theorem to take the square root of $T^2 = a - ib$, we have

$$\mathrm{TB} = (a^2 + b^2)^{1/4}\cos(\theta/2)$$

$$\mathrm{TD} = (a^2 + b^2)^{1/4}\sin(\theta/2) \tag{16}$$

where $a = \mathrm{LB}^2 + \mathrm{LB}'^2 + \mathrm{CB}^2 - \mathrm{LD}^2 - \mathrm{LD}'^2 - \mathrm{CD}^2$ and $b = 2(\mathrm{LB}\cdot\mathrm{LD} + \mathrm{LB}'\cdot\mathrm{LD}' + \mathrm{CB}\cdot\mathrm{CD})$. θ is the angle that satisfies

$$\cos\theta = a(a^2 + b^2)^{-1/2}$$

$$\sin\theta = b(a^2 + b^2)^{-1/2} \tag{17}$$

There are of course two roots of T^2, only one of which is given by eq 16. The other root involves the angle $(\theta + 2\pi)/2 = (\theta/2) + \pi$. This generates the same roots as eq 16 but with the signs of both TB and TD reversed. Since TB and TD are defined as the difference in the real (refractive) and imaginary (absorptive) parts of the retardation, changing the sign of both merely reverses the roles of the two eigenpolarizations in defining TB and TD. The second root introduces nothing new in a physical sense, since the signs of all birefringences and dichroisms are a matter of arbitrary definition. Only the relative sign of TB and TD have physical significance, and these may be obtained from eq 16.

TB and TD are often obtained algebraically as

$$\mathrm{TB} = \pm\left(\frac{(a^2 + b^2)^{1/2} + a}{2}\right)^{1/2}$$

$$\mathrm{TD} = \pm\left(\frac{(a^2 + b^2)^{1/2} - a}{2}\right)^{1/2} \tag{18}$$

but sign information is lost so care is required. If a system is simple, i.e., only one of the three quantities L', C, or L is operative, then TB and TD reduce to the ordinary birefringence and dichroism. If a material is not absorbing, then $\mathrm{TB} = (\mathrm{LB}^2 + \mathrm{CB}^2)^{1/2}$, a formula which is well-known in crystal optics. This notation differs slightly from that of a previous publication by the authors.[29] For convenience in translation, we have also indicated in Table II the symbols utilized by Gō[30] for the six physical quantities required to specify the vector $\mathbf{T}$.

We recall now the commutative properties of the Pauli spin matrices

$$\sigma_j \cdot \sigma_k = i\sigma_l = -\sigma_k \cdot \sigma_j \tag{19}$$

where j, k, and l are 1, 2, and 3 cyclically permuted. We have seen that the retardances L', C, and L are correlated with the matrices σ_1, σ_2, and σ_3. From this and eq 19 it may be anticipated that an optical system will generate artifactual signals according to the following:

$$L' + C = L \text{ artifact}$$
$$C + L = L' \text{ artifact}$$
$$L + L' = C \text{ artifact}$$

Since circular birefringence and circular dichroism tend to be orders of magnitude smaller than linear effects, the last of these relations is particularly troublesome for the measurement of CD and optical rotation in anisotropic media. These artifacts show up in the detailed formulas for the general sample.[4,11,20]

D. Stokes Vectors and Mueller Matrices

In the absence of depolarizing effects, the formulas that have been obtained in the previous section can be used to analyze the properties of any optical systems, but it is preferable to switch to an intensity representation. Practically all modern optical instruments use some device such as a photomultiplier tube that transduces light intensity into an electrical signal. Consequently a representation such as the Stokes vector is more directly connected with experiment. We represent the Stokes vector as s_0, s_1, s_2, s_3, where the components are the Stokes parameters. The order of the parameters is total intensity, 45° linear polarization, circular polarization, x or y axis linear polarization). We use this notation with some trepidation. The Stokes parameters have been represented in a variety of orders and with a wide variety of symbols.[4,5,7,8,13-19,22,30-42] The actual symbols used (e.g., P_0, P_1, P_2, P_3; I, M, C, S; I, Q, U, V) are not particularly important but changes in order can be quite confusing, especially in comparing elements of the 4 × 4 Mueller matrix. The Perrin order (s_0, s_3, s_1, s_2 in our notation) appears to be the most common, though ref 8, 30, 36, and 38 use the ordering which we propose. Our notation and numbering bring out the direct correlation between the Stokes parameters and the corresponding spin matrices. We do not do this because of a desire for formal elegance. One can literally "see" total intensity, 45° polarization, circular polarization, and 0° polarization by looking at the forms of the four spin matrices in the order 0–3. Other notations have no physical context. The parameters are defined in the usual way. s_0 is the total intensity, s_1 (with $s_2 = s_3 = 0$) indicates the presence of light polarized linearly at 45°, s_3 (with $s_1 = s_2 = 0$) indicates the presence of light polarized linearly at 0°, and s_2 (with $s_1 = s_3 = 0$) indicates the presence of circularly polarized light. If $s_2 = 0$, there is linear polarization at azimuth $\tan(2\alpha) = s_1/s_3$. In general the light may be construed as a combination of unpolarized and elliptically polarized light, with only the latter contributing to s_1, s_2, and s_3. The azimuth of the principal axis of the ellipse, α, is given by $\tan(2\alpha) = s_1/s_3$, and the ellipticity, β, by $\tan(2\beta) = s_2(s_1^2 + s_3^2)^{-1/2}$.

Textbooks on optics outline the ways that the Stokes parameters can be measured directly by using perfect polarizers and quarter-wave plates. The way in which they can be measured with phase-modulation spectroscopy comes from the following analysis. For completely polarized light the Stokes parameters may be calculated from the Jones vector with the formula (Chapter 4 of ref 17)

$$\mathbf{s}_i = \mathrm{m}^\dagger \sigma_i \mathrm{m} \tag{20}$$

where m is the corresponding Jones vector. We obtain

$$s_0 = m_1^2 + m_2^2$$
$$s_1 = m_1^* m_2 + m_1 m_2^* = 2\,Re\,(m_1^* m_2)$$
$$s_2 = -i(m_1^* m_2 - m_1 m_2^*) = 2\,Im\,(m_1^* m_2)$$
$$s_3 = m_1^2 - m_2^2 \tag{21}$$

The matrix operator that represents an optical instrument or experimental sample is a 4 × 4 Mueller matrix. In a previous publication[4] we showed that the Mueller matrix could be generated from the Jones matrix by the formation of a direct product followed by a transformation. We will here develop the connection in another way, which will be shown later to correspond very closely with the type of experiment done in modulation spectroscopy. Suppose that the Jones description of an experiment is given as

$$\mathrm{m_F} = \boldsymbol{J}\mathrm{m_1} \tag{22}$$

Then according to (21) the Stokes vector components of the emerging light are given by

$$(s_\mathrm{F})_i = \mathrm{m_F}^\dagger \sigma_i \mathrm{m_F} = \mathrm{m_1}^\dagger (\boldsymbol{J}^\dagger \sigma_i \boldsymbol{J}) \mathrm{m_1} \tag{23}$$

The operator $\boldsymbol{J}^\dagger \sigma_i \boldsymbol{J}$ in this formula is a 2 × 2 matrix and thus can be written in the form of a linear combination of the Pauli matrices including the identity

$$\boldsymbol{J}^\dagger \sigma_i \boldsymbol{J} = \sum_{j=0}^{3} M_{ij}\sigma_j \tag{24}$$

Substituting this result into eq 23 gives

$$(s_\mathrm{F})_i = \sum_{j=0}^{3} M_{ij}(\mathrm{m_1}^\dagger \sigma_j \mathrm{m_1}) = \sum_{j=0}^{3} M_{ij}(s_1)_j \tag{25}$$

but this is just the ith row of the matrix transformation from the incoming Stokes vector $\mathbf{s}_1$ to the outgoing Stokes vector $\mathbf{s}_\mathrm{F}$. The M_{ij} are therefore the elements of the transformation matrix in Stokes space, the Mueller matrix,[16,19] and we write

$$\mathbf{s}_\mathrm{F} = \boldsymbol{M}\mathbf{s}_1 \tag{26}$$

The rule then is simple. To find the M_{ij} element of $\boldsymbol{M}$, form the product $\boldsymbol{J}^\dagger \sigma_i \boldsymbol{J}$, and then express the result in the form of a sum of Pauli matrices. The coefficient of σ_j in the expansion is the desired element, M_{ij}.

As a simple example we evaluate the second row (M_{lk}, k = 0,3) for a material showing only optical activity. Then

$$\boldsymbol{J} = \begin{pmatrix} c & s \\ -s & c \end{pmatrix} \quad \boldsymbol{J}^\dagger = \begin{pmatrix} c & -s \\ s & c \end{pmatrix}$$

$$(\boldsymbol{J}^\dagger \sigma_1 \boldsymbol{J}) = \begin{pmatrix} c & -s \\ s & c \end{pmatrix} \begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix} \begin{pmatrix} c & s \\ -s & c \end{pmatrix} =$$

$$\begin{pmatrix} -\sin 2\theta & \cos 2\theta \\ \cos 2\theta & \sin 2\theta \end{pmatrix} = \cos 2\theta\ \sigma_1 - \sin 2\theta\ \sigma_3$$

where $s = \sin\theta$ and $c = \cos\theta$. Thus the elements of the

TABLE V. First Row of Mueller Matrix and Some Definitions

$$M_{00} = e^{-A_e}[X + (W/2)(\mathrm{T}\cdot\mathrm{T}^*)]$$
$$M_{01} = e^{-A_e}[U\cdot\mathrm{LD}' - V\cdot\mathrm{LB}' + W\cdot(\mathrm{LB}\cdot\mathrm{CD} - \mathrm{CB}\cdot\mathrm{LD}')]$$
$$M_{02} = e^{-A_e}[U\cdot\mathrm{CD} + V\cdot\mathrm{CB} + W\cdot(\mathrm{LB}\cdot\mathrm{LD}' - \mathrm{LB}'\cdot\mathrm{LD})]$$
$$M_{03} = e^{-A_e}[-U\cdot\mathrm{LD} - V\cdot\mathrm{LB} + W\cdot(\mathrm{CB}\cdot\mathrm{LD}' - \mathrm{LB}'\cdot\mathrm{CD})]$$
$$U = (\mathrm{TD}\sinh \mathrm{TD} + \mathrm{TB}\sin \mathrm{TB})/TT^*$$
$$V = (\mathrm{TB}\sinh \mathrm{TD} - \mathrm{TD}\sin \mathrm{TB})/TT^*$$
$$W = (\cosh \mathrm{TD} - \cos \mathrm{TB})/TT^*$$
$$X = (\cosh \mathrm{TD} + \cos \mathrm{TB})/2$$

row are 0, cos 2θ, 0, and $-\sin 2\theta$. Similar manipulations with the other rows yield the entire matrix as

$$\boldsymbol{M} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & \cos 2\theta & 0 & -\sin 2\theta \\ 0 & 0 & 1 & 0 \\ 0 & \sin 2\theta & 0 & \cos 2\theta \end{pmatrix}$$

a result which is well-known.

We now wish to apply this formula to the general case where $\boldsymbol{J}$ is given by eq 13. The first row of the general Mueller matrix is developed by decomposing the operator

$$\boldsymbol{J}^\dagger\sigma_0\boldsymbol{J} = e^{-A_e}[\sigma_0 \cos T^* + i(\mathbf{T}^*\cdot\sigma)(\sin T^*)/T^*] \times [\sigma_0 \cos T - i(\mathbf{T}\cdot\sigma)(\sin T)/T] = e^{-A_e}\left\{|\cos T|^2\sigma_0 + i\left[\frac{\cos T \sin T^*}{T^*}\mathbf{T}^*\cdot\sigma - \frac{\cos T^* \sin T}{T}\mathbf{T}\cdot\sigma\right] + (\mathbf{T}^*\cdot\sigma)(\mathbf{T}\cdot\sigma)\left|\frac{\sin T}{T}\right|^2\right\} \quad (27)$$

Note that σ is self-adjoint. Before dealing with this complicated expression, it will be useful to consider the significance of its terms. e^{-A_e} represents the mean transmission of the two eigenpolarizations. The first term in the brackets is a σ_0 term and contributes to M_{00}, which is the main transmission term. M_{00} is the ratio of total intensity out to total intensity in. This term keeps track of the fact that the two eigenpolarizations have different absorption coefficients and are attenuated at different rates as they pass through the sample. Another contribution to M_{00} arises later. The middle term in brackets is linear in the spin matrices and in the optical quantities L', C, and L. These might be called the direct contributions of the optical properties. For example, L and L^* are coefficients of the σ_3 term. This will bring LD effects into M_{03}. Similarly LD′ appears in M_{01} and CD in M_{02}.

The last term contains products of spin matrices and products of optical parameters. Because of the properties of spin matrices, products of identical terms (say, $(L'\sigma_3)(L\sigma_3)$) yield σ_0 terms and contribute to the first column of $\boldsymbol{M}$. Products of two different kinds (say, $(L'\sigma_1)(L\sigma_3)$) yield spin matrices of the third kind and thus lead to artifactual response as discussed earlier. The algebra of these product effects is given by the spin formula

$$(\sigma\cdot\mathbf{T}^*)(\sigma\cdot\mathbf{T}) = (\mathbf{T}^*\cdot\mathbf{T})\sigma_0 + i\sigma\cdot(\mathbf{T}^* \times \mathbf{T}) \quad (28)$$

The job of substituting (28) into (27), converting the formula to real form, and combining terms is rather tedious and will not be repeated here. The results, obtained by different methods, have already been published.[4,8] Instead we state directly the results for the first row of $\boldsymbol{M}$ in Table V.

This table should be studied carefully. The elements of the Mueller matrix for the general sample are complicated and can be grouped in a number of ways. The grouping of Table V in which the functions U, V, W, and X appear as factors has special advantages in the limit of small anisotropies and in the approach to this limit, where a small anisotropy is defined by $|T| \ll 1$. This mode of factoring was introduced by Disch and Sverdlik,[43] though our analysis and notation differ from theirs. These functions are dependent only on TD and TB, i.e., on the scalar magnitude of the physical birefringence and dichroism. In order to depict these functions we assume a value for TD of 0.2. This upper limit estimate is arrived at as follows. Since adequate light must penetrate the sample for spectra to be taken, A_e will not normally exceed 2–3. Typical maximal values of $\Delta A/A$ might be about 0.15, though larger values can be obtained with simple crystals where all chromophores can be identically oriented. We thus take 0.2 to be a typical upper limit for TD. Note the factor of 2 in the definition of LD in Table II. With this assumption U, V, and W can be plotted as functions of TB, which can be quite large. (X occurs only on the diagonal and is not important in polarization modulation spectroscopy.) The curves are shown in Figure 1. U approaches unity as TB and TD $\rightarrow$ 0. The U term represents the pure measurement of a dichroism or

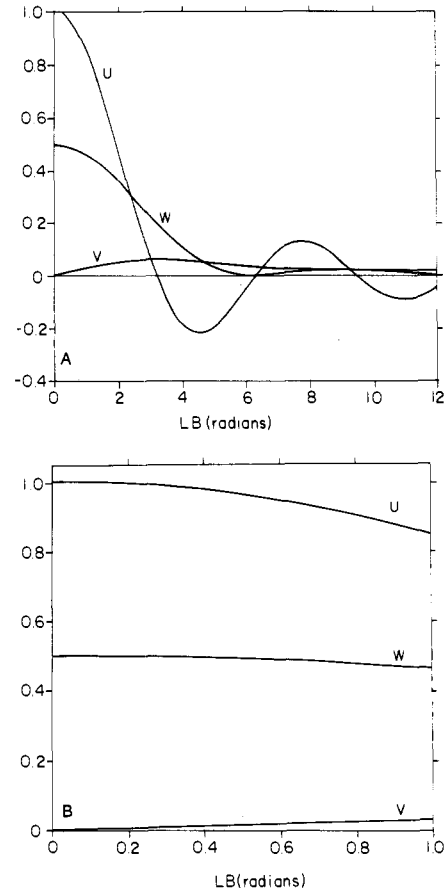

Figure 1. Values of the functions U, V, and W as a function of TB with TD set at 0.2. U, V, and W are defined in Table V. TB is the real part of the retardance of the medium, which reduces to LB or CB in simple cases. TD is the imaginary, or absorptive, part of the retardance, which reduces to LD or CD in simple cases. Formulas for TB and TD for the general case are given in eq 18 and Table II. The dominant contribution to TB is usually ordinary linear birefringence, so the plots are essentially an investigation of the effect of birefringence on the Mueller matrix elements. A and B are for large and small retardances, respectively.

TABLE VI. General Mueller Matrix[a]

	0	1	2	3
0	X $+W/2\cdot(T\cdot T^*)$	$-U\cdot LD'$ $-V\cdot LB'$ $+W\cdot(LB\cdot CD - CB\cdot LD)$	$U\cdot CD$ $+V\cdot CB$ $+W\cdot(LB\cdot LD' - LB'\cdot LD)$	$-U\cdot LD$ $-V\cdot LB$ $+W\cdot(CB\cdot LD' - LB'\cdot CD)$
1	$-U\cdot LD'$ $-V\cdot LB'$ $-W\cdot(LB\cdot CD - CB\cdot LD)$	X $+W\cdot(LB'^2 + LD'^2)$ $-W/2\cdot(T\cdot T^*)$	$-U\cdot LB$ $+V\cdot LD$ $-W\cdot(CB\cdot LB' + CD\cdot LD')$	$-U\cdot CB$ $+V\cdot CD$ $+W\cdot(LB\cdot LB' + LD\cdot LD')$
2	$U\cdot CD$ $+V\cdot CB$ $-W\cdot(LB\cdot LD' - LB'\cdot LD)$	$U\cdot LB$ $-V\cdot LD$ $-W\cdot(CB\cdot LB' + CD\cdot LD')$	X $+W\cdot(CD^2 + CB^2)$ $-W/2\cdot(T\cdot T^*)$	$-U\cdot LB'$ $+V\cdot LD'$ $-W\cdot(CB\cdot LB + CD\cdot LD)$
3	$-U\cdot LD$ $-V\cdot LB$ $-W\cdot(CB\cdot LD' - LB'\cdot CD)$	$U\cdot CB$ $-V\cdot CD$ $+W\cdot(LB\cdot LB' + LD\cdot LD')$	$U\cdot LB'$ $-V\cdot LD'$ $-W\cdot(CB\cdot LB + CD\cdot LD)$	X $+W\cdot(LB^2 + LD^2)$ $-W/2\cdot(T\cdot T^*)$

[a] The entire table is to be multiplied by e^{-A_e}, where A_e is the mean absorbance of the two eigen polarizations.

birefringence. We can define an artifact-free measurement on the general sample as one in which U = 1 and all other factors vanish. V approaches 0 for small TB (thin samples). W approaches 0.5, but one can see by inspection that W in the table is always multiplied by a factor which is less than the denominator of W. Thus the size of the W term cannot be greater than that of its numerator. This equals cosh (TD) − cos (TB) and approaches zero quadratically for small TB and TD. The oscillatory behavior of U generates channeled spectra, i.e., cyclic variations in retardance as the birefringence is changed, usually as a function of wavelength. As is well-known these oscillations are sinusoidal for a purely linearly birefringent sample. What we are talking about here, however, are the oscillations that would occur in an attempted measurement of CD or LD as a function of TB, which in turn depends on sample thickness.

Figure 1B shows the values of the functions at lower ranges of TB. *These curves suggest that a method of evaluating a pure optical effect in a system with mixed anisotropies is to make measurements as a function of sample thickness and extrapolate back to a thickness of zero. At any rate measurements of linear birefringence (the dominant factor in TB) should always be made on a system so that the order of magnitude of W and V can be estimated and a judgment made on the magnitude of the artifacts they produce.* The result is that when TB is less than 0.3 or so, the off-diagonal terms of M are dominated by the U terms. This is the limit discussed by Gō[30] and by Troxell and Scheraga,[38] where all six anisotropic parameters are segregated in the $\boldsymbol{M}$ matrix. These are the most favorable circumstances for measurement. For large TB, on the other hand, one observes channeled spectra when TB is varied. This is shown in Figure 1B. The variations in TB could arise, for example, from the dispersion of the retardance with wavelength. Twelve radians of retardance is in fact the appropriate value for a 0.1-mm-thick piece of quartz cut parallel to its optic axis, using visible light.

To develop the remainder of the $\boldsymbol{M}$ matrix we must expand the following expressions as linear combinations of Pauli matrices and identify the M_{ij} as indicated above.

second row $\boldsymbol{J}^\dagger\sigma_1\boldsymbol{J}$

third row $\boldsymbol{J}^\dagger\sigma_2\boldsymbol{J}$

fourth row $\boldsymbol{J}^\dagger\sigma_3\boldsymbol{J}$

This is done with $\boldsymbol{J}$ from eq 13 by using the product rule for Pauli matrices, eq 19, together with eq 28, and combining terms. The final matrix, utilizing the U, V, W, and X notation, is given as Table VI. The main task of modulation spectroscopy is to measure the elements of the matrix, $\boldsymbol{M}$, at least the three or four elements that are dominated by interesting optical effects.

E. Modulation Spectroscopy

The modern use of modulation techniques in polarization spectroscopy followed the original papers of Grosjean and Legrand.[43a] A review of the instrumentation and applications has been presented by Hipps and Crosby[43b] and by Drake.[43c] The basic polarization modulator consists of a polarizer, which we shall consider to provide pure x-axis polarization of the light, followed by a time-varying retarder with its axis set at 45° to the x axis. The Stokes vector for the light that emerges from a polarization modulator is found by simply applying the matrix of a 45° retarder to the Stokes vector for x-polarized light of unit intensity. The result is

$$\mathbf{s}_1 = (1, 0, \sin 2\S, \cos 2\S) \tag{29}$$

where § is the retardance (see, for example, ref 4). In a modulation experiment § is a function of time. With certain electrooptical devices it can assume a variety of forms (square waves, sawtooth waves, etc.), but in the photoelastic modulator,[44] which will be the focus of this discussion, § is a sinusoidal function of time

$$\S = (\delta_0/2) \sin ft \tag{30}$$

where f is 2π times the modulation frequency and δ_0 is the amplitude of the modulation, which can be adjusted by controlling the voltage of the circuit that drives the modulator. We note that the Stokes components sin (δ_0 sin ft) and cos (δ_0 sin ft) contain not only the fundamental frequency but its harmonics.

There are three basic arrangements for a polarization experiment, which are illustrated in Figure 2. The modulated light, represented by eq 29, is passed through the sample, and the output signal is then measured, either directly or after passage through an analyzing polarizer set at 45° or 90° to the initial polarizer. Another arrangement, more of theoretical than practical interest, uses a circular polarizer as analyzer. We shall now show how these arrangements permit the mapping of the Mueller matrix for the sample. The

TABLE VII. Polarizers

linear 0°	$\frac{1}{2}\begin{pmatrix}1&0&0&1\\0&0&0&0\\0&0&0&0\\1&0&0&1\end{pmatrix}$	$\begin{pmatrix}1&0\\0&0\end{pmatrix} = \frac{1}{2}(\sigma_0 + \sigma_3)$
linear 45°	$\frac{1}{2}\begin{pmatrix}1&1&0&0\\1&1&0&0\\0&0&0&0\\0&0&0&0\end{pmatrix}$	$\begin{pmatrix}1&1\\1&1\end{pmatrix} = \frac{1}{2}(\sigma_0 + \sigma_1)$
linear, arbitrary angle	$\frac{1}{2}\begin{pmatrix}1&\sin 2\alpha&0&\cos 2\alpha\\\sin 2\alpha&\sin^2 2\alpha&0&\sin 2\alpha\cos 2\alpha\\0&0&0&0\\\cos 2\alpha&\sin 2\alpha\cos 2\alpha&0&\cos^2 2\alpha\end{pmatrix}$	$\begin{pmatrix}\cos^2\alpha&\sin\alpha\cos\alpha\\\sin\alpha\cos\alpha&\sin^2\alpha\end{pmatrix} = \frac{1}{2}(\sigma_0 + \sin 2\alpha\,\sigma_1 + \cos 2\alpha\,\sigma_3)$
right circular	$\frac{1}{2}\begin{pmatrix}1&0&1&0\\0&0&0&0\\1&0&1&0\\0&0&0&0\end{pmatrix}$	$\begin{pmatrix}1&-i\\i&1\end{pmatrix} = \frac{1}{2}(\sigma_0 + \sigma_2)$

input to the sample is given by eq 29, so that its output is given by the Stokes vector

$$\mathbf{s_F} = \boldsymbol{M}\mathbf{s_I} = \begin{pmatrix} M_{00} + sM_{02} + cM_{03} \\ M_{10} + sM_{12} + cM_{13} \\ M_{20} + sM_{22} + cM_{23} \\ M_{30} + sM_{32} + cM_{33} \end{pmatrix} \tag{31}$$

where $\boldsymbol{M}$ is the Mueller matrix of the sample, and s and c are sin 2§ and cos 2§, respectively.

The first experimental arrangement of Figure 2 detects only the intensity, and therefore is a way of measuring the zeroth element of the vector in eq 31. This is tantamount to exploring the first row of the sample matrix. Detection of the individual terms of the intensity component, which is equivalent to detecting the zeroth, second, and third columns, is effected by Fourier analysis. The photomultiplier signal that is developed is proportional to

$$M_{00} + \sin(\delta/2 \sin ft)M_{02} + \cos(\delta/2 \cos ft)M_{03} \tag{32}$$

which is a sum of the first row responses. By use of the standard expansions[45]

$$\sin 2\S = \sin(\delta_0 \sin ft) = 2J_1(\delta_0)\sin ft + 2J_3(\delta_0)\sin 3ft + \ldots$$

$$\cos 2\S = J_0(\delta_0) + 2J_2(\delta_0)\cos 2ft + J_4(\delta_0)\cos 4ft + \ldots \tag{33}$$

the total signal can be resolved as a dc component, ac components at f and $2f$, and higher harmonics. The intensities of the dc, f, and $2f$ components, as detected by dc amplification and lock-in amplifiers, is given by

$$\begin{aligned} I_{dc} &= M_{00} + J_0(\delta_0)M_{03} \\ I_f &= (4/\pi)J_1(\delta_0)M_{02} \\ I_{2f} &= (4/\pi)J_2(\delta_0)M_{03} \end{aligned} \tag{34}$$

The $J_k(\delta_0)$ are known in principle but in practice are established as part of an instrument proportionality constant that is determined experimentally. Harmonic analysis thus permits the determination of the first row of the sample matrix except for M_{01}. This can be determined by rotating the sample or modulator by 45°. As will be discussed below, the rotation of a sample by 45° effectively converts L effects into L' effects and vice versa and interconverts M_{01} and M_{03}.

The second experimental arrangement of Figure 2 permits the measurement of the second row of the

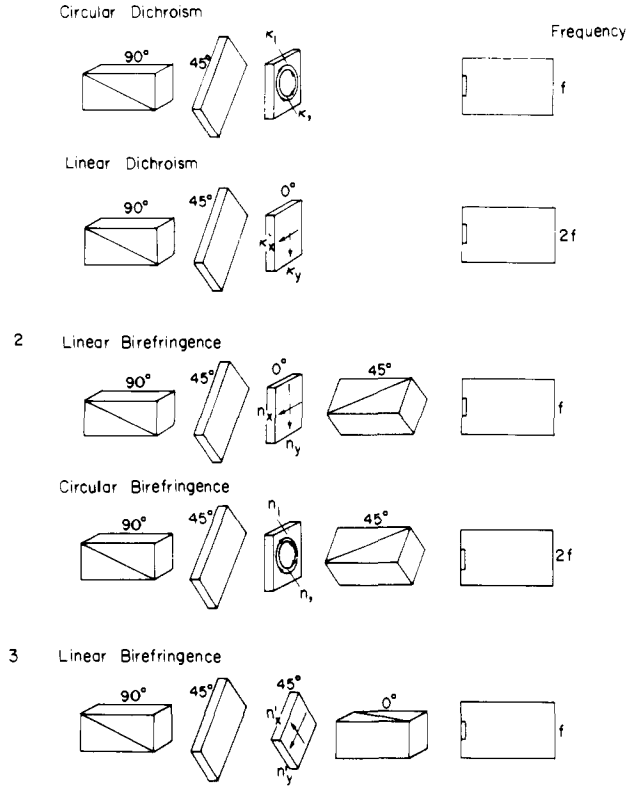

Figure 2. Three arrangements for polarization spectroscopy (see Experimental Measurements (J). From left to right, the optical elements are the polarizer, modulator, sample, optional analyzer, and photomultiplier. Figure adapted from ref 11.

sample matrix. The matrices for 45°, circular, and 90° polarizers are given in Table VII. It is easy to see from the simple structure of the 45° matrix that its output intensity, s, is the sum of the first two Stokes components of its input, divided by 2. Consequently the output of the entire arrangement is given by half the sum of the s_0 and s_1 elements of eq 31. Harmonic analysis of the resulting signal again takes the form of eq 34, but now contains the sum of the first- and second-row elements of the sample matrix (eq 35).

$$\begin{aligned} I_{dc} &= (M_{00} + M_{10}) + J_0(\delta_0)(M_{03} + M_{13}) \\ I_f &= (4/\pi)J_1(\delta_0)(M_{02} + M_{12}) \\ I_{2f} &= (4/\pi)J_2(\delta_0)(M_{03} + M_{13}) \end{aligned} \tag{35}$$

Since M_{00}, M_{01}, and M_{02} are determined by the first

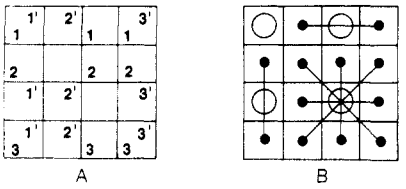

Figure 3. Measurement of Mueller matrix elements. (A) Schematic presentation of the elements of the Mueller matrix that can be measured by the arrangements of Figure 2. Elements marked 1, 2, and 3 are detectable by the corresponding arrangements of that figure. Elements marked 1′, 2′, and 3′ are detectable if the direction of the light path is reversed. (B) Effect of a rotation of the sample by 45° on the elements of the Mueller matrix. Black dots connected by tie lines indicate interconversion of matrix elements by the 45° rotation. Open circles indicate invariant matrix elements.

measurement, M_{10}, M_{20}, and M_{30} may be determined by difference.

It should be clear from this analysis and the form of the matrices for polarizers in Table VII that the use of a 0° (or 90°) polarizer (arrangement 3 of Figure 2) will give photomultiplier signals which are sums of terms coming from the zeroth and third rows of the matrix and that circular polarizers detect signals which are sums of the zeroth and second rows of the matrix. Detection with circular polarizers has not been included among the practical arrangements for modulation spectroscopy because they are not achromatic (see also the complications discussed in ref 42). Consequently circular polarizers are suitable for studies at a single wavelength, or a narrow region of wavelengths, but not for general spectroscopic investigations. The third arrangement of Figure 2 is not commonly used and may in fact lead to practical difficulties. As discussed in the legend of Figure 4, this arrangement passes a strong $2f$ signal in the absence of a sample, so that imperfections in the optics or electronics could lead to spurious effects.

A scan of the matrix elements detected by the three types of experiment is shown in Figure 3.

It is also possible to reverse the order of the optical elements in Figure 2, i.e., to interchange the positions of the light source and photomultiplier in the figure so that unpolarized light first passes through the detection polarizer (if there is one), then through the sample, and finally the modulator. Using the same methods as above, it is not difficult to show that the three experimental arrangements, used in reverse, develop the zeroth, second, and third *columns* of the sample matrix and that the rows can be distinguished by harmonic analysis. Thus if we perform all six experiments, all the

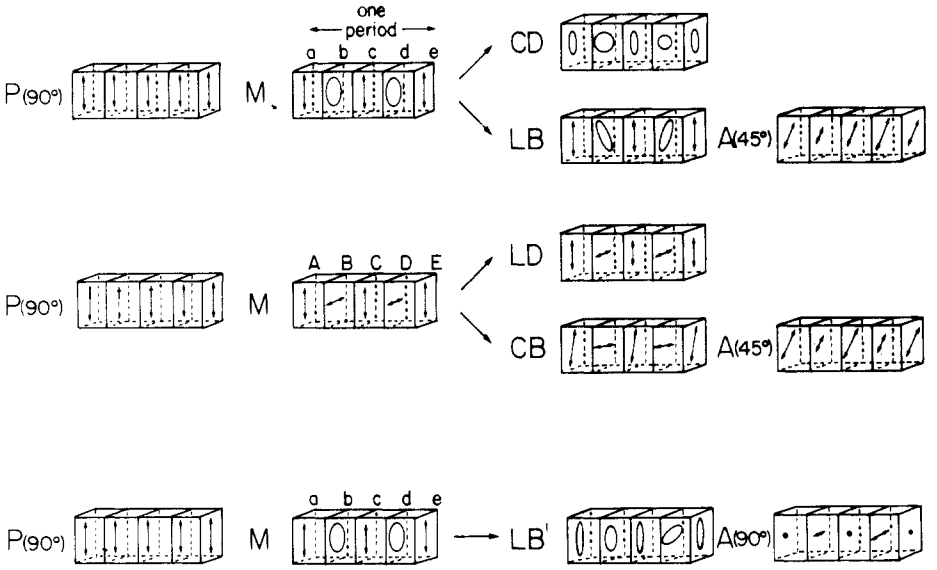

Figure 4. Response of a polarization spectrophotometer to five isolated optical effects: CD, LB, LD, CB, and LB′. The polarization figures in the boxes refer to the successive states of polarization as the retardation is varied through its cycle. In the upper part of the diagram, where the modulation spans quarter-wave retardation only, the symbols a, b, c, d, and e refer to retardations of 0, $\pi/2$, 0, $-\pi/2$, and 0, respectively. The intervening elliptical polarizations are not indicated in the drawing. In the middle section, where the modulation is half-wave, A, B, C, D, and E represent retardations of 0, π, 0, $-\pi$, and 0, respectively, with intervening circular and elliptical polarizations left out. The CD and LD signals are easy to understand. A circularly dichroic material absorbs right and left circularly polarized light to different extents, so that there is a maximum-minimum relationship of intensity between states b and d. As a result the transmitted intensity varies sinusoidally with the same frequency as the modulator, and the difference is directly detectable as an ac signal at the modulator frequency. One could say that the circularly dichroic material serves as a detector of the circularly polarized light in the incoming radiation. The same argument applies to LD measurements except that two cycles of linear polarization appear per modulator cycle, so that the LD signal appears at $2f$. For a pure LB signal the polarization is not affected for vertically polarized light, so that all the effects are seen with the alternating circularly polarized components b and d. These are converted into elliptical polarizations alternately oriented at ±45°. The analyzer, oriented at 45°, alternately sees the long and short axes of the elliptical polarization, leading to an ac signal at the fundamental frequency that is proportional to LB. For a sample showing pure CB, circularly polarized light is transmitted without change in polarization, so there is no signal at the fundamental frequency. On the other hand, the linearly polarized beams A, B, and D are tipped to polarizations slightly clockwise or anticlockwise depending on the sign of the optical rotation. As a result, one is turned toward the direction of transmission of a 45° analyzer, and the other away from it, leading to an alternating signal at $2f$. The LB′ apparatus detects LB′ at the fundamental frequency. In the LB case we had right and left circularly polarized light converted to elliptical polarization oriented at ±45°. In this case we have LB′ converting circularly polarized light to elliptical polarizations at 0° and 90°. It is just the previous case rotated by 45°. On the other hand, in the LB′ arrangement there is a very strong $2f$ signal, even in the absence of a sample, arising from the fact that the analyzer transmits one of the modulator polarizations and totally rejects the other. All the other arrangements have a null ac signal with no sample and ideal components. Since imperfections in the optics and electronics could allow some of the strong $2f$ signal to be detected at the fundamental frequency, it is not advisable to use this mode except for very strong LB′ systems. It is normally possible to rotate the sample or the polarizer modulator combination by 45° to convert LB′ into LB.

matrix elements have been measured except M_{11} and M_{22}. It turns out that M_{11} is obtainable by symmetry, but M_{22} really requires a circular polarizer for its measurement. The matrix is, however, overdetermined already, since it is dependent only on seven parameters. The diagonal elements, except for M_{00}, have so far not been of practical interest.

We close this section with a demonstration of the similarity between the formal method of deriving expressions for the sample Mueller matrix and the experimental procedures discussed in this section. We take the second experimental arrangement of Figure 2. If the Jones matrix for the sample is $\boldsymbol{J}$, then the matrix for the sample plus the detection polarizer is $\boldsymbol{P}_{45}\boldsymbol{J}$, where $\boldsymbol{P}_{45}$ is the 45° linear polarizer. Multiplying this matrix by its adjoint, in the manner used in the previous section, we have

$$(\boldsymbol{J}^\dagger\boldsymbol{P}_{45})(\boldsymbol{P}_{45}\boldsymbol{J}) = (\boldsymbol{J}^\dagger\boldsymbol{P}_{45}\boldsymbol{J}) = \tfrac{1}{2}(\boldsymbol{J}^\dagger\sigma_0\boldsymbol{J} + \boldsymbol{J}^\dagger\sigma_1\boldsymbol{J}) \quad (36)$$

where account has been taken of the fact that the operators for polarizers are self-adjoint and that, when ideal, they are idempotent, i.e., $\boldsymbol{P}^2 = \boldsymbol{P}$. Nothing is gained by polarizing light twice, though for real polarizers there will be some light loss that must be accounted for with an attenuation factor. The Jones matrices for polarizers for 45°, right circular, and 0° polarized light may be written in terms of Pauli matrices as is shown in Table VII. This leads to the form at the right of the table. We see that the use of these polarizers introduces Pauli matrices into eq 36 in the same way as the formalism for developing rows of the Mueller matrix of the sample. No polarizer is equivalent to σ_0 and develops the zeroth row; a 45° polarizer is equivalent to $^1/_2(\sigma_0 + \sigma_1)$ and develops the zeroth and first rows. Clearly circular and x/y polarizers develop the second and third rows of M, respectively, together with the zeroth row.

F. Symmetry Considerations

Another way of exploring further elements of the Mueller matrix is by sample rotation. It is clear that L and L' are defined only with respect to specific orientations of the sample, so that rotation introduces transformations between L and L' elements. By definition a rotation of the sample by −45° brings the 45° axis into conjunction with the x axis and introduces the transformations

$$\text{LD} \rightarrow -\text{LD}' \qquad \text{LD}' \rightarrow \text{LD}$$
$$\text{LB} \rightarrow -\text{LB}' \qquad \text{LB}' \rightarrow \text{LB}$$

All the other quantities in the matrix elements show cylindrical symmetry and are not affected by rotation about the direction of the light beam (TB, TD, CB, CD, a, b, etc.). Using the above rules, we find the following transformations:

before	after	before	after
M_{01}	M_{03}	M_{03}	M_{01}
M_{10}	M_{30}	M_{30}	M_{10}
M_{12}	M_{32}	M_{32}	M_{12}
M_{11}	M_{33}	M_{33}	M_{11}
M_{13}	$-M_{31}$	M_{31}	$-M_{13}$
M_{21}	M_{23}	M_{23}	M_{21}

The elements M_{00}, M_{22}, M_{02}, and M_{20} are invariant to rotation. The rule is that 3's are converted to 1's and 1's to 3's. When a 3 is converted to a 1, the matrix element is multiplied by −1. The pattern of connections is shown in Figure 3B, where the tie lines represent the interconversions. The result is that by using the three experimental arrangements with the modulator in front of the sample and rotating the sample by 45° all the elements but four can be measured. These are the four elements of the M_{2j} row. The six standard measurements of LD, LB, LD′, LB′, CD, and CB are possible with this arrangement if there are no noncommuting optical properties. For the general sample 12 measurements can be made, which, in principle, are more than enough to determine the 7 optical properties of the system. Reversing the direction of light through the sample permits the measurement of all but M_{22}. Apart from M_{00}, which gives the dc intensity, the diagonal elements are not of particular interest since none of the direct terms (U terms) occur there.

The reader should be reminded that the above arguments deal only with transmission measurements and cannot be applied to the Mueller matrix for nonforward scattering. Workers in the scattering field have recently devised very ingenious techniques for using multiple modulators at different frequencies to select for particular matrix elements through overtone and combination frequencies.[12] These techniques can also be applied to transmission studies of the kind discussed here. At the present time the relative advantages of using multiple modulators or rotating the sample and using an additional rotatable analyzer are not clear. The multiple modulators sharpen the discrimination but add a number of optical elements that may or may not increase the difficulty with instrumental artifacts. At the moment the multiple modulator technique is an exciting new development which is as yet untested for transmission spectroscopy.

At any rate the system is in principle overdetermined. There are only six parameters to be determined in the matrix since the average transmission of the sample can be determined independently and cancels out when the ratios are taken by the instrument (see the Experimental Measurement Section). Measurements with and without a 45° analyzer and at two sample orientations permit the measurement of the first two rows of the matrix, from which the six elements can be calculated. In practice, there may be artifacts introduced by other optical components, and the magnitudes of the parameters may be so disparate that the larger ones completely wash out the smaller. These problems are discussed in the section on mixed anisotropies and in the discussion of experimental apparatus.

G. Systems Showing Only One Kind of Anisotropy

Systems whose optical properties may be represented by just one of the optical pairs (L', C, or L) are easily investigated with polarization modulation spectroscopy. We discuss two cases, mainly as background for the next section. Since CB and CD or LB and LD normally go together, at least for spectroscopists, we present the matrices containing the pairs of conjugate effects, eq 37 for a chiral system and eq 38 for a linearly anisotropic system with $L' = 0$.

LD and LB are large effects and can usually be measured rather easily. The principal LD signal appears in M_{03} with no analyzer, and the principal LB

$$M_C = e^{-Ae}\begin{pmatrix} \cosh CD & 0 & \sinh CD & 0 \\ 0 & \cos CB & 0 & -\sin CB \\ \sinh CD & 0 & \cosh CD & 0 \\ 0 & \sin CB & 0 & \cos CB \end{pmatrix} \quad (37)$$

$$M_L = e^{-Ae}\begin{pmatrix} \cosh LD & 0 & 0 & -\sinh LD \\ 0 & \cos LB & -\sin LB & 0 \\ 0 & \sin LB & \cos LB & 0 \\ -\sinh LD & 0 & 0 & \cosh LD \end{pmatrix} \quad (38)$$

signal appears in M_{12} with a 45° analyzer. It should be noted that the dependence of the signal on LB is sinusoidal. Since LB can amount to many radians even for very thin specimens, one often sees channeled spectra in the mode which detects M_{12}. When this occurs it is a sign that the sample is too thick or that LB is so large that measurements of small quantities such as CB will be impossible. The size of the sample can be trimmed to diminish the magnitude of the LB signal (see Figure 1A) but, if LB is large, this will be effective only over a narrow range of wavelength.

In purely chiral systems CD is picked up in the fundamental mode and CB at $2f$ with a 45° analyzer. The optical rotation signal is also a sinusoidal function of CB, but this constitutes a curiosity rather than a problem since the effect can only be observed with very thick crystals with high optical activity. Commercial instruments nowadays provide the choice of the f or $2f$ mode for the measurement of CD or LD on the same instrument.

H. Systems with Mixed Anisotropies

We now consider the complications that arise when circular and linear effects are mixed. The usual object is to measure circular dichroism in the presence of linear dichroism and birefringence or vice versa. It might be questioned whether it has any meaning to discuss the linear and circular dichroism of a material that is in fact elliptically dichroic, but we shall adhere to the position, stated earlier in this section, that CD, LD, etc. may be defined by the way they appear in the matrices for infinitesimal systems. This provides an operationally effective definition of the six anisotropic quantities. The molecular interpretation of the six parameters is reserved for a later section.

Before looking at the equations that will govern the measurements, it is worthwhile to review the orders of magnitude of the quantities under consideration. As discussed earlier the absorptive quantities A, LD, and CD are naturally limited by the necessity for having a transmission through the sample that is sufficiently large to permit measurement. This places $A < 2$. For partially oriented systems linear dichroism is less than about 10% of the total absorption. When it is larger, we are entering the realm of dichroic polarizers. CD will normally be in the neighborhood of 10^{-3}–10^{-5} of the total absorption.

The range of birefringence depends on whether a transparent or absorbing medium is being studied. If the medium is transparent, the order of magnitude of $n - 1$ is about unity, and the upper limit to Δn is several tenths. There is, however, no limit to the thickness of the specimen. A 1-mm thickness of quartz cut parallel to the optic axis has an LB of about 105 radians and a CB of about 0.75 radian at a wavelength of 589 nm. This type of system is of interest to the crystal physicist or mineralogist. A chemist, on the other hand, usually wishes to obtain data on particular spectral transitions and normally works in regions of absorption, where the above-mentioned transmission limits are in effect. In the region of an absorption band CB is usually of the same order of magnitude as CD, but LB can be very much larger than LD. Within an absorption band CD arises only from the absorbing transition, while CB arises, not only from that transition but from the tails of all the "Cotton Effects" of all the transitions of the molecular system. These, however, are of both signs, and extensive empirical experience has shown that the background curve is small and that most of the optical rotation, like the circular dichroism, is dominated by the absorption band under investigation. The same kind of principle applies to LB and for the same reason. There is a zero-valued sum rule controlling the total contribution of all terms. It is found, however, that LB is often greater than LD. This is particularly true for studies of oriented polymer systems where form birefringence, solvent ordering, and other effects contribute, which have nothing to do with the chromophore under investigation. For the purpose of our estimates we shall make the assumption that LB ≅ LD but with the caveat that this may be incorrect for systems in which the chromophore makes up only a small part of the total system, i.e., the system also contains solvent, ligands, etc.

On the basis of the above discussion we now consider the following as reasonable estimates of the parameters for the study of mixed anisotropies: $A_e = 2 - 4$; LD = LB = 0.2; and CD = CB = 10^{-3}–10^{-5}. In setting up this example of experimental conditions the strengths of the CD and CB signals have been intentionally selected to be small compared to that of LD and LB, as is more often the case in practice. The purpose is to discuss the problems that arise in the measurement of a weak signal (CD, CB) in the presence of a much stronger, conflicting one (LD, LB). If the investigator is lucky enough to be working with a sample with $\Delta\epsilon_{\pm}/\epsilon \cong 10^{-2}$ and with weak LD and LB (some $n\pi^*$ transitions, d–d transitions), then the situation is much more favorable, but can easily be recalculated.

As can be seen in eq 37 and 38, the four quantities CD, CB, LB, and LD are localized in the matrix elements M_{02}, M_{13}, M_{12}, and M_{03}, respectively, when the systems are simple. These same matrix elements are the most useful for analyzing systems complicated by noncommuting properties. We consider now the explicit forms of these four matrix elements together with M_{00}, which is involved in the dc measurement that is an intrinsic part of the experiment. We have

$$M_{00} = X + W(\mathbf{T}\cdot\mathbf{T}^*)$$

$$M_{02} = W(\mathrm{LB}\cdot\mathrm{LD}' - \mathrm{LD}\cdot\mathrm{LB}') + U\cdot\mathrm{CD} + V\cdot\mathrm{CB}$$

$$M_{03} = W(\mathrm{CB}\cdot\mathrm{LD}' - \mathrm{CD}\cdot\mathrm{LB}') - U\cdot\mathrm{LD} - V\cdot\mathrm{LB}$$

$$M_{12} = -W(\mathrm{CB}\cdot\mathrm{LB}' + \mathrm{CD}\cdot\mathrm{LD}') - U\cdot\mathrm{LB} + V\cdot\mathrm{LD}$$

$$M_{13} = W(\mathrm{LD}\cdot\mathrm{LD}' + \mathrm{LB}\cdot\mathrm{LB}') - U\cdot\mathrm{CB} + V\cdot\mathrm{CD} \quad (39)$$

To simplify the results we will assume that LB′ and LD′ can be simultaneously eliminated by rotating the sample. This is true for systems that have been oriented by electric, magnetic, or flow fields. It is also true for uniaxial crystals and for monoclinic crystals if the di-

rection of propagation of the light is in the plane of the nonorthogonal angle. Under these circumstances the proper rotation of the crystal can eliminate LB′ and LD′ so that the W terms in M_{02} can be made to vanish. It is useful to examine the behavior of the quantities U, V, W, and X as TB and TD become small. This can be done for crystals by varying the thickness; for solutions it may be done by varying the concentration or degree of orientation. By a straightforward expansion of the trigonometric and hyperbolic functions we find that for small anisotropies

$$U = 1 - [(TD^2 - TB^2)/6] + ... = 1 - (a/6) + ...$$

$$V = \tfrac{1}{12}b(1 - (a/20) + ...)$$

$$W = \tfrac{1}{2} - (a/24) + ...$$

$$X = 1 + \tfrac{1}{2}(a^2 + b^2)^{1/2} \quad (40)$$

The quantities a and b were defined earlier (Table II). This shows that V approaches zero quadratically as a function of sample thickness (Figure 1), while U and X approach unity quadratically and W approaches 0.5 quadratically. With the values assumed for the parameters given above, a and b will be totally dominated by the values of LB and LD. This will give us values for U, V, W, and X of 0.987, 0.0067, 0.497, and 1.057, respectively. We note that even though the quantity W tends to 0.5, it is multiplied by terms that are quadratic in sample thickness or concentration and so tend to zero more rapidly than the U terms which are linear. Therefore the measurement of the four matrix elements specified in eq 39 will provide fairly accurate values for the four quantities even if they are of disparate magnitude. Thus it is possible in principle to measure CD or CB in the presence of linear anisotropies that are orders of magnitude greater.

The difficulty which is hidden in the above analysis is that a polarization modulator instrument measures the properties not only of the sample but of the entire optical train. Consequently, if the instrument has its own LD response, and this is true of most photomultipliers, then LB of the sample will interact with it to give spurious CD and CB signals. If optical components have slight strains or imperfections, they will contribute birefringence components that can interact with the LD of the sample or the photomultiplier. The only way to handle such problems is to use a birefringent plate in the apparatus to explore the dichroic properties of the apparatus and dichroic polarizers to explore the birefringence properties. Then the components can be oriented and compensators and depolarizers introduced to bring these difficulties to a minimum. These strategies will be discussed in the experimental section.

A sample for which the linear birefringence and dichroism axes do not coincide may also be investigated in principle. What one needs to do, after minimizing the artifacts in the manner just outlined, is to study all five key matrix elements as a function of sample angle. This is equivalent to seven matrix elements in the first two rows of the matrix. One then has sufficient data to solve the system, provided the experiments are sufficiently accurate. This is a long and complicated process, especially if wavelength is being scanned at the same time, and, to our knowledge, general systems of this kind have not been solved as yet.

I. Summary of the Phenomenology

We now review a few of the practical considerations that come out of the above analysis.

(1) The three experimental arrangements outlined in Figure 2 plus detection of DC, f, and $2f$ signals, where f is the angular frequency of the polarization modulator, permit the experimental evaluation of nine Mueller matrix elements. This number is extended to 12 by symmetry considerations and sample rotation. Placing the modulator after the sample in the optical train extends this number to 15.

(2) In practice the measurement of five matrix elements (M_{00}, M_{02}, M_{03}, M_{12}, and M_{13}) is sufficient if these are combined with sample rotation of 45°. Only the first two experimental setups of Figure 2 are required for this measurement.

(3) If only linear or circular anisotropy is present, CD, CB, LD, and LB are easily detectable as can be seen by the matrices of eq 37 and 38.

(4) In the general case the material is dichroic and birefringent, but the eigenwaves are elliptical and not necessarily orthogonal. The difference in phase retardation, TB, and the difference in the base e absorption coefficient, TD, are important ingredients of the theory. Even though the birefringence and dichroism are in fact elliptical, analysis in terms of linear and circular parameters is possible and useful.

(5) A useful form of the Mueller matrix is given in Table VI as a function of the fundamental seven-parameter set: LB′, LD′, CD, CB, LD, LB, and A. The elements also depend on functions of TB and TD that are defined in Table V. This form is useful for small signals and complex systems. As the total retardance becomes small because of the use of thinner specimens, lower concentrations, or weaker fields, U approaches unity and the U terms generate the desired quantity in a particular matrix element. The W terms are noncommuting terms. They arise because of the incompatibility of the optical phenomena. If one type of retardance is combined with a second type of retardance, it generates an artifactual signal for the third type of retardance. For example, LB and LD′ generate CD signals. By use of the relations at the bottom of Table III, $(LB \cdot LD' - LB'LD) = \sin 2(\chi - \theta)LD_\chi LB_\theta$. This factor is intrinsic to the sample and is not changed by orientation. It clearly vanishes, however, if the symmetry of the sample system is high enough that LB and LD have the same axes ($\theta = \chi$ or $\theta = \chi \pm (\pi/2)$). Even when present, W terms can be eliminated by varying thickness or concentration, since the W terms are quadratic in these variables and the U term is linear. The V terms describe "complementary" artifacts. For example, in measuring CB, CD combines with the total retardances to give a spurious signal and vice versa. V, however, goes rapidly to zero for thin specimens or dilute solutions (see Figure 1).

(6) A very serious source of error lies in the fact that the anisotropic properties of the sample can interact with noncommuting anisotropic properties of the instrument, resulting in artifacts. For example, the nonisotropic response of most PM tubes to polarized light leads to an effective LD, which can interact with a sample LB′ to give an apparent CD signal. Small birefringences in the optical train can also interact with linear dichroism in the sample. These effects can be

diminished by testing with elements with strong birefringence or linear dichroism, reorienting optical components and using depolarizers and compensators. References to the literature on this subject will be given in the experimental section.

(7) An order of magnitude calculation of the problem of measuring CD in the presence of LD and LB is not so unfavorable as is generally supposed, provided LD and LB have the same axis.

J. Experimental Measurement[4,11,13a,b,38,43a-c,44a]

1. Illustrations with Pure Measurements of CD, LD, CB, LB, and LB'

A typical optical train for a polarization spectrophotometer is shown in Figure 2. It consists of a linear polarizer (P), a photoelastic modulator (M), the cell compartment, an optional rotatable polarizer (A), and a photomultiplier connected to a lock-in amplifier so that signals of either the same or double the frequency of the modulator may be detected. Light from a monochromator passes through the entrance polarizer and then through the photoelastic modulator set with principal axes at 45° to that of the polarizer. For visible and near ultraviolet light, the modulator is a section of fused silica driven to sinusoidal oscillation by mechanical coupling to a piezoelectric quartz crystal. Both the quartz crystal and the silica section are cut to have an identical resonant frequency, which is nominally 50 kHz. This provides a periodically varying birefringence in the silica plate with an amplitude that is determined by the strength of the output of the driving oscillator. The modulator is described in ref 44.

As was discussed earlier, the retardation of (M) is given by § = ($\delta_0/2$) sin ft (eq 30), and the polarizer–modulator output is given by the Stokes vector (1, 0, sin 2δ, cos 2δ) (eq 29). As can be seen from the structure of this Stokes vector and the expansions of eq 33, the resulting radiation may be regarded as a superposition of circularly polarized light (modulated at the fundamental frequency and higher odd harmonics) and 0/90° linearly polarized light (modulated at 2f and the higher even harmonics). By tuning to the fundamental frequency, one detects the response of the sample to circularly polarized light, by tuning to 2f, the response to alternating 0/90° linearly polarized light. After leaving the sample, the light either goes directly to the photomultiplier, as in the first arrangement in the figure, or through an analyzer set at 45° or 90° to the original polarization, as depicted in the second and third arrangements.

We represent the functioning of the instrument by showing its response to the "pure" phenomena CD, LD, LB', CB, and LB in Figure 4. This figure provides a physical picture of some of the relations derived mathematically in the preceding sections. The sets of boxes contain polarization figures for the polarization states as a function of the retardance of the modulator in steps of $\pi/2$ or π. Successive figures show the evolution of each state of polarization as the light goes through the train of optical elements. See the figure captions for details. It will be noted that LD' is missing from the detectable phenomena of Figures 2 and 4. Examination of the Mueller matrices shows that this optical effect shows up only in response to the second component of the Stokes vector (±45° polarization), and this is absent from the modulator setup of Figure 2. One must either rotate the polarizer–modulator combination or the sample by 45°. It is preferable to do the latter. This leaves the orientations of the polarizer, modulator, and analyzer intact. These are usually carefully oriented to reduce artifacts before measurements are made.

Absolute measurements are not made in polarization spectroscopy, though this presumably is possible. Instead the instrument is calibrated with materials of known anisotropies. This has the result of canceling out a large number of optical and electronic factors that depend on wavelength and which slowly drift. For circular dichroism standardization is performed with epiandrosterone, camphor sulfonic acid, (+)-$[Co(en)_3]^{3+}$, or similar stable, reproducible substances with a prominent circular dichroism band. A procedure for calibration of circular dichroism that makes use of the linear dichroism produced by partial reflection from thin silica plates has recently been proposed.[44a] Silica components have the advantages of durability and reproducibility over chemical substances in solution. For linear dichroism a popular standard for calibration is a microscope coverslip, oriented at the Brewster angle, for which the transmission of orthogonal polarizations may be calculated from the refractive index. For this type of standard, which depends on selective partial reflection, it is necessary to correct for multiple internal reflections. Procedures for doing this, with the required formulas and tables, have recently become available.[44a] In our laboratory we use a Rochon polarizer oriented at 45° ± 3° from the polarization of the modulator. Use of the last equation of Table VII shows that the apparent linear dichroism of this system is sin (6°) = ±0.105 with a small nonlinearity correction which is easily calculated (see below). Another convenient procedure for the standardization of linear dichroism makes use of stretched films containing oriented dyes.[44b,c]

The signal processing is usually arranged so that the ratio of the ac to dc signals is recorded. This cancels out instabilities in the light source and electronic components. From eq 34 we see that for f and 2f detection these ratios are given by

$$\frac{I_f}{I_{dc}} = \frac{(4/\pi)J_1(\delta_0)M_{02}}{M_{00} + J_0(\delta_0)M_{03}} \tag{41a}$$

$$\frac{I_{2f}}{I_{dc}} = \frac{(4/\pi)J_2(\delta_0)M_{03}}{M_{00} + J_0(\delta_0)M_{03}} \tag{41b}$$

For the special, but very important, cases of measurements of CD and LD in the absence of interfering anisotropies, these relations become

$$\frac{I_f}{I_{dc}} = (4/\pi)J_1(\delta_0)\ \tanh\ (\mathrm{CD}) \cong (4/\pi)J_1(\delta_0)\mathrm{CD} \tag{42a}$$

$$\frac{I_{2f}}{I_{dc}} = \frac{(4/\pi)J_2(\delta_0)\ \tanh\ (\mathrm{LD})}{1 + J_0(\delta_0)\ \tanh\ (\mathrm{LD})} \cong (4/\pi)J_2(\delta_0)\mathrm{LD} \tag{42b}$$

where the rightmost expressions are for the usual case of small signals. For CD the value of δ_0 is adjusted to maximize J_1 (first-order Bessel function, maximum $J_1(x) = 0.58$ for $x = 1.8$). This is done by adjusting the output of the modulator driver to give a maximum CD signal with a circularly dichroic substance in the ap-

paratus. The driver is programmed to maintain this maximum as the wavelength is varied. For LD the modulator is adjusted to maximize J_2 (max $J_2(x) = 0.49$ for $x = 3.1$). The functions J_0, J_1, and J_2 are tabulated in ref 45.

The same relations apply for the general case (eq 41), but it is the matrix elements M_{02}, M_{03}, and M_{00} that enter into the equations rather than simple functions of CD and LD (see eq 34). Fuller and his associates have derived methods for measuring a number of matrix elements M_{ij} simultaneously. We give a brief discussion of their technique to show that the methods developed above for a conventional polarization modulation experiment can be readily applied to other situations. The following rules, which have been either explicitly or implicitly demonstrated in the preceding theoretical discussion, will be of help.

(1) A circular polarizer set at 0° is a linear retarder with LB = $\pi/2$ and LD = 0. From eq 38 we see that its matrix is

$$Q_0 = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 0 & -1 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix}$$

(2) The effect of Q_0 on a Stokes vector is to interchange s_1 and s_2. Its effect on a Mueller matrix is to interchange row 1, M_{1j}, with row 2, M_{2j}. The change in sign of row 2 will not be of concern in polarization modulation spectroscopy.

(3) The effect of polarizer $\boldsymbol{P}_j$, which produces the pure polarization described by the Stokes component, s_j, is to produce an intensity $(s_0 + s_j)/2$. Thus the effect of $\boldsymbol{P}_1$, a 45° polarizer, on s_0, s_1, s_2, and s_3 results in the intensity component $(s_0 + s_j)/2$. This addition is of course accompanied by the rejection of one of the two states of polarization described by s_j. $\boldsymbol{P}_j$, operating on a Mueller matrix, yields a first row that is the sum of the zeroth and jth rows divided by 2. These properties have been brought out both in the discussion of the experiental arrangements of Figures 2 and 4, where polarizers were used as detection of rows of the Mueller matrix of the sample, and in the theory of the Mueller matrix.

We can now describe the first apparatus of Fuller and his associates.[13a] The aim is to measure LD and LD′ simultaneously. The important matrix elements are M_{03} and M_{13} (see eq 38 or 62). This is done very simply by putting a quarterwave plate set at 0° between the modulator and the sample in the first arrangement of Figure 2. Schematically we have

$$\begin{pmatrix} 1 \\ 0 \\ 0 \\ 0 \end{pmatrix} \rightarrow \text{polarizer} \rightarrow \begin{pmatrix} 1 \\ 0 \\ 0 \\ 1 \end{pmatrix} \rightarrow \underset{\text{modulator}}{45^\circ} \rightarrow \begin{pmatrix} 1 \\ 0 \\ s \\ c \end{pmatrix} \rightarrow Q_0 \rightarrow \begin{pmatrix} 1 \\ -s \\ 0 \\ c \end{pmatrix} \rightarrow \text{sample}$$

where s and c are as in eq 29 and 30. The intensity of the photomultiplier is given by $M_{00} - sM_{01} + cM_{03}$. The M_{01} may be detected in precisely the same way as M_{02} in a CD experiment. Signal processing is arrayed so that the fundamental and overtone frequencies are detected at the same time. If the axis of birefringence matches that of the dichroism ($\theta = \chi$ in our notation), then the magnitude of the LD, LD_χ, and the angle χ can be followed as a function of time by using the formulas at the bottom of Table III. If θ and χ are not identical, LD and LD′ can still be determined in the thin-sample limit, provided LB_θ is not too much greater than LD_χ.

We shall not go into the details of the second experimental arrangement.[13b] The beam is split, and half is processed as just outlined. The aim for the other beam is to get information on LB and LB′, which are prominent in M_{21} and M_{23}. A circular polarizer would combine these elements in the first row (intensity), but circular polarizers are not easily found. However, the combination P_1Q_0 acts as a circular polarizer. Q_0 interchanges rows 1 and 2, and P_1 combines row 1 with the intensity row. The net result is the combination of row 2 with row 0. In the complete apparatus both the fundamental and overtone for both beams are analyzed simultaneously to give measurements of M_{01}, M_{03}, M_{21}, and M_{23}. Special cases are discussed where this leads to experimental values for LD, LD′, CB, and LB′.

Finally, it is often necessary to know the isotropic absorbance of the anisotropic system under investigation. If the method of orientation is by a flow, electric, or magnetic field, one can simply turn off the field and measure the transmission. In other cases, such as crystals or stretched films, this is not possible. In such cases one measures the transmission directly either by summing two polarized absorptions or by using unpolarized light. As stated in the discussion following eq 27, the apparent absorbance is the mean absorbance of the two eigenvectors for the system.

It is important to realize that dichroic systems do not obey the Lambert law, i.e., the intensity of the transmitted radiation is not a simple exponential function of the path length. Each of the eigenvectors will obey Lambert's law in most cases. They do so, however, with different absorption coefficients so that the polarization state of the light and the apparent absorbance depend on path length.

We shall discuss the evaluation of the isotropic absorbance from the apparent absorbance for the particular case of a strongly dichroic uniaxial system: $A_X = A_Y \neq A_Z$. Primes will indicate absorbances to the base e, and the dichroism will be evaluated as $(A_Z - A_X)$, which is customary for uniaxial systems. The analysis is thus suitable for stretched films, fibers, and uniaxial crystals. We define the following absorbances:

apparent absorbance

$$A_{app}' = -\ln (I/I_0)$$

isotropic absorbance

$$A_{iso}' = (A_X' + A_Y' + A_Z')/3 = (2\,A_x' + A_Z')/3$$

mean absorbance

$$A'_m = (A_Z + A_X)/2$$

dichroism

$$\Delta A' = A_Z' - A_X'$$

Note that A'_m is the quantity that appears as A_e in eq 27 and in Tables II, III, and VI.

A formula for A_{app} is readily obtained since the ratio of output to input intensity for unpolarized light is

simply M_{00}. The reader can readily verify that for pure LD and LB the birefringence part of M_{00} cancels out, leaving $M_{00} = e^{-A_m'}$ cosh LD or $A_{app}' = A_m' - \ln$ (cosh LD) $= A_m' - \ln$ (cosh $(\Delta A'/2)$).

From the identity $(A_Z + A_X)/2 = (A_Z + 2A_X)/3 + (A_Z - A_X)/6$ we have $A_m' = A_{iso}' + (\Delta A'/6)$ so that $A_{iso}' = A_{app}' - (\Delta A'/6) + \cosh(\Delta A'/2)$. Conversion to base 10 absorbances yields $A_{iso} = A_{app} - (\Delta A/6) + \ln \cosh(2.303\Delta A/2)$. Though differing in appearance this is identical with the formula derived by Norden for this case.[3] The correction can be large for strong linear dichroism. When polarization modulation techniques are used, ΔA is often small. On the other hand, if the linear dichroism is sufficiently strong that ΔA can be measured by subtracting separately measured values of A_X from A_Z, the use of this formula is mandatory. If the system is not uniaxial, absorbance measurements must be made for at least two propagation directions.

2. Applications and Artifacts

We now explore several cases where artifacts or nonlinearity are introduced into the measurement. The examples are selected in order to bring out some of the more serious pitfalls that may be encountered, as well as some fundamental principles which have not been stressed previously. Though the general formalism applies to all types of anisotropic media, biaxial as well as uniaxial, measurements are most frequently made on uniaxial systems and the following discussion will be restricted to this case.

Below we will work with the following notation. The angles are defined in a plane perpendicular to the direction of the light path: α = orientation of the polarizer in the polarizer–modulator combination; χ = orientation of the axis of the uniaxial sample (for example, one of the principal axes for LD of the sample); and θ = orientation of the strain axis of an external optical element.

Nonlinearity of Strong Signals. We will start by considering a medium displaying LD and LB, but no circular anisotropy, and it will be further assumed that the instrument is strain free. Using the experimental arrangement of Figure 2 and measuring at twice the modulation frequency, we can calculate the instrument signal to be

$$\frac{I_{2f}}{I_{dc}} = 4/\pi \frac{\cos(2(\chi - \alpha))\ \tanh LD}{1 - J_0(\delta_0)\cos(2(\chi - \alpha))\tanh LD} \qquad (43)$$

by a straightforward application of the matrices.[4] The signal is maximized when $\alpha = \chi$, so that the optic axis may be located by rotating the sample or the polarizer–modulator. When this is achieved, LD can then be determined. Since J_0 is about −0.3 when J_2 is at its maximum, the second term in the denominator becomes insignificant for small values of LD, and it is just for these small values that tanh (LD) may be approximated by LD. Specifically for LDs of 0.17 and 0.40, tanh (LD) is overestimated by 1 and 5%, respectively, when the linear approximation is used. For these cases the magnitude of the J_0 term in the denominator is about 0.05 and 0.12, respectively, which is significant relative to 1.0. Thus somewhere above an LD of 0.1, depending on the accuracy of the experiment, the linear relationship between the detected signal and the linear dichroism begins to break down and data processing requires more elaborate treatment. A trick which could be used to avoid the J_0 interference is to set the modulator amplitude so that $J_0 = 0.0$.[46] At this point, however, the J_2 function is no longer at its maximum. Thus if one wanted to use this procedure over a wide spectral range, a special wavelength program for the δ_0 amplitude would be required in order to maintain the calibration of the instrument.

A better procedure has been developed by Norden and Seth.[44a] Equation 41b or 42b may be written in the form

$$\Delta A_{app} = \frac{K_1 \tan k(LD)}{1 + K_2 \tan k(LD)}$$

where ΔA_{app} is the instrumental measurement I_{2f}/I_{dc}.

In this form K_1 and K_2 are instrumental constants and can represent not only the Bessel functions of eq 41 and 42 but any instrumental imperfections such as tracking of the modulator program. K_1 and K_2 are potentially functions of wavelength. The formula can be inverted to give LD in terms of the experimental quantity ΔA_{app}.

$$LD = \tfrac{1}{2} \ln \frac{K_1 + \Delta A_{app}(1 - K_2)}{K_1 - \Delta A_{app}(1 + K_2)}$$

This relation was used to determine the instrumental constants of two commercial circular dichroimeters with the gratifying result that K_1 and K_2 were essentially independent of wavelength.[44a]

Strain in the Optical Components. Before we discuss the effects of strain (i.e., linear birefringence) on the measurement of CD and LD, a few general remarks have to be made. The signal detected at the photomultiplier is the intensity variation. Pure birefringence effects, both linear and circular, by their nature, change only the polarization of light and do not affect its intensity. Thus a birefringent element alone, following the polarizer–modulator combination, generates no signal. But if a polarizer or, more generally, a linear dichroism is inserted between the birefringence and the detector, the polarization oscillations induced by the birefringence will be transformed into intensity oscillations. Thus with the appropriate optical arrangement of birefringence followed by linear dichroism, CB and LB can be measured conveniently in the f and $2f$ mode. (Figure 2, ref 4, 11, and 47) In particular, in the f mode, LB is optimized if the polarizer following it has its polarization axis at 45° to the LB axis, while no signal is produced if the axes are parallel. These remarks also apply if the birefringence under consideration is an undesirable artifact of an optical component of the instrument. One cannot conclude too hastily that strain in all elements which follow the sample will cause no difficulty in the measurement of CD or LD, because all photomultipliers have at least a slight dependence of sensitivity on the direction of linear polarization. This is equivalent to a small linear dichroism signal. End-on photomultipliers should always be oriented to minimize the artifact produced by their detection of strain birefringence in an empty instrument.

Measuring CD in the Presence of LD and Strain. We now consider artifacts in the CD mode due to strains in the optical elements preceding the sample. We assume a uniaxial medium with LD axis at χ and

with all optical effects sufficiently small that only the linear term in the expansion of the sample matrix is required (see eq 62 and 67). In addition, a strain matrix is included that precedes the sample. The birefringence magnitude is LB_θ^e and its angle is θ, where the superscript e stands for external. The resulting signal in the f mode is

$$\frac{I_f}{I_{dc}} = (4/\pi)J_1(\delta_0)\frac{CD - LB_\theta^e LD_\chi \sin(2(\chi - \theta))}{1 - J_0(\delta_0)LD_\chi \cos(2(\chi - \theta))} \quad (44)$$

This demonstrates that the LD_χ of the medium couples with the strain of the optical component to induce an apparent CD signal. In a spectroscopic study the signal will track the LD absorption curve of the substance and appear as a spurious CD band, even in an achiral system. Because the LD of an oriented system is often 2 or 3 orders of magnitude larger than its CD, even a small strain can cause disastrous effects (see the discussion later in this section).

There are, however, a number of ways to minimize the problem. The first and most effective is to propagate light down the axis of the uniaxial system. (This does not work for biaxial samples.) This, however, is often impossible (stretched films, transverse flow, etc.), and in any case it only provides direct information on one diagonal component of the CD tensor (see section III.B.4).

A second method can be applied if the sample and the element producing the strain can be oriented relative to one another. In this case the angle $(\chi - \theta)$ can be brought to zero, which eliminates the problem as may be seen from eq 44. Another method is to insert a variable strain device (e.g., a clamped window) before the sample and to adjust its orientation and strain to cancel out the indigenous strain of the other components. In spectroscopic work this method may cause difficulty because of the difference in dispersion of the birefringence of the various components, but in practice components tend to be made of the same materials. In UV–vis work, for example, one has fused silica in the cell, lenses, and windows, and quartz in the Rochon prism.

Chen et al. have used a second modulator with a different frequency placed directly after the sample in order to remove spurious signals originating from polarization effects in components subsequent to the sample.[48] A final method, used by Tunis–Schneider and Maestre,[49] is to average two measurements taken with sample orientations differing by 90°. As can be seen by substitution in eq 44 above, this results in equal and opposite contributions of the strain artifact that cancel in the averaging.

In conclusion we will briefly review several papers dealing with artifacts and the dangers of measuring circular dichroism in linearly anisotropic systems.

As far back as 1969 Disch and Sverdlik[43] realized that the apparent CD spectrum of an oriented system differs from that of the true spectrum because of the effects of linear dichroism and birefringence. They calculated the effects that would be observed (underestimation of CD, red and blue shifts, spurious bands) for a number of examples. Tunis-Schneider and Maestre introduced their sample rotation method in 1970 (see above). This has been used by other authors since its publication and is claimed to work well in cases of organic molecules in various orienting media such as stretched films.[50] In that same year Hofrichter demonstrated the use of a birefringence compensator and gave an extensive discussion of artifacts.[11]

In 1971 the problem of coexisting circular and linear anisotropies was further explored by Troxell and Scheraga.[38] They made use of the Stokes vector formalism and the power series of the general Mueller matrix as formulated by Gō.[30] Their discussion of the second order matrix of the combined sample and instrument provides a very instructive and practical view of the way that instrument anisotropies combine with sample anisotropies to give spurious effects. Our own paper[4] generalized these results by deriving the explicit form of the Mueller matrix for the most general case.

Norden and co-workers have dealt considerably with the problem of obtaining accurate circular dichroism data and have presented an analysis of the artifacts and correction factors in CD spectrometers that result from linear anisotropy.[52,53] They measured the retardation, produced by ambient strain, of a Pockels cell and a photoacoustic modulator. These turned out to be 0.1 and 0.02 radians, respectively! Using a formula equivalent to eq 44 above, they concluded that for a molecule like DNA, the LD is sufficiently large and the CD sufficiently small that the changes in CD induced by orientation are not measurable. They also found that the birefringence axis of the modulator drifts with time, which makes it difficult to set the angle of the birefringence so that the artifact is minimized.

Lately Shindo and co-workers have given a thorough discussion of the artifacts in CD spectroscopy.[54–57] They show how the general Mueller matrix formalism[4] is very powerful in the design, analysis, and evaluation of polarization instruments. These points have also been stressed by Tsankov et al.[58] and Schoenhofer et al.[8] As one example of the extensive analyses of Shindo et al., we cite their conclusions about the so-called LCICD (liquid crystal induced circular dichroism) effect. They point out that the data supporting the existence of this effect may be insufficient in several cases.[55,56] They do not deny the possibility that achiral molecules are optically active in cholesteric liquid crystals (see section IV.D and references cited therein (144–151). This would, in fact, be a form of induced circular dichroism that is well-known in other fields, e.g., binding to proteins, Pfeiffer effect, etc. On the other hand, from an error analysis of commercial CD spectrometers, they conclude that merely obtaining a CD signal from such systems does not adequately demonstrate the existence of the effect.

We close this section with two comments. The first is that the investigation of media with mixed anisotropies should always be accompanied by controls and checks for artifacts. This can be done by rotating optical elements or the sample and by introducing sources of LD, LB, and CB and checking the results. Film polarizers and thin sections of quartz will produce these effects very strongly. Another procedure that has been tried is the independent measurement of CB and CD and a quantitative comparison via the Kronig–Kramer's transform.[58a] Errors in other optical properties caused by CD are not normally very important. Second, the problems discussed in this section are superposed on the intrinsic difficulty of unraveling the Mueller matrix

elements in complicated cases. The instrument itself does not determine CD or LD in the first arrangement of Figure 2. It determines M_{02} and M_{03}. This section has dealt with methods of determining those matrix elements. If the system is complex, one then must turn to section II.H for further analysis. As was shown there, it will frequently be possible to resolve the problem for systems where the axes for linear birefringence and linear dichroism are parallel, but the general case with strong interference may defy analysis.

III. Molecular Interpretation

A. Applicability of the Lattice Gas Formalism

With methods developed by Jones and others, the phenomenology section has shown that there is a one-to-one correspondence between the transfer function of an infinitesimal layer of optical material and two-dimensional matrix operators. The eight independent parameters of a complex 2 × 2 matrix correspond to the eight independent optical properties that may be measured for the direct transmission of light through an infinitesimal sample. Additional parameters are required for scattering at an arbitrary angle.[13,34] In cases where linear and circular anisotropies do not exist simultaneously, it is easy to show which elements of the matrix correspond to each optical effect. Since there is no obvious way of discussing the optical activity of a sample that has elliptical polarization eigenvectors, we have simply *defined* all eight parameters to match their equivalent roles in the general Jones matrix for an infinitesimal sample. For example, the skew symmetric part of the matrix corresponds to optical rotation (CB/2). These same parameters are then carried over into the transfer matrix for Stokes parameters as well. Because phase information is lost in the Stokes representation, there remain seven independent parameters in the Mueller matrix. The mean refractive index is the missing parameter.

This approach takes care of a difficult problem as far as pure phenomenology is concerned, but it arises again in the question of the molecular interpretation of these optical quantities. In order to relate experiment to molecular theory it is necessary to solve the field equations for anisotropic systems. The molecular properties then come in as quantum mechanical formulas for susceptibilities. This is an extremely difficult area when systems are absorbing, linearly anisotropic, and chiral. Rigorous formulas exist for isotropic refraction and absorption (Ewald–Oseen method[21,22,59]) or for purely chiral systems.[23,60] There has also been considerable progress in obtaining practicable solutions of Maxwell's equations in a general anisotropic medium, particularly by the Russian school.[61] Finally there have been elegant advances in the interpretation of crystal spectroscopy using the exciton approach.[62–64] None of these have led, however, to a set of interpretive formulas that may be utilized directly to obtain molecular parameters in the general case. On the other hand, quantum mechanical formulas have been derived for the optical properties of an oriented ensemble of noninteracting molecules (the lattice gas model), and the purpose of this section is to inquire into the circumstances under which these formulas may be used to interpret the experimental quantities, defined as phenomenological matrix elements.

Our approach is to consider the formulas for absorptive processes only, including linear and circular dichroism. These are of most interest in chemical applications, and we assume that the dispersive properties may be obtained by the appropriate Kronig–Kramers transforms. The three basic formulas are, therefore, the lattice gas relations for absorption, linear dichroism, and circular dichroism. These formulas have been widely stated in the literature. We make use of our own publications[65–67] to avoid problems of notation.

1. Absorption[65,66]

Absorption is defined by

$$\epsilon = (\lambda\rho/K)D_{0m} \tag{45}$$

where $K = 3(2303)hc/8\pi^3N = 0.9186 \times 10^{-38}$ and D_{0m} is the dipole strength given by $D_{0m} = \mu_{0m}\cdot\mu_{m0}$, where μ_{0m} is the electric transition moment of the jump to excited state m. $\rho(\lambda)$ is the shape function for the band. It is defined so that its integral over the band equals unity: $\int\rho(\lambda)\,d\lambda = 1$. The useful form of this equation for the determination of molecular matrix elements from experimental data involves integration over the absorption band. This requires some kind of band resolution for overlapping bands

$$D_{0m} = K\int(\epsilon/\lambda)\,d\lambda \cong K/\lambda_{max}\int\epsilon\,d\lambda \tag{46}$$

2. Linear Dichroism, Oriented Molecules[65]

We begin by using a molecularly defined coordinate system. This will be symmetry determined if the molecule has symmetry, and one of the axes can be taken to correspond with the direction of the transition moment in a simple nondegenerate absorption. In this case, if v is a unit vector in the direction of the electric field of the radiation, then[66]

$$\epsilon_v = (\lambda\rho_v/K)\mathbf{v}\cdot\boldsymbol{D}_{0m}\cdot\mathbf{v} \tag{47}$$

Here $\boldsymbol{D}_{0m}$ is the dipole strength tensor. As an example, the xy or 12 element of this tensor is $(D_{xy})_{0m} = 3(\mu_x)_{0m}(\mu_y)_{m0}$. The shape function ρ is subscripted since it depends in general on orientation. Indeed the splitting and shape changes of degenerate systems provide one of the most interesting observations that can be made with linear dichroism measurements. The factor of 3 is introduced into D because the average of the tensor expression over all orientations is $^1/_3$ the trace of D, which leads back to eq 45. If we first take v to be in the x direction and then in the y direction and evaluate the difference, the formula for linear dichroism of the $0 \rightarrow m$ band (LD from the phenomenology section) is obtained.

$$\Delta\epsilon_{x-y} = (\lambda/K)[\rho_x(D_{xx})_{0m} - \rho_y(D_{yy})_{0m}] \tag{48}$$

where ρ_x and ρ_y are separately normalized shape functions. On integrating, the formula for the matrix elements is obtained

$$(D_{xx})_{0m} - (D_{yy})_{0m} = K\int\frac{\Delta\epsilon_{x-y}}{\lambda}\,d\lambda \tag{49}$$

If the transition is nondegenerate, then the x axis could have been taken along the direction of the transition moment so that $(D_{xx})_{0m} = 3D_{0m}$. If the transition mo-

ment makes an angle θ with **v**, then $(D_{xx})_{0m} = 3\cos^2\theta\, D_{0m}$. These formulas are based on very simple geometric considerations, but it is rare that one can make direct use of the molecular coordinate system. In the case of molecules oriented by flow, stress, or electromagnetic fields, orientation is not complete and what is seen in the laboratory is a distribution over orientations. In the case of crystals, there may be a number of molecules of different orientations in the cell. In addition, the orientation of the light beam may make an arbitrary angle with the crystal axes. At any rate there is always a linear relation between the orientation of a vector **u** in the laboratory and molecular coordinate systems.

$$\mathbf{u}^{\mathrm{L}} = \boldsymbol{O}\mu^{\mathrm{M}} \tag{50}$$

$\boldsymbol{O}$ is the transformation matrix from one coordinate system to the other.[68] Using the 1,2,3 notation for coordinates we have

$$(\mu_1^{\mathrm{L}})_{0m} = \sum_j O_{1j}(\mu_j^{\mathrm{M}})_{0m} \tag{51}$$

O_{1j} must be averaged over the molecular distribution function. For example, the xy component of the dipole strength tensor in the laboratory frame is given by

$$(D_{12}^{\mathrm{L}})_{0m} = \sum_{j,k=1}^{3} \langle O_{1j}O_{2k}\rangle (\mu_j^{\mathrm{M}})_{0m}(\mu_k^{\mathrm{M}})_{m0}$$

A very important practical case is the quantity LD, discussed in the phenomenological section. This is proportional to $D_{XX} - D_{YY}$ in the laboratory frame and is given by

$$\Delta\epsilon_{x-y}^{\mathrm{L}} = \frac{2\lambda\rho}{K}\sum_{j,k}\langle O_{1j}O_{1k} - O_{2j}O_{2k}\rangle (\mu_j^{\mathrm{M}})_{0m}(\mu_h^{\mathrm{M}})_{m0} \tag{52}$$

The coefficient $\langle O_{1j}O_{1k} - O_{2j}O_{2k}\rangle$ depends exclusively on the orientational distribution of the molecules in the system and is called the orientation function for LD. Orientation functions have been evaluated for most important distributions.[3] With the help of symmetry it is often possible to determine the components of the dipole strength tensor in molecular coordinates from the above relations. LD′ in the molecular frame is given by

$$\Delta\epsilon_{xy}^{\mathrm{M}} = \frac{2\lambda\rho}{K}(\mu_x^{\mathrm{M}})_{0m}(\mu_y^{\mathrm{M}})_{m0} \tag{53}$$

For a nondegenerate transition it is always possible to make this vanish by a suitable choice of axes and this is usually done. This topic will be resumed in section B.

3. Circular Dichroism

The circular dichroism of an isotropic system of noninteracting molecules is given by[65,66]

$$\Delta\epsilon_{\mp} = \left(\frac{4\lambda\sigma}{K}\right)R_{0m}; \qquad R_{0m} = K/4\int(\Delta\epsilon_{\mp}/\lambda)\,\mathrm{d}\lambda \tag{54}$$

where R_{0m}, the rotatory strength, is defined by $R_{0m} = Im\{\mu_{0m}\cdot\mathbf{M}_{m0}\}$. $\mathbf{M}_{m0}$ is the magnetic moment matrix element associated with the transition from state 0 to m, σ is the shape function for the circular dichroism of the band, and K has its previous significance. For the case of a molecule with a definite orientation, eq 54 must be replaced by the formula[67]

$$\Delta\epsilon_{\mathbf{k}} = \left(\frac{4\lambda\sigma_k}{K}\right)\mathbf{k}\cdot\boldsymbol{R}_{0m}\cdot\mathbf{k} \tag{55}$$

where **k** is a unit vector in the direction of propagation of the light, $\sigma_k(\lambda)$ is the orientation-dependent shape factor, and $\boldsymbol{R}_{0m}$ is the rotatory strength tensor. In diad form it is given by

$$\boldsymbol{R}_{0m} = -\frac{3e}{2mc}\,\mathrm{Im}\,\{\mu_{0m}\times(\mathbf{pr})_{m0}\} \tag{56}$$

for molecules small compared with the wavelength of light. Other forms of this formula exist where the electric dipole is replaced by the dipole velocity or momentum operators. We shall not pursue the relative merits of these formalisms, which have been much discussed in the literature. **pr** is the diad product of the momentum and position operators. Cross products of diads are clumsy to work with, and we have found it preferable to accomplish the cross multiplication via the antisymmetric matrix

$$\boldsymbol{U} = \begin{pmatrix} 0 & -\mu_3 & \mu_2 \\ \mu_3 & 0 & -\mu_1 \\ -\mu_2 & \mu_1 & 0 \end{pmatrix}_{0m} \tag{57}$$

The subscripts on the lower right of the brackets indicate that all of the quantities within are matrix elements between the designated states. The rotatory strength tensor is then given as

$$\boldsymbol{R}_{0m} = -\frac{3e}{2mc}\,\mathrm{Im}\,\{U_{0m}\cdot(\mathbf{pr})_{m0}\} \tag{58}$$

For the usual case where light is considered to be propagated along the z axis, we have[67]

$$R_{ZZ} = \frac{3e}{2mc}\,\mathrm{Im}\,\{(\mu_y)_{0m}(p_xz)_{m0} - (\mu_x)_{0m}(p_yz)_{m0}\} \tag{59}$$

Equations for the x and y axes may be obtained by permuting the indices cyclically. We give formulas for two off-diagonal elements

$$(R_{xy})_{0m} = \frac{3e}{2mc}\,\mathrm{Im}\,\{(\mu_z)(p_yy) - (\mu_y)(p_zy)\}_{0m;m0}$$

$$(R_{yx})_{m0} = \frac{3e}{2mc}\,\mathrm{Im}\,\{(\mu_x)(p_zx) - (\mu_z)(p_xx)\}_{0m;m0} \tag{60}$$

from which the other four off-diagonal elements can be obtained by cyclic permutation. The rotatory strength tensor is not symmetric, but it is easily shown that only its symmetric part contributes to circular dichroism in eq 55. The circular dichroism tensor depends on matrix elements that differ from those of absorption and ordinary dichroism. This is the motivation for circular dichroism studies on oriented molecules. Circular dichroism has a different sensitivity to molecular conformation and provides additional details about the electronic excitation process.

For most applications eq 55 must be converted to laboratory coordinates. The method is the same as that discussed for linear dichroism. Though the molecular distribution function is identical for the averaging of linear dichroism and circular dichroism, the so-called orientation functions for the two types of dichroism differ because linear dichroism is associated with the

polarization vectors of the light (eq 47), while circular dichroism is associated with the propagation vector of the light (eq 55). Molecular distribution functions have been derived for systems in electric, magnetic, and flow fields. The distribution is of course accurately known for solved crystal structures. A review of the theory and experimental methods of obtaining orientation functions for all of these methods has been given by Norden.[3]

4. Evaluation of the Assumptions

Equations 45, 47, 54, and 55 are the mainstays of the interpretation of electronic absorption spectroscopy. They permit the conversion of experimental measurements into the quantitative determination of excitation matrix elements. In principle there are 11 matrix elements that can be obtained which are equivalent to the three components of the electric moment, the three components of the magnetic moment, and the five quadrupole components. As we shall see, however, magnetic and quadrupole moments do *not* arise directly in the theory except for the isotropic case and special molecular symmetries.

The question is when these basic and comparatively simple equations can be used to interpret experimental results. To decide on this we will now enumerate the restrictive assumptions that are made in their derivation: (1) They are all derived with the assumption of simple, pure states of the polarization of radiation, which do not obtain in the type of experiment we are considering. (2) The molecules are assumed to be independent; i.e., excitations on one chromophore do not interact with those on other chromophores. (3) The molecules are assumed to be small relative to the wavelength of light. (4) Strong anisotropic effects such as nontransverse radiation fields or physical separation of rays are not considered. (5) The intensity is attenuated by absorptive processes only; scattering losses are not significant.

We shall now show that only item 2, chromophore interaction, is a serious practical worry. We do not consider item 4 in the above list further since we have seen in the phenomenology section that it is not possible to disentangle the signals which result from thick, highly anisotropic samples. If one is faced with such specimens, it would be better to separate the beams and study the polarizations of the eigenvectors. This is a viable alternative to ordinary transmission spectroscopy. If molecules are not small compared with the wavelength of light (item 3), which occurs in polymers and large biological molecules, then a more general formulation must be used. Explicit work has been done in the case of helical polymers.[69,70]

We will also not discuss the case of scattering samples (item 5) since the interest of this review is in standard transmission spectroscopy. Methods of working with scattering samples and interpreting the results have been discussed by Maestre and co-workers.[71]

Item 1, the assumption that the theoretical quantities and the phenomenological coefficients may be identified with one another, requires more discussion. The derivation of eq 45 is obtained by calculating the probability of an absorptive quantum jump of a molecule in a thin lamina (Lambert–Beer method). Though the radiation is assumed to be linearly polarized, the molecules are randomly oriented, and the absorption of the assembly of molecules in the thin layer is obtained by averaging over all orientations of the molecules. This is equivalent to using nonpolarized light. Hence formula 1 is apparently restricted to the attenuation of the intensity of nonpolarized light in an absorbing medium. The circular dichroism formulas are obtained as follows.[66] By use of perturbation theory, the absorption of left circularly polarized light by a thin lamina containing isolated molecules is obtained. The result for pure right circularly polarized light is also calculated, and then the latter is subtracted from the former. This gives an equation for ΔI, the differential absorption of left and right circularly polarized light. Equations for linear dichroism are obtained in the same way, except that two orthogonal states of linear polarization are used instead of the two states of circular polarization.[66]

It turns out that the absorption and linear and circular dichroism obtained in this way are identical with those defined as phenomenological coefficients in the Jones or Mueller calculus. To see this we obtain the Mueller matrix for an infinitesimal lamina of a general sample showing all optical effects. The general Mueller matrix may be written as

$$\boldsymbol{M} = e^{-\mathrm{H}} \tag{61}$$

with

$$\boldsymbol{H} = \begin{pmatrix} A_e & \mathrm{LD}' & -\mathrm{CD} & \mathrm{LD} \\ \mathrm{LD}' & A_e & \mathrm{LB} & \mathrm{CB} \\ -\mathrm{CD} & -\mathrm{LB} & A_e & \mathrm{LB}' \\ \mathrm{LD} & \mathrm{CB} & -\mathrm{LB}' & A_e \end{pmatrix} \tag{62}$$

This was first shown by Gō[30] and later derived by us from the general Jones matrix.[4] For an infinitesimal layer the exponential may be expanded to terms linear in the thickness of the sample

$$\boldsymbol{M} = \boldsymbol{E} - \boldsymbol{H} \tag{63}$$

We use $\boldsymbol{E}$ for the unit matrix because I already stands for the intensity and the input vector. If the input vector to the lamina is $\mathbf{s}_1$, the output vector may be written as

$$\mathbf{s}_\mathrm{F} = \boldsymbol{M}\mathbf{s}_1 \tag{64}$$

and the change in the Stokes vector occasioned by passing through the lamina is

$$\mathbf{s}_\mathrm{F} - \mathbf{s}_1 = -\boldsymbol{H}\mathbf{s}_1 \tag{65}$$

Since we are only interested in intensities, we obtain the first row as

$$(s_\mathrm{F})_0 - (s_1)_0 = -\sum_j H_{0j}(s_1)_j \tag{66}$$

The quantity on the left is clearly the change in intensity. It is convenient to use normalized Stokes vectors; i.e., all components are divided by the intensity I. We then obtain

$$\delta I/I = -\sum H_{0j}(s_1)_j \tag{67}$$

where the components $(s_1)_j$ are normalized to unit intensity.

What we are doing now is calculating the effect of a general infinitesimal sample, containing all optical effects, on simple polarization vectors, using eq 67. For unpolarized light the Stokes vector is (1,0,0,0), and we obtain

$$\delta I/I = -A_e \tag{68}$$

Recall that A_e contains the infinitesimal path element dZ, so that this is the classical Lambert expression. If one uses left circularly polarized light, the Stokes vector is (1,0,−1,0), and we obtain

$$\delta I/I = -A_e + \mathrm{CD} \tag{69}$$

For right circularly polarized light the result is the same except for the sign of the CD term, so that the difference may be expressed as

$$\Delta I_{\mp}/I = 2\mathrm{CD} \tag{70}$$

This is precisely the identification that is made for circular dichroism in the theoretical derivation of eq 54 and 55 since CD = (ln 10/2)ΔA. Similar identifications may be verified for LD and LD′ as is obvious from the structure of the matrix H. The main point is that the presence of a noncommuting mixture of optical properties does not interfere with identification of the various optical effects and that the transfer coefficients A_e, CD, LD, and LD′ defined phenomenologically turn out to be identical with the quantities calculated quantum mechanically.

On the other hand, interaction with surrounding molecules (item 2) takes place in all condensed systems. If the surrounding molecules are transparent to the probing radiation, the interaction problem takes the form of the evaluation of the internal field correction. Apart from cubic or isotropic systems, where the Lorenz–Lorentz formula may be used, this factor is not known and is usually roughly approximated or ignored. One does not usually know the local geometry of surrounding molecules in solutions, films, liquid crystals, etc., so that a calculation cannot even be made. These considerations limit the accuracy of calculations of intensities, or conversely they limit the precision of the determination of dipole or rotatory strengths. It is generally conceded that the accuracy with which these quantities can be determined is not better than 10% or so in condensed media. Further causes for inaccuracy come from the fact that eq 45, 47, 54, and 55 make use of the Born–Oppenheimer factorization and the fact that the measurement of intensities is often not precise, especially in the case of overlapping bands.

A more interesting situation develops when the absorbing chromophore is surrounded by other absorbing chromophores. Two special cases of interest are crystals and polymeric molecules in solution or films. The case of polymers has been very thoroughly investigated.[72] They usually can be treated as linear systems, often of known structure. Polypeptide and DNA helices are the most extensively investigated examples. In these cases the entire polymeric system is treated as an assembly of coupled excitations. Degenerate and nondegenerate bands are included so that exciton bands and their interactions with one another are present as components of the theory. In addition, in some calculations, the polarizability tensors of the groups are approximated so that there is an attempt to cover the complete set of electronic excitations. As a result all interactions *within* the molecule are taken into account. In such cases chromophore interaction, far from being an annoying source of error, is often the main interest of the investigation, since most if not all of the characteristic spectroscopic features of polymer molecules arise from the interactions (band splitting, hypochromism, circular dichroism). The idealized formulas presented above then apply to excitations that are delocalized over the entire molecule. An example of a helical polymer will be presented in section IV.A.

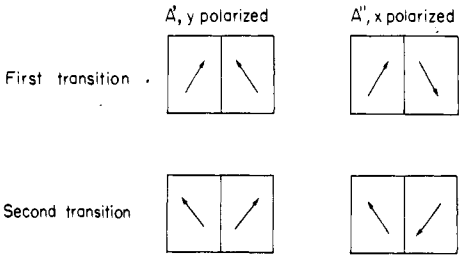

Figure 5. Band splitting with a unit cell possessing a mirror plane as its only element of symmetry. Mixing of the first transition with the second will change both the total intensity of absorption and the polarization of both bands.

The other case of extensive interaction arises in the case of crystals of absorbing molecules. In crystals there are, in general, elements of point symmetry as well as translational symmetry. There are two main results of these symmetries on chromophore interactions. The first is the Davidov splitting of the translational excitons into species related to the point symmetry. This leads to polarization modes of the excitons and to selection rules that are well understood.[62,63] We note here only that the polarization of a given level of an exciton band is determined by the symmetry of the arrangement of chromophores in the unit cell, and not by the polarization of the bands in the chromophores themselves. Polarizations in crystals are parallel or perpendicular to mirror planes, parallel or perpendicular to rotational or screw axes, etc. and are not determined by the orientation of transition moments in the chromophores (except for intensity). This will be seen in an example given below. One result of this is that the determination of the direction of transition moments in the chromophore often requires the accurate measurement of the intensity of absorption bands in the crystal, and not the direction of maximum absorption.

The second effect is that a crystal absorption band arising from an exciton with a given point symmetry interacts with excitons of all other electronic absorption bands of the same symmetry. This can lead to serious errors in evaluating the matrix elements for molecular excitation, such as the energy, dipole strength, polarization, etc. As an example, we consider a crystal with two molecules per unit cell related by a plane of symmetry (Figure 5). The only features that are shown in the figure are the electric transition moments. For simplicity the molecules that generate the moments are suppressed. The mirror plane is indicated by a central vertical line in the unit cell. The two linear combinations possible for a pair of chromophores are represented in the columns of the figure together with their polarizations and symmetry species. The two rows represent different electronic excitations with different polarizations in the molecules. There should in fact be a row for every electronic excitation of the molecule. If we neglect the third dimension for simplicity, the direction of the transition moment of the first band would hopefully be found by comparing the intensities of the absorption of x and y polarized light. We would have in fact

$$\tan^2 \theta = I_y/I_x \tag{71}$$

However, because of the interactions of the bands, the absorption of the A′ and A″ species of the band under investigation will borrow intensity from the A′ and A″ branches, respectively, of all other electronic transitions of the molecule. Hypochromicity (or hyperchromicity) is a tensor property that not only changes the intensity of absorption but also changes the apparent polarization of absorption. A further complication arises from the fact that the energy of interaction of the bands, and thus of all related phenomena, depends on the direction of propagation of light through the crystal. These effects are well understood, but their analysis is complicated and often inaccurate. Attempts at calculating crystal spectra for molecules with known spectral properties (dipole strength, transition moment directions) has been rather successful.[64,73] The more difficult problem of extracting molecular spectral properties has been attacked in a number of ways by various authors. Chen and Clark have in fact even attempted to invert the full crystal theory, which involves performing retarded dipole sums over the crystal as a function of direction of propagation.[74] This is done in an iterative fashion until self-consistency is attained.

The main message of this discussion is that the lattice gas formulas presented above are only of qualitative application to crystals in general. We have dealt only with absorption and polarized absorption. The problems associated with the circular dichroism are even more difficult. Crystal spectra are amenable to simple interpretation only if interactions are sufficiently weak that exciton interaction effects are effectively absent. Fortunately there are such systems. The weak d–d transitions of a number of inorganic complexes constitute an important class of systems that can be studied, and an example of this type of application will be given below. Other systems are crystals of biological molecules where the chromophores are often diluted by a large volume fraction of nonabsorbing groups and also host–guest systems where the nonabsorbing host molecules provide a wide separation between the absorbing guest molecules. Evidence for significant chromophore interaction can be sought experimentally by comparing crystal spectra with solution spectra and by examining the crystal spectra for evidence of gross changes in band shape. Rough calculations are also helpful. For example, it is easily shown by using the dipole approximation that a strong transition ($\epsilon = 10^4$) roughly 5 A from a weak transition ($\epsilon = 100$) interacts with the latter with an energy of only 50 cm^{-1}.

Finally the circular dichroism formula must not be applied to cholesteric liquid crystals. Equations 58–60 are based on the assumption that the dimensions of the interacting system are small compared to the wavelength of light. It is well established that the characteristic chiral properties of cholesteric phases arise from the long-range chirality of orientation of the molecules.[74a,75] The repeat distance in the liquid crystal is often of the same order as the wavelength of light.

B. Interpretation of the Formulas

1. Orientational Averages

Molecular orientation is normally produced by flow, stress, or electromagnetic fields or by the formation of crystals or liquid crystals. Crystals are distinguished from the other cases by the fact that only a few discrete molecular orientations are present, whereas the other physical situations must be described by continuous distribution functions. We first address the cases of continuous distributions and take up the crystalline case later.

Orientational averages come into the problem via equations like 47, 52, or 55, which we put into the general form

$$T_v = \mathbf{v}\cdot\boldsymbol{T}^L\cdot\mathbf{v} = \mathbf{v}^\dagger \boldsymbol{O}\boldsymbol{T}^M\boldsymbol{O}^{-1}\mathbf{v} \tag{72}$$

Here $\boldsymbol{T}^L$ is the tensor for the electric dipole strength or rotatory strength in the laboratory frame, $\boldsymbol{T}^M$ is the tensor in the molecular frame, and $\mathbf{v}$ is a unit vector that corresponds to the direction of polarization of light for linear dichroism and to the direction of propagation of light for circular dichroism. T_v is proportional to the intensity of absorption associated with $\mathbf{v}$. As discussed above, the transform from molecular to laboratory coordinate frames can be accomplished by eq 50. $\boldsymbol{O}$ is the matrix of direction cosines

$$\boldsymbol{O} = \begin{pmatrix} X_x & X_y & X_z \\ Y_x & Y_y & Y_z \\ Z_x & Z_y & Z_z \end{pmatrix} \tag{73}$$

where X_y, for example, is the cosine of the angle between the X axis in the laboratory and the y axis in the molecular frame.

We now consider a very important simple case. This arises when the molecule being oriented has effective cylindrical symmetry about an axis. This is true of molecules with 3-fold or higher rotation axes, helices, ellipsoidal proteins in flow fields, molecules oriented by their electric or magnetic moments, etc. In this case the molecular tensor property takes the form

$$\boldsymbol{T}^M = \begin{pmatrix} a & & \\ & a & \\ & & b \end{pmatrix} \text{ or } \boldsymbol{T}^M = \begin{pmatrix} b & & \\ & a & \\ & & a \end{pmatrix} \tag{74}$$

where the z or x axis, respectively, has been selected as the cylindrical axis. The latter form is used in stretched-film analysis. Equation 74 should be obvious on symmetry grounds and will be demonstrated below. This formula can be rewritten as

$$\boldsymbol{T}^M = a\boldsymbol{I} + (b-a)\begin{pmatrix} 0 & & \\ & 0 & \\ & & 1 \end{pmatrix} \tag{75}$$

where $\boldsymbol{I}$ is the unit tensor. Note that the first term is isotropic and is unaffected by a transformation to the laboratory coordinate system. Transforming to the laboratory frame with $\boldsymbol{O}$ yields

$$\boldsymbol{T}^L = a\boldsymbol{I} + (b-a)\boldsymbol{O}\begin{pmatrix} 0 & & \\ & 0 & \\ & & 1 \end{pmatrix}\boldsymbol{O}^{-1}$$

and on multiplying out the matrices we find

$$\boldsymbol{T}^L = a\boldsymbol{I} + (b-a)\begin{pmatrix} (X_z)^2 & (X_z)(Y_z) & (X_z)(Z_z) \\ (Y_z)(X_z) & (Y_z)^2 & (Y_z)(Z_z) \\ (Z_z)(X_z) & (Z_z)(Y_z) & (Z_z)^2 \end{pmatrix} \tag{76}$$

Finally, adding the quantity $(b-a)/3$ to the first term and subtracting it from the second, we obtain

$$\boldsymbol{T}^L = T_{iso}\boldsymbol{I} + \tfrac{2}{3}\left(\frac{b-a}{3}\right)\boldsymbol{S} \tag{77}$$

T_{iso} is the isotropic dipole or rotatory strength, given

by $(2a + b)/3$ $\boldsymbol{I}$, and $\boldsymbol{S}$ is the orientation tensor. Its components are the order parameters

$$S_{IJ} = (3 \cos \theta_{Iz} \cos \theta_{Jz} - \delta_{IJ})/2 \quad (78)$$

where θ_{Iz} is the angle between axis 3 in the molecular frame and axis I in the laboratory frame. These may also be defined more generally for the case where cylindrical averaging is not appropriate.[75]

These formulas are simple to apply if the averages are known. For example the Z–X linear dichroism is directly proportional to $(D_{ZZ} - D_{XX})$ (see eq 48), and from eq 77 this is

$$(D_{ZZ} - D_{XX})^{L} = \tfrac{2}{3}(D_{zz} - D_{xx})^{M}\langle S_{ZZ} - S_{XX}\rangle \quad (79)$$

In many cases the molecular distribution is known and may be calculated from a model. (See ref 3 for a review of known distributions.) In this case linear dichroism methods can be used to determine the dichroism in the molecular frame. This permits the calculation of directions of transition moments. In other cases the direction of transition moments is known and the formula permits the determination of order parameters. The formula may also be applied to the rotatory strength associated with a given direction, say, the Z direction, in the laboratory.

$$R_{ZZ}^{L} = R_{iso} + \tfrac{2}{3}\left(R_{zz} - \frac{R_{xx} + R_{yy}}{2}\right)^{M}\langle S_{ZZ}\rangle \quad (80)$$

The first term is the isotropic rotatory strength, which is not affected by orientation, and the second term is the orientational part. Measurement of this second component permits the evaluation of matrix elements that are not accessible with ordinary measurements of circular dichroism.

If the molecular distribution function is uniaxial in the laboratory frame (stretched films, orientation with electric and magnetic fields, nematics), we then have $S_{XX} = S_{YY} = -(1/2)S_{ZZ}$. This arises from the fact that $S_{XX} + S_{YY} + S_{ZZ} = 0$ for an orthogonal coordinate system and $S_{XX} = S_{YY}$ for the uniaxial case. Equation 79 then takes the form

$$(D_{ZZ}^{L} - D_{XX}^{L}) = (D_{zz}^{M} - D_{xx}^{M})\langle S_{ZZ}\rangle \quad (81)$$

The special case described by eq 74–81 is very important and is used to interpret a large fraction of the work on systems oriented by flow, electric, and magnetic fields and by fiber formation. We now outline briefly a more general viewpoint, though without details or derivations, since these would be too specialized for this review. Four cases of orientational distributions will be described.

The problem to be solved is set up in eq 72. The rotatory or dipole strength tensor, defined relative to molecular axes, is to be transformed to laboratory axes and averaged over a distribution of molecular orientations. By use of (72) and (73) the matrix multiplications lead to the formula

$$T_{JK}^{L} = \sum O_{Jj} T_{jk}^{M} O_{kK}^{-1}$$

where $O_{Jk} = O_{kJ}^{-1} = \cos(Jk)$; $J, K = X, Y, Z$; and $j, k = x, y, z$. $\boldsymbol{T}$ is the dipole or rotatory strength. Only three angles, the Euler angles (25, 27, 68), are required to transform one Cartesian coordinate system into another so all of the direction cosines in $\boldsymbol{O}$ are, in general, functions of these three angles. The angles are alternately symbolized by φ, θ, and ψ or α, β, and γ, where φ and θ are the angles describing the orientation of the z axis in the laboratory frame.

A statistically distributed ensemble of molecules is describable in terms of a normalized distribution function $f(\phi,\theta,\psi)$, which gives the probability that the molecular axes are related to the lab axes by ϕ, θ, and ψ. If the optical effects are linear and additive, then

$$\langle T_{JK}\rangle = \sum_{jk} T_{jk} \int f \cos(J_j) \cos(K_k)\, d\Omega$$
$$= \sum_{jk} \langle \cos(J_j) \cos(K_k)\rangle T_{jk}$$

where the angular brackets indicate an ensemble average.

The formula above would be a cumbersome way to evaluate an averaged coordinate transformation. It was written out only to show that the transform is a quadratic form in the direction cosines. The averaged products are called the second moments of the distribution function. It follows from the above that the orientational properties of absorbance, linear dichroism, and circular dichroism (as well as optical rotation and birefringence) depend only on the second moments of the orientational distribution.

Dichroic measurements usually take the form of spherical harmonics. Because only second moments are involved and $\boldsymbol{D}$ and $\boldsymbol{R}$ are symmetric, only the five second-order spherical harmonics arise. We wish to avoid the complex form of spherical harmonics (the Y_{LM}) and tabulate instead the real form (or d orbital form) of these five functions.

symbol	formula	corresponding d orbital
C_{20}	$(3\cos^2\theta - 1)/2$	$2z^2 - x^2 - y^2$
C_{21}	$\sin\theta \cos\theta \cos\phi$	xz
C_{22}	$\sin^2\theta \cos 2\phi$	$x^2 - y^2$
S_{21}	$\sin\theta \cos\theta \sin\phi$	yz
S_{22}	$\sin^2\theta \sin 2\phi$	$2xy$

For light propagating in the Z direction LD and LD′ have the angular dependence of C_{22} and S_{22}, respectively (eq 48 and 53). For light propagating perpendicular to an uniaxial system, LD has the angular dependence of C_{20} (eq 81). For oriented systems, $R_{zz} - R_{iso}$ has the angular dependence of C_{20} (eq 80). If the molecule has real or effective cylindrical symmetry, then f is a function of the polar angles θ and φ only and can be expanded as a series in the spherical harmonics[75a,b]

$$f = A_{00} + \sum_{\substack{J=i \\ M\le J}} (A_{MJ}^{C} C_{MJ} + A_{MJ}^{S} S_{MV})$$

where the A_{MJ}'s are coefficients and the superscript indicates the presence of the sin or cosine of $M\phi$. If the quantity being averaged has the form of a single spherical harmonic, as in the examples above, then a single term of the expansion contributes to the average because of the orthogonality of the spherical harmonics.

We can now proceed to the four cases of orientational distribution functions.

Case 1. The molecule is effectively cylindrically symmetric, and the macroscopic system is uniaxial. Examples are long molecules in electric or magnetic fields, fibers, and certain crystal structures such as the cobalt complexes discussed in section IV.C. Because of the postulated conditions, $D_{xx} = D_{yy}$ in the molecule and $S_{xx} = S_{yy}$ in the laboratory (refer to eq 79)

$$(\epsilon_Z - \epsilon_X) = 3\epsilon_{iso}\left\langle \frac{3\cos^2\alpha - 1}{2}\right\rangle_{MOL}\left\langle \frac{3\cos^2\theta - 1}{2}\right\rangle_{LAB}$$

$$\frac{\Delta\epsilon}{\epsilon_{iso}} = \frac{\Delta A}{A_{iso}} = 3\left\langle \frac{3\cos^2\alpha - 1}{2}\right\rangle_{MOL}\left\langle \frac{3\cos^2\theta - 1}{2}\right\rangle_{LAB}$$

$\Delta\epsilon/\epsilon_{iso}$ is called the relative linear dichroism. For cylindrically symmetric molecules it is a product of a geometrical factor in the molecule, which gives the average orientation of the absorbing transition moments in the molecular frame times an average of the orientation of the molecule in the laboratory frame. The latter is called the orientation function for the particular measurement, and it normally has the form of one of the spherical harmonics. The average in the molecule can be over internal orientations if the molecule is flexible and over different transitions if they overlap.

Some steps have been skipped, but the jump from formulas in the elements of D to $\Delta\epsilon$ and ΔA is possible because of the canceling of common proportionality constants.

For this system there is no LD for light propagated along the axis of the uniaxial system but it can be shown that

$$\text{(CD)}\quad \frac{\Delta\epsilon_Z - \Delta\epsilon_{iso}}{\Delta\epsilon_{iso}} = \left(\frac{R_{ZZ} - R_{iso}}{R_{iso}}\right)\left(\frac{3\cos^2\theta - 1}{2}\right)$$

This is again the product of a molecular factor which is dependent on the geometrical arrangement of transition moments and an orientation function for the molecule in the laboratory. This type of measurement was performed by Holzwarth and his co-workers on helices of polypeptides and nucleic acids (see section IV.A, Figure 8).

Case 2. The molecules are effectively cylindrical, but the medium is biaxial rather than uniaxial. A very important example of this case is flow birefringence and flow linear dichroism. If one assumes that the molecules are stiff, the Peterlin distribution is applicable.[75a,76,77,77a] If the X axis is defined as the direction of laminar flow and the Y axis as the direction of the gradient, then for measurements with light propagating down the Z axis

$$\text{(LD)}\quad \Delta\epsilon_0/\epsilon_{iso} = \frac{\Delta A_0}{A_{iso}} = \left\langle \frac{3\cos^2\theta - 1}{2}\right\rangle_{MOL}\left\langle \sin^2\theta\cos 2\varphi\right\rangle_{LAB}$$

$$\text{(LD')}\quad \frac{\Delta\epsilon_{45}}{\epsilon_{iso}} = \frac{\Delta A_{45}}{A_{iso}} = \left\langle \frac{3\cos^2\theta - 1}{2}\right\rangle_{MOL}\left\langle \sin^2\theta\sin 2\varphi\right\rangle_{LAB}$$

The subscripts 0 and 45 are customary and refer to the angle to which the polarization modulator must be set to make the measurement. Again the results are a product of an internal optical factor for the molecule and an orientation function. In this case, however, the orientation functions are C_{22} and S_{22} rather than C_{20}.

Formulas for the linear dichroism and birefringence with light along the Y axis and for CD or optical rotation have been derived.[11,47,77a,b]

Case 3. The macroscopic system is uniaxial, but the molecule does not have effective cylindrical symmetry. Stretched films[78] and nematic liquid crystals[79,79a] are important examples. This case is discussed in detail in section IV.B. The reduced linear dichroism is no longer a simple product of an optical geometrical factor and an orientation function but a sum of at least two such terms (see eq 101, which can be rewritten as the following):

$$\Delta A/A = \tfrac{1}{2}(\epsilon_x - \epsilon_y)(S_{xx} - S_{yy}) + \left(\epsilon_Z - \frac{\epsilon_x + \epsilon_y}{2}\right)S_{zz}$$

In this case there are two orientation functions that are spherical harmonics, $\langle C_{22}\rangle$ and $\langle C_{20}\rangle$. In practice other methods of analysis are used.[3,78,79b]

Case 4. The molecule does not have effective cylindrical symmetry, and the macroscopic system is not uniaxial. We now have three molecular axes that are all different. In this case all three Euler angles enter the problem. A powerful general approach is to use the Wigner matrix elements and their simple transformation properties. In this case the distribution function is expanded in the Wigner functions.[80]

$$f = \sum_{N=0}^{\infty}\sum_{M,M'=-N}^{+N} A^N_{MM'}\,\mathcal{D}^N_{M,M'}(\alpha,\beta,\gamma)$$

where the $\mathcal{D}^N_{MM'}(\alpha,\beta,\gamma)$ are the Wigner functions whose formulas and elegant transformation properties are tabulated in books on group theory and angular momentum.[25,68,80]

Only five of the nine functions with $N = 2$ contribute to the optical properties, and it can be shown that these transform like the spherical harmonics. This method requires the formulation of the problem in terms of irreducible tensors, which are complex quantities, and has seldom been used for the orientational spectroscopy of electronic or infrared absorption bands. There is, however, a well-developed literature for the use of irreducible tensors in the field of liquid crystals and in the theory of the NMR of oriented systems.

2. Molecular Cylindrical Averaging

Many of the most important applications to oriented systems permit averaging about a long axis or symmetry axis of the molecule, and we shall now discuss the simplifications that arise for this case. Note that the averaging is done in the molecular frame so that biaxial systems in the laboratory frame are not excluded, e.g., flow fields (case 2 above). The tensors for absorption and circular dichroism may be most easily averaged over all orientations by introducing the vector transformation for a rotation about the z axis by an angle α

$$x' = cx - sy \qquad y' = sx + cy$$

where s and c are the sine and cosine of α. This substitution is made for the x and y components of all the components of $\boldsymbol{D}$ and $\boldsymbol{R}$, and the result is averaged uniformly over all values of α. As a result, all of the off-diagonal elements of both tensors vanish; the xx and yy components of the averaged tensor are the means of the xx and yy components of the unaveraged tensor, and the zz components are not changed.

The results of cylindrical averaging are given in Table VIII for both the dipole strength and the rotatory strength tensors. Cylindrical averaging converts the system to a uniaxial symmetry so that the optical ten-

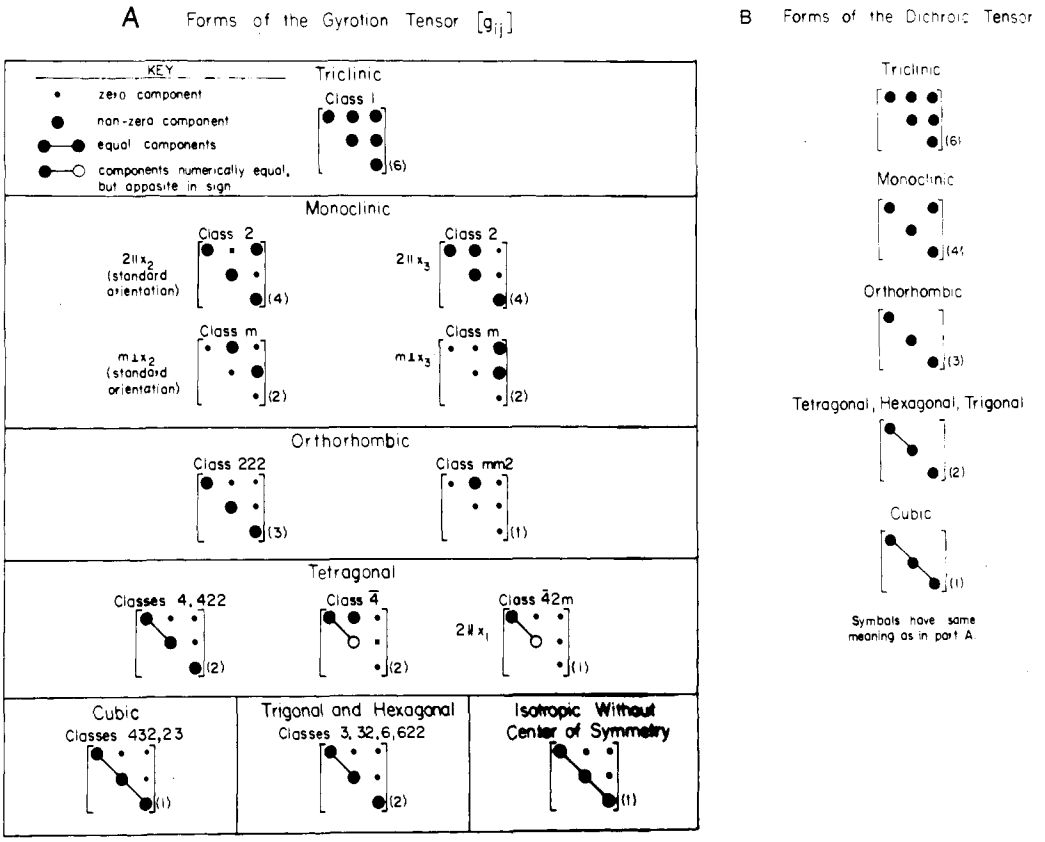

Figure 6. Structure of optical tensors. Symmetry considerations predict that certain tensor elements must vanish, must be equal to other tensor elements, or must be opposite in sign and equal in magnitude to other tensor elements. These relationships are depicted in A for the gyration tensor (CD and CB) and in B for the linear dichroism–linear birefringence tensor. The figures are adapted after Nye.[83]

TABLE VIII. Cylindrically Averaged *D* and *R* Tensors

$$D_\perp = D_{xx} = D_{yy} = {}^1/_2[(\mu_x)(\mu_x) + (\mu_y)(\mu_y)]_{0m,m0}$$
$$D_\parallel = D_{zz} = [(\mu_z)(\mu_z)]_{0m,m0}$$
$$R_\perp = R_{xx} = R_{yy} = (3e/4mc)\ Im\ [(\mu_z)(p_yx) - (\mu_z)(p_xy) + (\mu_x)(p_zy) - (\mu_y)(p_zx)]_{0m,m0}$$
$$= {}^3/_2\ Im\ [(\mu_z)(M_z)]_{0m,m0} + (3e/4mc)\ Im\ [(\mu_x)(p_zy) - (\mu_y)(p_zx)]_{0m,m0}$$
$$R_\parallel = R_{zz} = (3e/2mc\ Im\ [(\mu_y)(p_zz) - (\mu_x)(p_yz)]_{0m,m0}$$

sors have the same form as for uniaxial crystals, as may be seen by referring to Figure 6, which gives the form of the optical tensors as a function of symmetry. After the averaging, the only distinction that remains is between parallel and perpendicular elements of the tensors, $D_\parallel$, $D_\perp$, $R_\parallel$, and $R_\perp$. It must always be remembered that this notation refers to the direction of the electric field of the radiation for $\boldsymbol{D}$ and the direction of propagation for $\boldsymbol{R}$. The formulas are applicable for any coordinate system with z along the long axis, though it will often be useful to take advantage of symmetry or other considerations that will further simplify the formulas. For example, for any given transition it is possible to make either μ_x or μ_y equal to zero. It is also seen that the formulas for the components of $\boldsymbol{R}$ are not invariant with respect to an interchange of x and y. This is because of the chiral nature of $\boldsymbol{R}$ and its dependence on the handedness of the coordinate system, which is changed when x and y are interchanged. Finally we note that a term of the xx and yy components of $\boldsymbol{R}$ contains the z component of magnetic moment. This is the result of averaging about the z axis. It should be recalled that complete averaging of the rotatory strength over all orientations reduces all of the **pr** components to components of the magnetic moment in the form of $\mu\cdot\mathbf{M}$.

We close with a summary of the types of cylindrical symmetry that may arise with partially oriented systems. Electric and magnetic field orientation is frequently applied to long molecules that assume effective cylindrical symmetry in the molecular coordinate system by averaging about the long axis. The system is also symmetric about the direction of the field. Thus the optical properties are "uniaxial" in both the laboratory and (averaged) molecular frames. One order parameter, that connecting the Z axis of the laboratory with the z axis of the molecule, enters the equations for absorptive and refractive optical properties. These remarks also apply to molecules with a high-order symmetry axis ($n > 2$) in stretched films (see section IV.B).

For long rigid molecules in flow, such as biopolymer helices, the optical properties are cylindrically symmetric in the molecular framework, but the system is biaxial in the laboratory frame. The order parameters deal with the orientation of the z axis of the molecule with the three laboratory axes and have capital letters as subscripts, e.g., S_{ZZ}.

With stretched films the system is uniaxial in the laboratory frame but not necessarily symmetric in the molecular frame. The order parameters describe the orientation of the three molecular axes relative to the direction of stretch (Z axis) and have lower case letters as subscripts, e.g., S_{zz}.

3. *Determination of Spectroscopic Matrix Elements*

It would be desirable if all the matrix elements appearing in $\boldsymbol{D}$ and $\boldsymbol{R}$ could be evaluated directly from experiment. This is not possible for the general case,

though it can often be done if there are symmetry elements present. We assume that a distribution function is known so that optical elements determined in the laboratory frame can be converted to the molecular frame. For a given nondegenerate transition it is always possible to choose a coordinate system such that μ_y equals zero. μ_x then becomes $\mu_\perp$, the component of μ that is perpendicular to the axis. $\mu_\perp$ and $\mu_\parallel$ can be determined by dichroic experiments. For this case $R_\parallel$ equals $-(\mu_x)_{0m}(p_yz)_{m0}$, so that $(p_yz)_{m0}$ can be obtained. On the other hand, $R_\perp$ equals $[(\mu_z)(M_z) + (\mu_x)(p_zy)]_{0m,m0}$, which contains the two unknown quantities $(M_z)_{m0}$ and $(p_zy)_{m0}$, which cannot be resolved.

If the system has helical symmetry, a rotation axis, or a transverse mirror plane, so that transition electric dipoles can be classified as parallel or perpendicular, then the system becomes determinate. For a parallel band we have $D_\perp = R_\parallel = 0$. There are only two nonvanishing matrix elements: μ_z, which can be determined from $D_\parallel$, and M_z, which may be determined from $R_\perp$. For a perpendicular band, which will be degenerate for rotation or screw axes of higher order than two, μ_x is determined by $D_\perp$, $(p_yz)_{m0}$ from $R_\parallel$, and $(p_zy)_{m0}$ from $R_\perp$. Because of symmetry these values would also apply to (μ_y), (p_zx), and (p_xz), though with sign changes.

In practice the completely empirical approach to the determination of matrix elements outlined above is rarely adopted. Instead a model is set up for the system (for example, helical excitons for a helical polymer), the elements of $\boldsymbol{D}$ and $\boldsymbol{R}$ are calculated, and these values are then compared with experiment.

4. Crystals: The Case of D_3 Complexes

We now proceed to a discussion of crystals. General group theoretical arguments were given by Jahn, Bhagavantam, and others many years ago, which permit the evaluation of the number and kind of nonvanishing elements of tensor properties of crystals.[81-83] This type of result has been presented by Nye[83] in an especially perspicuous way as tensor diagrams. Diagrams for linear dichroism (or birefringence) and circular dichroism (or optical rotation), adapted from Nye, are shown in Figure 6. The elements of these tensors are the macroscopic counterparts of the elements of the rotatory strength tensor of the unit cell. We note that the gyration tensor is symmetric, while the rotatory strength tensor derived from quantum mechanics is not, but remind the reader that it is only the symmetric part of a tensor $\boldsymbol{R}$ which contributes to the product $\mathbf{k}\cdot\boldsymbol{R}\cdot\mathbf{k}$, since $\mathbf{k}\cdot\boldsymbol{A}\cdot\mathbf{k}$ vanishes when $\boldsymbol{A}$ is antisymmetric. As we shall see shortly, the rotatory strength tensor is simplified even further when the symmetry properties of a given excitation are also imposed on its matrix elements.

Because so much experimental work has been done, we will use as our example a series of uniaxial crystals of cobalt tridentate complexes with D_3 symmetry. In so doing we are following the steps of Saito and coworkers, who have not only discussed the theory but established many of the experimental results.[84] These systems also satisfy the requirement, discussed in the previous section, of being very weak transitions. The essentially negligible coupling between these very weak d–d transitions and other transitions makes it possible to consider the transitions as localized and not coupled

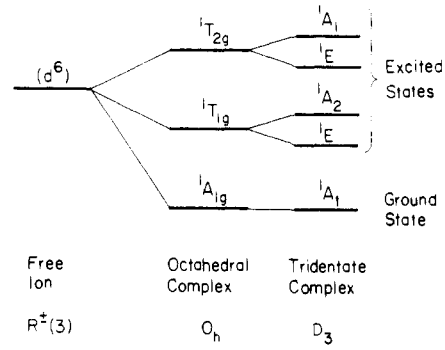

Figure 7. Correlation diagram for the singlet states of $[Co(en)_3]^{3+}$.

to the stronger, delocalized transitions of the system. Our discussion will differ from that of Kuroda and Saito[85] in two respects. First, they accounted for the variable polarization direction of circularly polarized light by averaging the field vectors over the transverse plane. This appears to give the correct answer for the transitions and symmetry they discuss, but it is simpler and more correct to use eq 55, which is based on the direction of propagation. Second, their formalism is based on a tensor that contains products of the components of the electric and magnetic moments rather than electric moments and the nine components of **pr**. This is, in fact, an allowable procedure for the transitions they discuss, which have small or negligible quadrupole components. We prefer to treat the general case that includes all types of transitions with no real increase in complexity.

Kuroda and Saito have assembled data for a number of Co^{3+} compounds, all of which may be assumed to possess D_3 symmetry in isolation. At the moment we are only interested in the manner in which symmetry affects the components of the rotatory strength tensor and will omit all structural details. In some crystals the D_3 axis of the complex is parallel to the optic axis of the crystal. In others the D_3 axis makes an angle α with the axis of the crystal, but the complexes are part of a higher order screw axis of the lattice. We shall first take up the form of the tensor in the coordinate system of the complex with the Z axis parallel to the D_3 axis of the complex. Matters are considerably simplified by considering the parentage of the electronic states in higher symmetry. In the octahedral symmetry of a hexammine complex, the 5-fold degeneracy of the d orbitals is removed and the six electrons reside in the degenerate d_{xy}, d_{yz}, and d_{zx} orbitals in the configuration t^6 (species A_{1g}). The excitations we shall be considering promote one of the electrons from a t orbital to an e orbital to give the configuration t^5e. In group O_h the six states that are produced from the products of the t and e orbitals are divided into two degenerate levels T_{1g} and T_{2g}. A correlation diagram adapted from Saito's book is given as Figure 7. T_{1g} is connected to the ground state by magnetic moment selection rules and T_{2g} by quadrupole moments. There are no allowed electric dipole transitions. This splitting is rather large and of the order of 10 000 cm^{-1}. In D_3 symmetry the T states split as follows: $T_{1g} \rightarrow A_2 + E$ and $T_{2g} \rightarrow A_1 + E$. This splitting is small, and the bands are not resolved with ordinary absorption spectroscopy. Electric dipole transitions are allowed to the A_2 and E states of D_3 but are very weak because they are mildly perturbed d–d transitions. Following previous authors we

shall concentrate on the nearly degenerate A_2 and E states. The $A_1 \rightarrow A_2$ transition is magnetically allowed, quadrupole forbidden. Transitions to E are in principle both magnetically and quadrupole allowed; but because of their parentage via a small perturbation on the octahedral T_1, the states we are considering should be magnetically allowed and almost quadrupole forbidden.

In D_3 the rotatory strength tensor is diagonal. An n-fold axis with $n > 2$ is equivalent to cylindrical averaging. Since for the A_2 transition both (μ_x) and (μ_y) are zero and since cylindrical averaging also averages the xx and yy components, the rotatory strength tensor for this transition may be obtained immediately from Table VIII.

$$\boldsymbol{R}_{A_1 \rightarrow A_2} = {}^3/_2 \, \mathrm{Im} \begin{pmatrix} (\mu_z)(M_z) & & \\ & (\mu_z)(M_z) & \\ & & 0 \end{pmatrix}_{A_1A_2;\, A_2A_1} \tag{82}$$

To consider the $A_1 \rightarrow E$ transition we divide the E state into its partner states. One of these may be taken to have μ_x allowed, presumably arising largely in the ligands. For this state we have $(\mu_y) = (\mu_z) = (p_zx) = (p_xz) = (p_yx) = (p_xy) = 0$. These relations may be established by considering the components of **p** as polar vector components and working out the symmetry of the products or by using carefully constructed group tables such as those in Wilson et al.,[86] where partner relationships between various bases are indicated by their order. Similarly for the y-polarized transition we have $(\mu_x) = (\mu_y) = (p_yz) = (p_zy) = (p_yx) = (p_xy) = 0$. The rotatory strength matrices for the separate partners of the E transition are given by (eq 59)

$$\boldsymbol{R}_{A_1 \rightarrow E_x} = \frac{3e}{2mc} \, Im \begin{pmatrix} 0 & & \\ & (\mu_x)(p_zy) & \\ & & -(\mu_x)(p_yz) \end{pmatrix}_{A_1E_x;\, E_xA_1}$$

$$\boldsymbol{R}_{A \rightarrow E_y} = \frac{3e}{2mc} \, Im \begin{pmatrix} -(\mu_y)(p_zx) & & \\ & 0 & \\ & & (\mu_y)(p_xz) \end{pmatrix}_{A_1E_y;\, E_yA_1} \tag{83}$$

It is preferable to combine these transitions by adding their matrices, since they must be equivalent because of the equivalence of any pair of orthogonal axes in the plane perpendicular to the 3-fold axis. One may choose any orientation of the x and y axis system that one wishes, but the radiation will select the proper linear combination to give one polarization in a direction that is transverse to the direction of propagation of the radiation (the ordinary ray) and another, not necessarily transverse, but perpendicular to the first (the extraordinary ray). The result is that the xx and yy elements of $\boldsymbol{R}$ have the same numerical value and may be calculated from either of the degenerate transitions. This gives, as the rotatory strength of the E transition

$$\boldsymbol{R}_{A_1 \rightarrow E} = \frac{3e}{2mc} \times$$

$$Im \begin{pmatrix} -(\mu_y)(p_zx) & & \\ & (\mu_x)(p_zy) & \\ & & (\mu_y)(p_xz) - (\mu_x)(p_yz) \end{pmatrix}_{A_1E;\, EA_1} \tag{84}$$

Table VIII could have been used directly to obtain eq 84 by applying it to each of the degenerate transitions and adding the result. In taking the longer route of evaluating the matrix elements for the x and y polarizations separately and then summing, we have gained some insights into the selection rules and the manner in which electric dipoles are coupled with elements of **pr**. All the matrix elements are experimentally determinate in the above formula, just as in the cylindrically averaged case. Because x and y are related by symmetry in this representation, there are only three independent matrix elements. For example we could choose (μ_x), (p_zy), and (p_yz).

A further approximate step can be taken. Elements like p_zy can be expanded by the identity

$$p_zy = \frac{yp_z + zp_y}{2} + \frac{yp_z - zp_y}{2} \tag{85}$$

Note that all of the operators commute. The rightmost term is clearly the x component of the angular momentum and leads to magnetic moment matrix elements. The first term is related to the yz quadrupole moment by the formula

$$(yp_z)_{m0} + (zp_y)_{m0} = \frac{im\omega_{m0}}{e} Q_{yz} \tag{86}$$

The theory of the circular dichroism of these D_3 complexes has shown that the rotatory strength is developed by combining the large magnetic moments of the d–d transitions of the central ion with small electric moments developed either by coupling with transitions in the ligands or by perturbation by the static ligand field. Because these borrowed electric moments are so small, a large magnetic moment is required. Since we are interested in oriented complexes, we must replace this statement by requiring that the transition must be associated with either a large magnetic moment or a large quadrupole moment. In the case we are considering we know that the transition had no quadrupole moment in octahedral symmetry and that the perturbation in going to D_3 is small. (This is not necessarily true for all D_3 complexes.) Consequently we may approximate the **pr** matrix elements by their angular momentum parts. When this is done the rotatory strength takes the form

$$\boldsymbol{R}_{A_1 \rightarrow E} =$$

$${}^3/_2 \, Im \begin{pmatrix} (\mu_y)(M_y) & & \\ & (\mu_x)(M_x) & \\ & & (\mu_x)(M_x) + (\mu_y)(M_y) \end{pmatrix}_{A_1E;\, EA_1} \tag{87}$$

This is the result of Kuroda and Saito[85] written in a somewhat different form. As can be seen, the approximation of dropping the quadrupole contributions is equivalent to assuming that $(p_zx) = -(p_xz)$ and $(p_zy) = -(p_yz)$. Since these matrix elements are both in principle determinable by experiment, this equality can be checked. One problem is that the E and A_2 transitions overlap so that a clear-cut experimental test may not be feasible.

The $\boldsymbol{R}$ tensors given in eq 82 and 84 (or 87) may be used directly for complexes in which the 3-fold axis of the complex is parallel to the axis of the uniaxial crystal. For those crystals in which the complex axis makes an angle α with the crystal axis, it is necessary to bring the $\boldsymbol{R}$ tensor into the crystal frame. Switching now to a

phenomenological representation, the $\boldsymbol{R}$ tensors may be written as

$$\boldsymbol{R}_{A_1\to A_2} = \begin{pmatrix} a & & \\ & a & \\ & & 0 \end{pmatrix}_j \quad \boldsymbol{R}_{A_1\to E} = \begin{pmatrix} b & & \\ & b & \\ & & 2b \end{pmatrix} \tag{88}$$

where a and b are the quantities that one tries to obtain by experiment and which are related to quantum theory by $a = (\mu_z)(M_z)$ (eq 82) and $b = (\mu_x)(M_x) = (\mu_y)(M_y)$ (eq 87). We are here making the approximation that quadrupole effects are negligible, which is probably quite accurate for these transitions of cobaltammine complexes. If this is not true, then the equation on the right above would have to be replaced by

$$\boldsymbol{R}_{A_1\to E} = \begin{pmatrix} b & & \\ & b & \\ & & c \end{pmatrix}$$

with elements correlating with eq 84. One could then demonstrate the presence of quadrupole effects by showing experimentally that the circular dichroism observed axially is not twice that observed perpendicularly. One has, of course, the experimental problem of band resolution mentioned above.

To return to the problem of nonparallel axes, if the axis of the complex is oriented at the polar angles α and β (latitude and longitude, respectively), then we can find its form in the crystal axis system by transforming by a rotation of α about the y axis and a rotation of β about the z axis.[68] After this, since the crystal is uniaxial, we may average about the angle β. We shall not go through these steps, which are standard in the literature, but only present the result. Given a diagonal matrix of the form

$$\begin{pmatrix} A & & \\ & A & \\ & & B \end{pmatrix}$$

molecular axes

this transformation, followed by averaging over β, leads to

$$\begin{pmatrix} \frac{(A+B)+(A-B)C^2}{2} & & \\ & \frac{(A+B)+(A-B)C^2}{2} & \\ & & A-(A-B)C^2 \end{pmatrix}$$

crystal axes (89)

where $C = \cos\alpha$ and α is the angle between the D_3 axis and the optic axis of the crystal. For the A_2 transition $A = a$ and $B = 0$ (eq 88), so that

$$\boldsymbol{R}_{A_1\to A_2} = \begin{pmatrix} \frac{a(1+c^2)}{2} & & \\ & \frac{a(1+c^2)}{2} & \\ & & a(1-c^2) \end{pmatrix} \tag{90}$$

For the E transition $A = b$ and $B = 2b$, giving the result

$$\boldsymbol{R}_{A_1\to E} = \begin{pmatrix} \frac{b(3-c^2)}{2} & & \\ & \frac{b(3-c^2)}{2} & \\ & & b(1+c^2) \end{pmatrix} \tag{91}$$

Values for $\cos\alpha$ may be obtained from crystal structures;[84] theoretical expressions for a and b are given in eq 82 and 87 (or 84). Also, since we have transformed to crystal axes, the R_{zz} component refers to propagation down the optic axis and the other two components to perpendicular propagation. Equations 89–91 contain the phenomenological relations derived by Kuroda and Saito[85] but are more general. The application of these formulas will be given in the section on experimental examples.

We will now go on to a brief discussion of the transition that is derived from the octahedral $A_{1g} \to T_{2g}$ transition. This is quadrupole allowed but magnetically forbidden. In this case we expect the rotatory strength to develop from the combination of small electric moments of the ligands and large quadrupole components of the d–d transitions of the cobalt ion. The $A_1 \to A_1$ transition is electric dipole forbidden in D_3 symmetry and should have no dipole or rotatory strength unless the D_3 symmetry is removed in the crystal. The rotatory strength tensor for the $A_1 \to E$ transition is again given by eq 84, since this applies to any E excited state in D_3 symmetry. The difference is that we now expect the components of **pr** to be highly quadrupolar with only a small, and perhaps negligible, magnetic part. Making this approximation and using eq 86, we obtain

$$\boldsymbol{R}_{A_1\to E} = -\frac{3\omega_{m_0}}{4e} \times \mathrm{Re}\begin{pmatrix} -(\mu_y)(Q_{zx}) & & \\ & (\mu_x)(Q_{zy}) & \\ & & (\mu_y)(Q_{xz}) - (\mu_x)(Q_{yz}) \end{pmatrix}_{A_1E;\,EA_1} \tag{92}$$

In this instance we can partially check our approximation. If the $A_1 \to E$ transition has no magnetic moment, then the rotatory strength of this transition should disappear in solution, since quadrupole contributions vanish on averaging over all orientations. Phenomenologically, quadrupole and magnetic dipole rotatory strength tensors differ in that the trace of the former vanishes. This is possible for the magnetic dipole rotatory strength, but does not occur for asymmetric molecules. The phenomenological form of the quadrupole tensor for D_3 symmetry is

$$\begin{pmatrix} b & & \\ & b & \\ & & -2b \end{pmatrix}$$

with $b = (\mu_x)(Q_{yz}) = -(\mu_y)(Q_{zx})$.

Quadrupole contributions have largely been ignored in the literature, but they have been discussed by Barron[87] and have actually been calculated by Kruchek,[88] using a model that couples the central quadrupole with the anisotropic bond polarizabilities of the ligands.

We close this section with the comment that it is not really necessary to divide transitions into magnetic and quadrupole categories, though this may be convenient (as in $A_1 \to A_2$) when high symmetry produces a clear subdivision. The matrix elements actually involved in the development of circular dichroism are the elements of **pr** such as $p_x z$. These are just as easy to calculate as magnetic and quadrupole moments, and it has been the practice of our laboratory to evaluate all the significant components of **pr** and then to combine these later to form magnetic and quadrupole moments. The

point is that the elements of **pr** are directly related to the manner in which radiation interacts with molecules to produce circular dichroism. This is not true in general of either the magnetic or quadrupole moments.

IV. Applications and Examples

In this section we review briefly a wide variety of examples of polarization spectroscopy in the fields of biological, inorganic, and organic chemistry. The purpose is to demonstrate the diversity of the method and its widespread use in structural chemistry as well as in the theory and interpretation of excited states. These examples were selected on the basis of our own familiarity and should not be construed as evaluations of the status of polarization spectroscopy in these various fields. We had the choice of restricting ourselves to the relatively narrow regions of our own expertise or providing a less professional but more general coverage. We chose the latter in the hope of stimulating more transfer of information among the three major areas of application, which have so far developed in mutual isolation.

A. Biopolymers

The natural state of many biological systems often consists of ordered arrays of molecules. Moreover, proteins, nucleic acids, polysaccharides, viruses, and other biological molecules or aggregates are large and can be readily oriented in electromagnetic, flow, or stress fields. As a consequence, studies of LD and CD on ordered arrays of biopolymer systems are numerous and varied.

The aim of most of these investigations has been to obtain structural information on the biological systems, though there has been very productive work in the areas of helical excitons, energy transfer, and other specialized electronic properties of large aggregates of chromophores. In the main, however, the approach is to combine experimental information on the LD and CD of biopolymers in solution and in oriented systems with theories of interacting chromophores to obtain structural information. This requires detailed knowledge of the spectroscopic properties of the monomeric chromophores (quantum states, energies, transition moments, wave functions), but because of the high importance placed on biological structural problems, there is a great deal of such information available.[89-91] The following is a sampling of the kind of questions asked. Are bases tilted in DNA?[92,93] How are α-helices oriented in membrane proteins?[94] How are nucleic acid helices oriented in phages?[95,96] What is the orientation of DNA in chromatin?[97-100] What is the orientation of absorbing units in chlorophyll proteins and retinal membranes?[101,102]

1. Methods of Orientation

Electric Fields. This has been one of the favored orientational techniques. The orientational distribution function is of the Langevin type with polarizability and permanent dipole contributions. The method has been thoroughly discussed in the monograph of Fredericq and Houssier[103] and in a recent symposium.[104]

Magnetic Fields. This procedure is useful only for very large systems or for very high magnetic fields.[105,106] It has the advantage of producing negligible perturbations of structure even for flexible molecules.

Flow Fields. Flow fields can be generated in Couette devices (rotating, concentric cylinders) or by pumping through capillaries or narrow gaps (Poiseille or related flow). Distribution functions as a function of gradient have been developed for rigid molecules modeled as ellipsoids[75a,76,77,77a] and for long flexible chains.[77b,107]

Film Stroking or Stretching. Thin fluid films of macromolecular solutions may be oriented by repeated stroking in a fixed direction. The orientational distribution is not known, but parallel and perpendicular bands can be identified.[109] They may also be oriented in stretched solid films[110] as discussed in the next section.

Transport through Gels. Very long polymers that are being transported through gels by electric fields must find channels parallel to the direction of flow. This provides a kinetic mechanism of orientation, which is at present being investigated.[111-115] The strong orientation associated with the electrophoretic migration of DNA in gels has subsequently been demonstrated by polarization spectroscopy.[115a-c]

Natural Orientation. Many biological systems are naturally anisotropic and can be studied directly. Microspectroscopic techniques are often required because of the smallness of specimens. Examples are molecules in fibers, proteins in membranes, muscle tissue, chloroplasts, etc. The aim is usually the experimental determination of order parameters (section III.B) that may be compared with structural models.

2. Specific Examples

From the large amount of literature in this field we shall choose only a couple of examples. The first is the classical work of Holzwarth and his associates on the optical properties of oriented helices. The idea was to synthesize the results of careful measurements of absorption, linear dichroism, and circular dichroism. For polypeptides the interpretation was based on the assumption that the absorption bands near 200 nm have the following components: an $n\pi^*$ band toward the red side of the absorption maximum; two strong bands polarized parallel and perpendicular to the helix that stem from helical excitons; and a helical "couplet", detectable only with CD, which is strongly predicted by helical exciton theory.[67,69,70,116,117,117a]

Orientation was achieved in nonaqueous solvents by an electric field and in aqueous solution by streaming through a packed array of capillaries. The optical path was parallel to the electric field or flow, which is equivalent to the axis of a uniaxial system. As a result only D_{XX} (or D_{YY}) and R_{ZZ} could be determined. The order parameter for these experiments was not known but was determined from the intensities of the parallel and perpendicular bands, which were of known polarization. Quantitative curve fitting was applied to all three spectral properties: A, LD, and CD. Figure 8 shows the results for the α-helix.[118] The data used in the analysis included the vacuum circular dichroism data of Johnson and Tinoco,[117a] the isotropic absorption, and electric-field-induced linear and circular dichroism.[118] Other helical systems were also studied in this way.[119,120] These studies remain the most definitive investigation of the optical properties of helical systems.

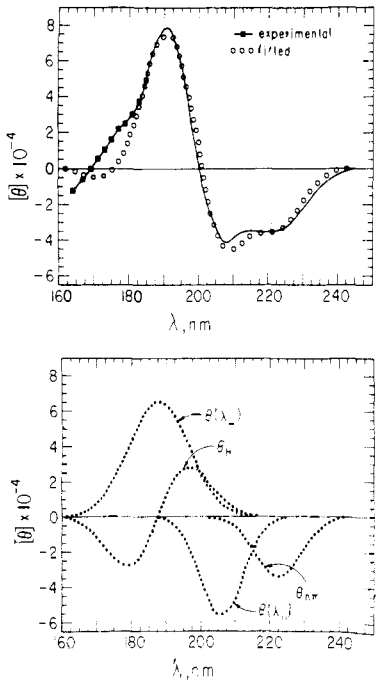

Figure 8. Circular dichroism spectrum of α-helical poly(γ-methyl-L-glutamate). Reprinted with permission from the work of Mandel and Holzwarth[118] (Copyright 1972 American Institute of Physics) and Johnson and Tinoco.[117a] (Copyright 1972 American Chemical Society.) The upper panel contains the experimental and fitted curves. The filled squares represent the vacuum circular dichroism data of Johnson and Tinoco. Below are the resolutions of the four components described in the text. Absorption and linear dichroism were also fitted to give a concordant set of parameters. The units of CD are deg·cm^2·dmol^{-1} on a residue basis.

A second example is more qualitative and shows the type of information that is available from the study of linear dichroism of a complex biological system. Figure 9 is a plot of the LD spectrum of tobacco mosaic virus (TMV) that has been oriented by flow.[47] The virus consists of single-stranded RNA bound to protein to form a long, stiff rod that is easily oriented. There are three nucleic acid bases per protein molecule. An interesting feature that immediately strikes the eye is in the region of 280–290 nm. The sharp reversals in LD arise from the interplay of the L_a and L_b of tryptophan, which have almost orthogonal transition moments. It is possible to estimate the order parameter of the tryptophan residues from these curves. With TMV it is possible to obtain rods of protein in the absence of nucleic acids. The LD of these rods may be used to approximate the LD of the protein in the full nucleoprotein complex. Subtraction then leads to an estimate of the LD of the nucleic acid moiety. This provides a value for the order parameter of the nucleic acid bases in the complex. The spectrum is dominated by the peptide bond in the neighborhood of 200 nm, which leads to information on the orientation of peptide groups and helices. The minimum–maximum combination, which is seen at 210–190 nm, arises from an array of helices with axes perpendicular to the phage axis. Thus the usual pair of LD bands is inverted relative to the spectrum of axially oriented α-helices. Our current knowledge of the structure of this virus is based mainly on diffraction techniques,[121] but linear dichroism remains an excellent technique for observing changes in structure that occur in solution. For complete spectra, including protein and difference spectra, see ref 47 and 11.

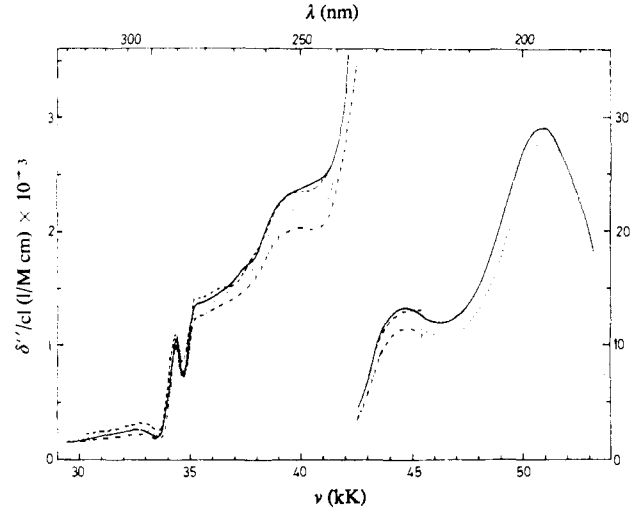

Figure 9. LD spectrum of TMV. Reprinted with permission from ref 47. Copyright 1973 Israel Academy of Sciences and Humanities. The variations in the curves arise from two different preparations and varying gradient and buffer concentration. The symbol δ'' is equivalent to LD/2.

B. Linear Dichroism of Stretched Films

A method for which there is very extensive experimental and theoretical literature employs stretched films as an orienting medium.[3,78,79b,123,125-127] The films are usually sheets of poly(vinyl alcohol), polyethylene, or other suitable material stretched in one direction to several times their normal length. It is assumed that the stretching is uniaxial and produces a uniaxial optical system.[78] With Z the direction of stretch and X the thin dimension of the film, we then have $A_X = A_Y$, where A_I indicates the absorbance for light propagated perpendicular to the film and polarized in the I direction. A_Y and A_Z are the measurable polarized absorption. The substance under investigation is dissolved in the film. Essentially identical results are obtained by dissolving the absorbing molecule either before or after the stretching, indicating that it is the stretched state rather than the process of stretching that determines the anisotropic properties. Experimental procedures are discussed in ref 78, 123, 125–127, 3, and 79b.

Work in this field differs from other areas of polarized absorbance in that the common practice is to measure A_Z and A_Y as conventional polarized spectra, rather than measuring ΔA or LD directly as discussed in previous sections. The results are, however, so closely related to the theme of this review that we include a summary, even though polarization modulation spectroscopy is infrequently used (see, however, ref 3). Relatively large differences in A_Z and A_Y are required for this procedure to be accurate, but this can normally be attained.

One aspect of comparing polarized absorbances rather than ΔA or LD is that the order parameters of eq 78, 79, and 81 are no longer uniquely convenient measures of anisotropy. Instead it is a common practice to use the elements of an orientational tensor

$$\boldsymbol{K}(Z) = \begin{pmatrix} K_{xx} & K_{xy} & K_{xz} \\ K_{yx} & K_{yy} & K_{yz} \\ K_{zx} & K_{zy} & K_{zz} \end{pmatrix} \qquad (93)$$

where, for example, $K_{xy} = \langle \cos [xZ] \cos [yZ] \rangle$ and lower case letters designate molecular axes. A specific set of axes will be selected below. Thus the $K_{\alpha\beta}$ are averages of the products of the direction cosines of the three molecular axes with the direction of stretch. Matrices $\boldsymbol{K}(Y)$ and $\boldsymbol{K}(X)$ can be defined, which are formed from products of direction cosines of the molecular coordinates with the film Y and X axes but these need not be used explicitly. We have in general

$$\boldsymbol{K}(X) + \boldsymbol{K}(Y) + \boldsymbol{K}(Z) = \boldsymbol{I} \tag{94}$$

where $\boldsymbol{I}$ is the unit matrix. Also for a uniaxial medium $\boldsymbol{K}(Y) = \boldsymbol{K}(X)$ and $\boldsymbol{K}(X)$ and $\boldsymbol{K}(Y)$ can be eliminated from the formulas via the relation

$$\boldsymbol{K}(Y) = \boldsymbol{K}(X) = \tfrac{1}{2}(\boldsymbol{I} - \boldsymbol{K}(Z)) \tag{95}$$

For this reason the identifier Z will be dropped, and $\boldsymbol{K}$ is understood to refer to the direction of stretch.

We may now use eq 72 to analyze the linear dichroic properties of films with $\boldsymbol{T}^{\mathrm{L}}$ and $\boldsymbol{T}^{\mathrm{M}}$ representing the absorbance tensor in the film and molecular frames, respectively, and $\boldsymbol{O}$ the matrix relating the two sets of coordinates.

$$A_{\mathrm{v}} = \mathbf{v}\cdot\boldsymbol{O}\boldsymbol{A}^{\mathrm{M}}\boldsymbol{O}^{-1}\cdot\mathbf{v} \tag{96}$$

For polarization in the Z direction, $\mathbf{v} = (0,0,1)$ and

$$\boldsymbol{A}_{ZZ} = \sum_{\alpha,\beta} O_{Z\alpha}A_{\alpha\beta}O_{\beta Z}^{-1} = \sum_{\alpha,\beta} K_{\alpha\beta}A_{\alpha\beta} \quad \alpha,\beta = x,y,z$$
$$= \mathrm{Tr}\,\{\boldsymbol{K}\boldsymbol{A}\} \tag{97}$$

Since $\boldsymbol{O}$ is orthogonal, we have put $O_{\beta Z}^{-1} = O_{Z\beta}$ and replaced $O_{Z\alpha}O_{Z\beta}$ with $K_{\alpha\beta}$ in accordance with eq 93. It is recognized that $\boldsymbol{A}$ is a function of wavelength and may be the sum over several bands in the case of overlapping bands. Also in replacing $O_{Z\alpha}O_{Z\beta}$ with $K_{\alpha\beta}$, averaging over the orientational distribution of the absorbing molecule has been performed, $K_{\alpha\beta} = \langle O_{Z\alpha}O_{z\beta} \rangle$.

$\boldsymbol{K}$ is a symmetric matrix and can be diagonalized. The axes of the coordinate system for which $\boldsymbol{K}$ is diagonal have been called the orientational axes.[78] By convention the z orientational axis is the one that has the greatest average component along the direction of stretch, the x axis the least, and the y axis the intermediate component. From here on it is assumed that the orientation axes have been selected as the molecular coordinate system. In this system the off-diagonal elements of eq 97 vanish and we have

$$A_Z = \sum_{\alpha=x,y,z} K_{\alpha\alpha}A_{\alpha\alpha} = \sum_{\alpha=x,y,z} K_\alpha A_\alpha \tag{98}$$

In the above the diagonal elements of the orientational tensor $\boldsymbol{K}$ and the molecular absorbance tensor $\boldsymbol{A}$ have been shortened to one index, as is customary. In this coordinate system the other experimental quantity, A_y, is given by

$$A_Y = \sum K(Y)_{\alpha\alpha}A_{\alpha\alpha} = \tfrac{1}{2}\sum(1 - K_\alpha)A_\alpha \tag{99}$$

Here $K(Y)$ has been eliminated by the relations

$$\langle \cos^2 (X\alpha) \rangle + \langle \cos^2 (Y\alpha) \rangle + \langle \cos^2 (Z\alpha) \rangle =$$
$$K_\alpha(X) + K_\alpha(Y) + K_\alpha(Z) = 1$$

the laws of direction cosines, and

$$K_\alpha(X) = K_\alpha(Y) = [1 - K_\alpha(Z)]/2$$

from eq 95.

We now distinguish two cases. If there is sufficiently high symmetry (C_{2v}, D_{2h}, or higher), the use of coordinates based on molecular symmetry axes will diagonalize both the orientation tensor $\boldsymbol{K}$ and the molecular absorbance tensor $\boldsymbol{A}^{\mathrm{M}}$. A nondegenerate transition moment will contribute to one of the three diagonal elements, A_α. A 2-fold degenerate transition will contribute equally to two of the elements A_α. The problem with this type of molecule is not to determine the angle of the transition moment in the molecular frame but to decide in which of the three possible directions it lies. This is often not a very difficult problem. One may have additional information, e.g., π–π^* transitions lie in the nodal plane of the π system. The TEM method (Thulstrup, Eggers, Michl) takes advantage of this feature. It also makes use of spectral superpositions to resolve the A_Z and A_Y into the three spectra A_x, A_y, and A_z and to determine the orientation factor. In addition, the use of polarized IR spectra on the same specimens can provide information on K_z and K_y if the directions of the IR transition moments are known. The details of these resolution techniques would take us too far afield, so the reader is referred to papers where they are described in detail.[3,79b,125–127]

For molecules of lower symmetry, the transition moment directions do not coincide with the directions of the orientation axes. Assume that a transition, i, has direction cosines $\cos \phi_x$, $\cos \phi_y$, and $\cos \phi_z$ with the orientation axes. The absorbance tensor in the orientation axis coordinate system may be regarded as being transformed from a molecular coordinate system in which the transition moment i is aligned along the z axis

$$\boldsymbol{A}_i^{xyz} = \boldsymbol{O}' \begin{pmatrix} 0 & & \\ & 0 & \\ & & A_i \end{pmatrix} \boldsymbol{O}'^{-1}$$

where $\boldsymbol{O}'$ is the matrix that effects the transformation. A_i is the absorbance of transition i with the polarization of light aligned along its transition moment, and $\boldsymbol{A}_i^{xyz}$ is the absorbance tensor in the coordinate system at the orientation axes. Because of the form of eq 98, only the diagonal elements of $\boldsymbol{O}'$ in the orientation axis coordinates are required. We have already seen that the squares of direction cosines appear on the diagonal for this type of transformation. Hence the absorbances for Z- and Y-polarized light are

$$A_{iZ} = \sum_\alpha K_\alpha \cos^2 \phi_\alpha A_i \equiv K_i A_i$$

$$A_{iY} = \sum_\alpha \tfrac{1}{2}(1 - K_\alpha) \cos^2 \phi_\alpha\, A_i \equiv \tfrac{1}{2}(1 - K_i)A_i$$

and summing over transitions for the general case of overlapping bands

$$A_z(\lambda) = \sum K_i A_i(\lambda)$$
$$A_y(\lambda) = (1/2)\sum(1 - K_i)A_i(\lambda) \tag{100}$$

The problem of solving such spectra is considerably more difficult than the high-symmetry case: methods of procedure have been outlined in a number of papers.[3,78,79b,123] Tactics include superpositions of spectra which null absorption bands, use of symmetry, shape, or other known characteristics of the molecules in question, and the use of ancillary spectral properties such as infrared dichroism, polarized fluorescence, etc. Successful studies are quite numerous, and again we refer the reader to the literature for more details.

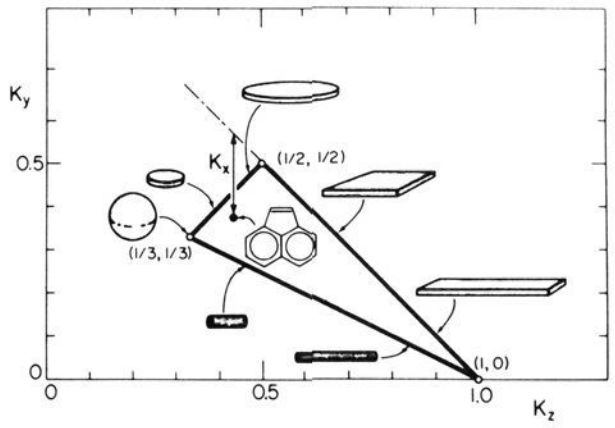

Figure 10. Orientation triangle. Reprinted with permission from ref 125. Copyright 1980 American Chemical Society. The vertical distance from the line $K_y = 1 - K_z$ gives K_x. Typical shapes corresponding to various points on the perimeter of the triangle are shown.

Because $K_x + K_y + K_z = 1$ there are only two independent orientation functions (normally K_z and K_y). As a result, the orientational properties of a molecule can be represented on a planar diagram. Because we also have $K_z \geq K_y \geq K_x$ by convention, all molecules and all degrees of orientation must occupy a limited area of the K_z,K_y plane. A totally random distribution (isotropic film) is represented by the point ($^1/_3$,$^1/_3$). A totally oriented molecule (z parallel to Z) is represented by the point (1,0). If the x axis is perpendicular to Z but the orientation is otherwise random, then $K_y = K_z$ and the point ($^1/_2$,$^1/_2$) is occupied. All other orientations must occupy the triangle formed by these three points as shown in Figure 10. In section III.B we discussed the special case of molecules that are effectively cylindrically symmetric either by possession of a ν-fold axis with $\nu > 2$ or by virtue of rotational averaging. These systems occupy a special place in the K_z,K_y diagram. If the symmetry axis tends to align with Z, then $K_x = K_y$ and the systems fall on the line $K_z + 2K_y = 1$, between the points ($^1/_3$,$^1/_3$) and (1,0). Rod-shaped molecules tend to have this orientation in stretched films as well as in electric, magnetic, and hydrodynamic fields. If the symmetry axis tends to antialign with the direction of stretch (symmetry axis is x axis), then $K_z = K_y$. These systems fall on the line between ($^1/_3$,$^1/_3$) and ($^1/_2$,$^1/_2$). This is characteristic of the orientation of disks. Finally, all systems with the x axis totally aligned perpendicular to the Z axis have $K_x = 0$ and $K_z + K_y = 1$. These points fall between ($^1/_2$,$^1/_2$) and (1,0). These features are illustrated generally in Figure 10 and with many examples in Figure 11. The construction is called the orientation triangle.

The K parameters are trivially connected with the S parameters ($S_{ij} = {}^1/_2(3K_{ij} - \delta_{ij})$), so the entire analysis, including the orientation triangle, can be formulated in terms of S_{zz} and S_{yy}.[3,127] Polarization modulation measurements give $A_Z - A_Y$ directly. Then from eq 100 the reduced linear dichroism is[3]

$$\frac{\Delta A}{A} = \frac{A_Z - A_Y}{A} = \frac{\sum K_i A_i - {}^1/_2\sum(1 - K_i)A_i}{A} = \sum\frac{({}^3/_2 K_i - {}^1/_2)A_i}{A} = \frac{S_{xx}A_x + S_{yy}A_y + S_{zz}A_z}{A} = \frac{S_{xx}\epsilon_x + S_{yy}\epsilon_y + S_{zz}\epsilon_z}{\epsilon} \quad (101)$$

A_x and ϵ_x, etc., refer to absorbances and extinction coefficients for light polarized along the molecular x axis, etc. Note that if deviations from Beer's law can be ignored, ratios of absorbances can be converted to ratios of extinction coefficients, since path length and concentration cancel out. Measuring LD directly by polarization modulation techniques is much more accurate and sensitive than measuring absorption spectra and subtracting them. Equation 101 provides a formalism for analyzing LD data.

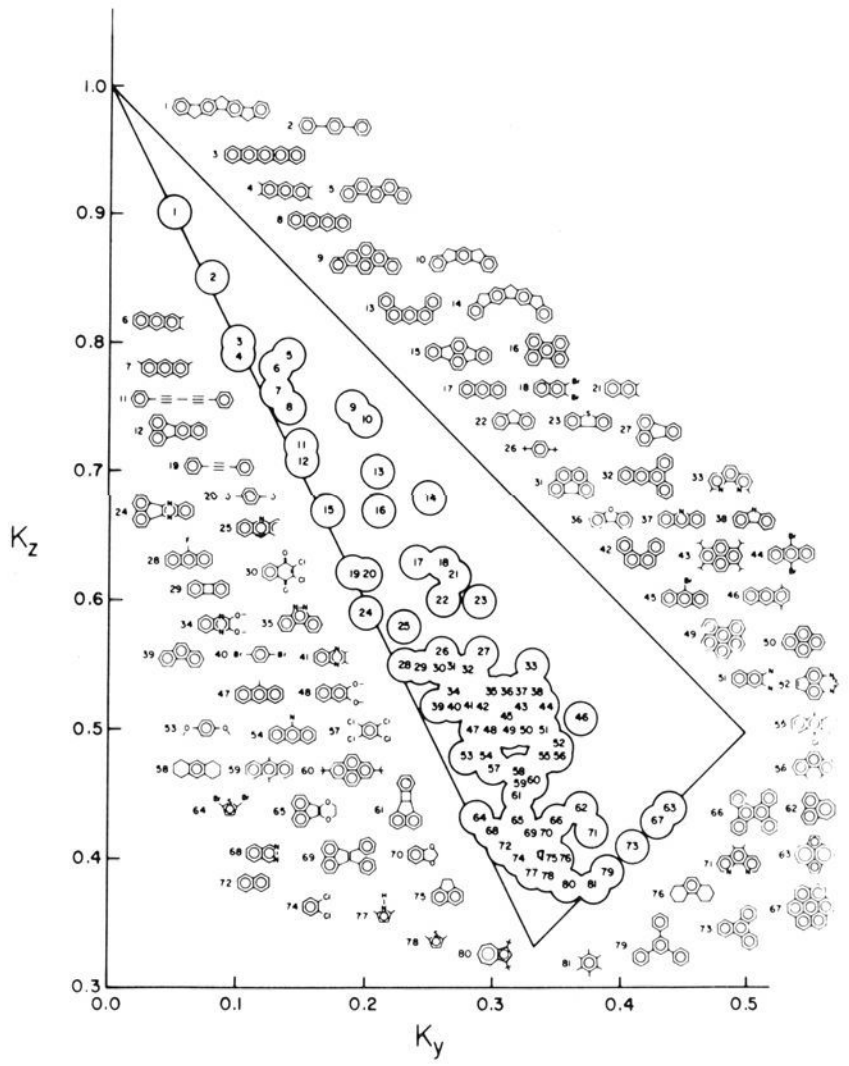

Figure 11. Orientation factors for aromatic molecules in stretched polyethylene films. Reprinted with permission from ref 126. Copyright 1982 American Chemical Society. Note permutation of coordinate labels from Figure 10. "There is a mistake in the original figure (ref 126) in that the formulas for phenazine (**37**) and acridine (**25**) are interchanged, and the point 66 is misplaced (it should overlap the point 67)" (personal communication from the authors).

The two main problems of polarization spectroscopy are the unscrambling of optical effects as outlined in the phenomenology section and the evaluation of the distribution function. We have discussed the stretched film case at some length because work in this area has shown what can be done without a theoretical distribution function. For the optical properties which are the concern in this article (LD, LB, CD, CB), it is not necessary to know the distribution function itself but only its second moments as exemplified by the K_α or $S_{\alpha\beta}$. Though many of the film procedures are specialized for uniaxial samples, they should prove useful in the analysis of data when other means of orientation are employed.

The alignment of solute molecules in stretched films depends on specific molecular interactions with the oriented solute environment and cannot easily be modeled in terms of generalized orientation functions. The same situation prevails in crystals and liquid crystals, though in the former case X-ray diffraction studies can provide precise orientational distribution functions, usually one or more δ functions in angular

space. In contrast, the orientation by electric, magnetic, and flow fields is adaptable to treatment by general physical forces (orientation via permanent electric dipoles and electric polarizability,[103,104] magnetic susceptibility,[105,106] equivalent ellipsoids in flow fields,[76] etc.).

Most workers in the field of stretched films treat the orientation functions as an empirical quantity and perform ancillary experiments and data analysis to obtain the second moments of the distribution, which is sufficient to relate laboratory measurements of LD or CD to the molecular tensors. This is the approach outlined in this section. There have, however, been a number of theoretical discussions of the mechanism of orientation of both solutes and solvent matrices in stretched films and liquid crystals. These will not be discussed here because they have just been extensively reviewed and discussed in ref 79b.

C. Single-Crystal Circular Dichroism Spectra of Coordination Compounds

There have been a large number of experimental and theoretical studies of the electronic origin of optical activity in coordination compounds[84,128-134] but, despite a wealth of information, the mechanisms are not yet fully understood. As pointed out by Geiser and Güdel,[135] this situation arises partly from a communication problem. On the one hand, the experimentalists find the theory intractable,[84,132,134,136-138] while, on the other hand, the theorists are usually trying to interpret the solution CD spectra, which do not offer an adequate basis for theoretical analysis. An initial attempt to overcome this difficulty was made as early as 1963 by McCaffrey and Mason,[128] who presented the first example of single-crystal circular dichroism spectroscopy by propagating the probing light beam along the axis of hexagonal crystals of Λ-(+)$_D$-2[Co(en)$_3$]Cl$_3$·NaCl·6H$_2$O (en = 1,2-ethanediamine). In this instance the light beam is parallel to the D_3 axis of the complex, permitting the direct observation of the rotatory strength of the $A_1 \rightarrow E$ transition of this complex. See section III.B.4 for a discussion of the electronic states of D_3 systems and of their polarization properties.

There have been many subsequent studies, especially of [Co(diamine)$_3$]$^{3+}$ ions. The technique usually is to combine observation along the axis of a uniaxial crystal with CD measurements on samples of randomly oriented microcrystals.[84,85,128] The form of the CD tensor for uniaxial systems is

$$\begin{pmatrix} A & & \\ & A & \\ & & B \end{pmatrix}$$

Measuring along the axis provides a measurement of B. Measurements of randomly oriented crystals determines one-third the trace, $(2A + B)/3$. It is thus possible to combine these measurements to get an indirect determination of A and eventually the rotatory strength of the $A_1 \rightarrow A_2$ transition, which cannot be observed with light propagating along the axis. The purpose of this strategem is to avoid making off-axis measurements on the crystal, because of the potential interference of LD artifacts (see section II.H). We now outline this method.

Because of the strong overlap of the visible A_2 and E bands of cobaltammine complexes, it is not possible to measure the circular dichroism of either band separately. Instead, CD measurements are sums of the contributions of both bands. From eq 55 this means that the CD tensor will be of the form

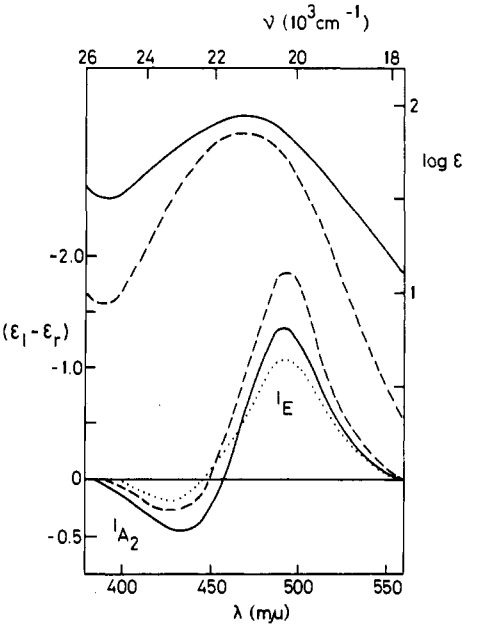

Figure 12. Circular dichroism and absorbance of [Co(en)$_3$]$^{3+}$:[138a] (—) (+)$_D$-Co(en$_3$)(ClO$_4$)$_3$ in a KBr disk; (- -) (+)$_D$-Co(en$_3$)Cl$_3$·3H$_2$O in aqueous solution; (···) same compound in KBr disks.

$$\Delta\epsilon = (4\lambda/K)[\sigma_{A_2}\boldsymbol{R}(A_2) + \sigma_E\boldsymbol{R}(E)] \quad (102)$$

where σ_{A_2} and σ_E are shape factors for the A_2 and E bands and $\boldsymbol{R}(A_2)$ and $\boldsymbol{R}(E)$ are their respective rotatory strengths. K is a constant factor (eq 45). This is the tensor equivalent of the standard solution equation

$$\Delta\epsilon = (4\lambda/K)(\sigma_{A_2}R_{A_2} + \sigma_E R_E)$$

where $R(A_2)$ and $R(E)$ are the usual rotatory strengths given by one-third the trace of $\boldsymbol{R}(A_2)$ and $\boldsymbol{R}(E)$. (The band shapes, σ, may vary with the direction of propagation, but this complication is usually neglected.) The rotatory strengths of the A_2 and E bands largely cancel in isotropic systems, producing typical "couplet" spectra as outlined in Figure 12. The forms for $\boldsymbol{R}(A_2)$ and $\boldsymbol{R}(E)$ for crystals in which the D_3 axis of the complex is parallel to the crystal axis are given in eq 82 and 87. Using these, we rewrite eq 102 in the form

$$\Delta\epsilon = \begin{bmatrix} a+b & & \\ & a+b & \\ & & 2b \end{bmatrix} \quad (103)$$

where a and b contain not only the matrix elements for the rotatory strength but the shape and constant factors as well.

$$a = (6\lambda/K)\sigma_{A_2}\,\mathrm{Im}\,((\mu_z)_{oA_2}(M_z)_{A_2o})$$

$$b = (6\lambda/K)\sigma_E\,\mathrm{Im}\,((\mu_x)_{oE}(M_x)_{Eo})$$

It is clear from eq 103 that the relative contributions of the a and b terms vary as a function of light path, and this allows the resolution of the a and b components. The method outlined above makes use of measurements along the optic axis for which $\Delta\epsilon_\parallel = 2b$ and measurements of a polycrystalline sample that provides an average over all crystal orientations

$$\Delta\epsilon_{av} = \tfrac{1}{3}\mathrm{Tr}(\Delta\epsilon) = \tfrac{1}{3}(2a + 4b) \quad (104)$$

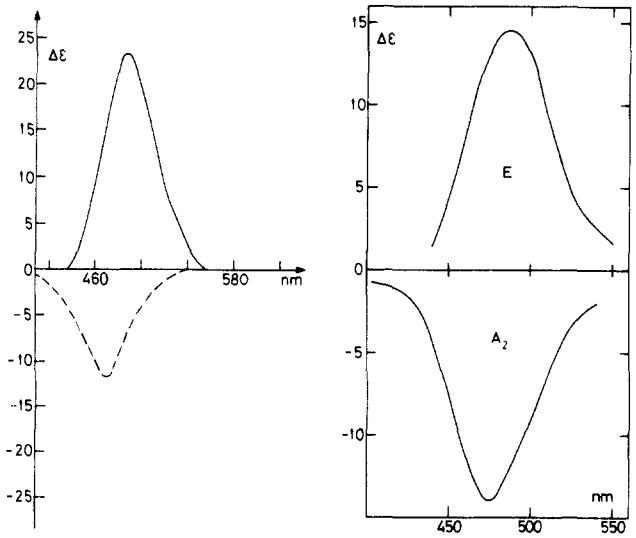

Figure 13. Polarized and resolved spectra of the $[Co(en)_3]^{3+}$ chromophore in a hexagonal crystal.[139] On the left the solid curve represents data taken with the light beam parallel to the C_3 axis at the complex, and the dotted curve perpendicular to this axis.

Combination of the two experimental results ($\Delta\epsilon_{av} - {}^2/_3\Delta\epsilon_{\parallel} = {}^2/_3 a$) permits the indirect determination of the A_2 spectrum.

Solution spectra are also given by eq 104, but the shape factors and rotatory strength components may be different because of the different molecular environment of the chromophore.

The method just outlined avoids LD and LB artifacts by using isotropic samples and optic axes. As discussed in section III.A.4, the weak transitions of coordination compounds are favorable to crystal studies, and it should be possible to measure circular dichroism perpendicular to optic axes when the birefringence is small. Because LD and LB have parallel axes in uniaxial crystals, the *W* artifact of M_{02} (Table V or VI) vanishes in the crystal itself, and one only has the usual worry of minimizing the interaction of these elements with linear anisotropies of the measuring apparatus (strain, effective LD of the photomultiplier, etc.). With small birefringence, the *V* artifact will also be small (Figure 1B). For this case the parallel and perpendicular measurements yield the results

$$\Delta\epsilon_{\parallel} = 2b \qquad \Delta\epsilon_{\perp} = a + b \qquad a = (\Delta\epsilon_{\perp} - \Delta\epsilon_{\parallel})/2$$

Such parallel and perpendicular observations were made on uniaxial crystals of the Λ-$(+)_D$-$[Co(en)_3]^{3+}$ chromophore[139] (see Figure 13A). Taking, for example λ = 500 nm, the numerical data are (in units of dm^3/Mcm)

$$\Delta\epsilon_{\parallel} = 18.3 \qquad \Delta\epsilon_{\perp} = -4.0$$

$$b = 18.3/2 \qquad a = -4.0 - (18.3/2) = -13.2$$

If we wish to convert to equivalent averaged circular dichroisms, we have

$$\Delta\epsilon(E) = {}^1/_3\ \mathrm{Tr}\ (\Delta\epsilon(E)) = 4b/3 = 12.2$$

$$\Delta\epsilon(A_2) = {}^1/_3\ \mathrm{Tr}\ (\Delta\epsilon(A_2)) = 2a/3 = -8.8$$

In this way one obtains the resolved curves of Figure 13B. The crystal spectra should be contrasted with the solution spectrum of the complex shown in Figure 12.

Next we consider a case in which the direction of light propagating through the crystal does not coincide with the symmetry axes of the molecular unit; i.e., it is not parallel or perpendicular to the C_3 axis. We assume that there is only one complex ion per unit cell or, if more than one, that they have parallel C_3 axes. For this case it is most convenient to use eq 55 directly in the molecular coordinate system. Since **k** is usually perpendicular to a crystal face, it corresponds to an indexed crystal axis. Once the crystal structure is known, it is an easy task to calculate the three direction cosines of **k** *in the molecular coordinate system.* We label the direction cosines α, β, and γ, referring to the *x*, *y*, and *z* molecular axes.

For the A_2 band

$$\Delta\epsilon(A_2) = (\alpha,\beta,\gamma)\begin{pmatrix} a & & \\ & a & \\ & & 0 \end{pmatrix}\begin{pmatrix} \alpha \\ \beta \\ \gamma \end{pmatrix}$$

$$= a(\alpha^2 + \beta^2) = a(1 - \gamma^2) \qquad (105)$$

For definitions of *a* and *b*, see eq 103 and following text.

For the E band

$$\Delta\epsilon(E) = (\alpha,\beta,\gamma)\begin{pmatrix} b & & \\ & b & \\ & & 2b \end{pmatrix}\begin{pmatrix} \alpha \\ \beta \\ \gamma \end{pmatrix}$$

$$= b(\alpha^2 + \beta^2 + 2\gamma^2) = b(1 + \gamma^2) \qquad (106)$$

In both cases only γ, the *z* direction cosine of the propagation vector, enters into the final equation.

We consider now a real example, the CD spectrum of $(+)_D$-$[Co(tn)_3]Br_3$ (tn = 1,2-propanediamine) in its monoclinic crystal with light propagating along the crystallographic *b* axis.[140] All the direction cosines are listed in ref 140. We note only that $\gamma^2 = 0.80$. Thus $\Delta\epsilon(A_2) = (1 - 0.80)a = 0.20a$ and $\Delta\epsilon(E) = (1 + 0.80)b = 1.80b$. We recall that for light parallel to molecular axis *z* the CD is 2*b*, therefore 90% of the maximum CD signal of the E band is developed along the *b* axis and 20% of the maximum A_2 signal.

Thus

$$\Delta\epsilon_{b\text{-axis}}(A_2 + E) = 0.20a + 1.80b \qquad (107)$$

as reported.[140] There are two unknowns in eq 107, so an independent measurement must be made, either on a polycrystalline sample or in another direction in the crystal. This may not always be practicable for monoclinic crystals. As discussed in section II.H, the *W* artifact in M_{02} (Table V) cannot be eliminated in crystals of low symmetry except in special directions or by studying optical effects as a function of crystal thickness.

Another important class of crystals has been discussed and analyzed by Saito and his co-workers.[84,85] These are uniaxial crystals in which the D_3 axes of the complex are not parallel to the unique axes of the crystals. This case was discussed in eq 89–91. It presents no new problems. Since the angle of the C_3 axis with the optic axis is known, there are still only two unknowns, *a* and *b*. The equations are a bit more complicated, but the method of solution is the same as outlined for the simple uniaxial case discussed above. See eq 90 and 91.

Measuring the polarized spectroscopy of oriented systems almost invariably provides more information than standard liquid- or gas-phase spectroscopy. This is especially true of systems with low symmetry but

applies as well to systems of high symmetry, as the examples in this section show. Chiral coordination complexes are often very weakly perturbed from a parent O_h symmetry. The result is a strong overlapping of bands that, to a first approximation, tend to have equal and opposite rotatory strengths. Because of this cancellation, one does not know even the order of magnitude of the rotatory strengths of the individual bands and therefore cannot determine the values of the spectroscopic matrix elements. This type of circular dichroism band is called a couplet, and it is known that the amplitudes of the peak and trough depend not only on the values of the rotatory strengths but also on the splitting between the positive and negative bands. The latter is unknown because it is usually too small to be detected by conventional spectroscopy if, as is frequently the case, the band separation is less than the bandwidth. It is also known for this case that the band splitting is not equal to the wavelength separation between the peak and trough of the solution CD curve. For small splittings this is a measure of bandwidth and not of the splitting itself. These problems are eliminated in principle by studies on crystals or other systems of known orientation. Separation of the spectra into two component spectra not only provides values for dipole and rotatory strengths but a measure of the band splitting, which is clearly an important component in understanding the interactions of complex ions. If circular dichroism studies are supplemented by crystal linear dichroism studies, it is possible to obtain values for the three components of both the electric and magnetic transition moments for the $A_1 \rightarrow A_2$ and $A_1 \rightarrow E$ transitions. This presupposes negligible quadrupole effects as outlined in section III.B.4. The theory of the circular dichroism of complex ions is not as yet in a complete state, and detailed experimental results on the rotatory and dipole strength tensors are especially useful. See ref 132 and 134 for an account of the theory, including complex ions of more general symmetry types than the D_3 systems discussed above.

D. Liquid Crystals

A very effective way of achieving the partial ordering of solute chromophores is by dissolving them in liquid crystals. Linear dichroism in a uniaxial optical system can be established by forming solutions in nematic liquid crystal phases,[79,79a,140a,b,141,142] a technique that has been called LCLD (liquid crystal linear dichroism). It is also possible to study the CD or ORD of molecules that have been ordered in this way, and we refer the reader to the papers of Kuball and Altschuh and their collaborators whose studies have been exceptionally thorough on both the experimental and theoretical side.[79,79a,140a,b,143]

In addition to these uniaxial systems, which are especially convenient for observing the linear and circularly dichroic tensors of oriented molecules, there is also the possibility of using cholesteric liquid crystals as solvents. Here the macroscopic helical structure of the cholesteric liquid phase can induce a circular dichroism in dissolved guest chromophores.[144-146] This has been called the LCICD effect (liquid-crystal-induced circular dichroism). Conversely, the dissolution of chiral molecules into an achiral nematic phase can induce a transformation to a cholesteric system. Theoretically

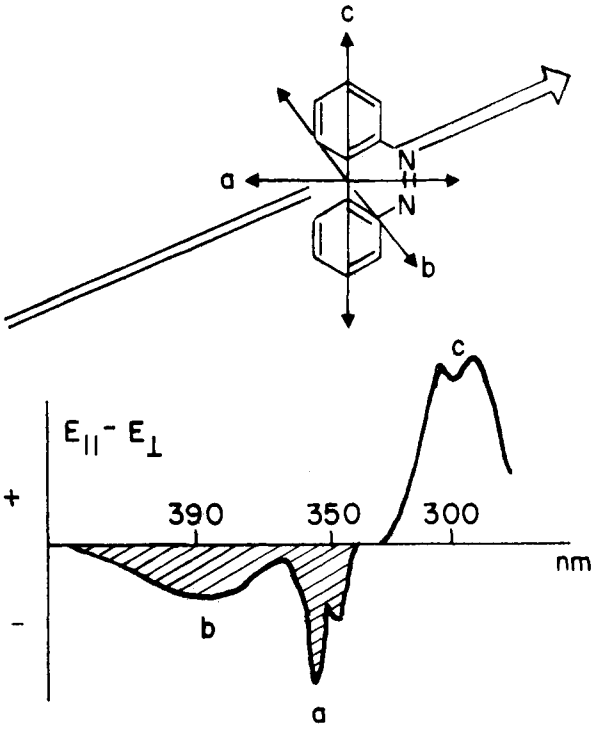

Figure 14. Linear dichroic spectrum of 9,10-diazaphenanthrene showing three axes of polarization in the molecule. Axis c is assumed parallel to the nematic axis. The *a* and *b* axes are in plane and out of plane, respectively. Figure adapted from Samori.[141]

one would predict two mechanisms for the generation of CD in a cholesteric phase with either or both being operative, depending on the geometry and interactions of the particular system. The first is via local chromophore–solvent interactions with the chiral phase. This is a well-known phenomenon with solutions of achiral molecules in chiral solvents that are ordinary liquids, but the effect should be accentuated with partially ordered solutes. The second is by the formation of long-range helical arrays of the dissolved chromophore in the helical matrix of the cholesteric phase. A recent analysis[55] has indicated that instrumental anisotropies may have contributed to some measurements of LCICD, and for this reason we shall confine our examples to a discussion of nematic systems with the emphasis on LCLD.

Figure 14 is a demonstration figure presented by Samori[141] to show the linear dichroism expected for 9,10-diazaphenanthrene, which has three mutually orthogonal transitions in the near ultraviolet when the long axis of the molecule is preferentially oriented along the axis of a nematic liquid crystal. The orientation function for a guest molecule in a nematic liquid crystal depends in a delicate way on the interactions of the molecule with its environment and on the perturbations of the nematic structure caused by the inclusion of the guest molecule. There is therefore no universal orientation function, and the orientation parameters must be obtained by methods like those described in the stretched-film section above (see especially the references to Thulstrup, Michl, Eggers, and Nordén). In cases where the directions of the transition moments are already known, the orientation parameters can be determined fairly readily. This is very important information for the theory of liquid crystals. The orientation parameters are correlated with the anisotropy of

molecular forces in the liquid crystal, and quantitative orientational distributions are required for testing models for the forces between nonspherical molecules (see ref 75, 147, and papers by Stone, Zannoni, Luckhurst, van der Meer, and Vertogen and by Cotter in ref 148).

As a second example we mention the study of the LD and ORD of the $n\pi^*$ transition of the C=C—C=O chromophore of testosterone propionate dissolved in the compensated nematic phase of mixtures of cholesteryl chloride and cholesteryl laurate.[79,140b] The data are accompanied by an extensive interpretation and theoretical discussion. This is an especially interesting spectroscopic problem because of the comparative richness of the vibronic structure of this absorption band. There is considerable theoretical interest in the resolution of the polarization and CD properties of vibronic progressions that arise from vibrations of different symmetry.

Finally an applied interest in the linear dichroism of liquid crystals has been generated in connection with the development of liquid crystal display devices, and an extensive technical literature has developed.[142,149-151]

V. Conclusions

Until about 20 years ago, electronic transitions were rarely studied by using polarized light. After the pioneering investigations of Cotton[152] and Kuhn,[153] circular dichroism studies were limited to a few scattered measurements. Linear dichroism studies on crystals and other oriented systems were limited mainly to the papers of Simpson and his students,[154] who had determined the directions of the transition moments of a few important chromophores. The field, however, remained in the hands of experts in spectroscopy.

This has all been changed in the past two decades. Circular dichrometers now exist in virtually every research department of chemistry, biochemistry, and biophysics, and linear dichroism measurements are now routinely made by biophysical, organic, polymer, and inorganic chemists as well as by electronic spectroscopists. This progress stems mainly from important instrumental advances with respect to the accuracy, sensitivity, and convenience of making measurements. The central feature of these instrumental advances is the use of polarization modulation, which (1) permitted the direct observation of LD, LB, CD, and CB and (2) replaced DC circuitry with lock-in amplifier technology, thereby vastly increasing the signal-to-noise ratio. It was evidently the seminal papers of Grosjean and Legrand[43a] that guided both research laboratories and instrument manufacturers into a wave of new developments.

Certain types of measurements are now standard and direct. CD and CB can be easily measured in isotropic samples. LD and LB are easily measured in achiral samples and usually in chiral samples as well if the linear polarization effects are very much stronger than the circular polarization effects, which is usually the case. Difficulties arise in attempting to measure circular anisotropies in the presence of strong linear anisotropies or whenever circular and linear anisotropies are of the same order of magnitude.

There are, in general, three stages in the development of polarization spectra. The first is the measurement itself. In terms of the methods discussed in this review, this means the evaluation of one or more of the transfer functions of the sample, i.e., one or more of the Mueller matrix elements. It is now generally realized that the important barrier to good measurements is the interaction of the optical anisotropies of the sample with those of the instrument to produce spurious effects. These artifacts were discussed in the section on experimental apparatus in section II.

The next stage is the unscrambling of the Mueller matrix element when this is necessary. Some procedures for handling this problem were discussed in the phenomenology section (section II). Measurements of the chiral properties of nonuniaxial samples is difficult, and sometimes impossible, except for certain directions of propagation of light. The linear birefringence should always be known when attempts are made to interpret the chiral properties of a sample that is linearly anisotropic. Studies in which the path length is varied can often detect and eliminate artifacts, etc.

The third stage is the interpretation of the basic optical properties (LD, LB, CD, CB). Usually this means making use of standard formulas relating these quantities with quantum mechanical matrix elements of the operators for the electric dipole, magnetic dipole, and electric quadrupole. These formulas can only be used if the lattice gas approximation is applicable. If there is extensive interaction between chromophores on different molecules, the lattice gas model can lead to results that are seriously in error. This aspect of the problem was discussed in section III.

Once these hurdles have been overcome, the results are usually applied in either of two ways. In the first the orientation and structure of the molecules under investigation are known. This permits the evaluation of the matrix elements for the electronic excitation and leads to information on the electronic structure of the excited state. Evaluation of a number of matrix elements leads to much more stringent tests of wave functions than a simple measurement of the position and intensity of the absorption band. In the second type of application it is assumed that the electronic state of the chromophore is understood, and the aim of the study is to determine either the orientation of the molecules or their structure. This type of study is especially important for biological systems and in the study of high polymers.

Many workers who decide to perform polarization studies are not aware of the pitfalls. With a good instrument and a favorable cleavage, it is just as easy to get a reading from a circular dichrometer on a triclinic crystal as on an ordinary solution, and the uninitiated may make the naive assumption that only circular dichroism is being measured. In many cases such a simplistic interpretation is perfectly applicable, but caution is absolutely essential when CD or CB is being measured in the presence of linear anisotropies. The purpose of this review has been to look at the general case, with all its difficulties, and to use this more general vantage point to establish what experimental situations are likely to cause problems and, to the extent that we are able, to suggest methods for overcoming them.

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Glossary

A_e	absorbance on base e scale
A′, A″	symmetry representations of group C_s
A_1, A_2, E	symmetry representations in group D_3
$\boldsymbol{A}^{M}$	molecular absorbance tensor
c	velocity of light
D_{om}	dipole strength of o → m transition
$\boldsymbol{D}_{om}$	dipole strength tensor
$\mathcal{D}^{N}_{MM'}$	elements of Wigner transformation matrices
E, **D**, **B**, **H**	Maxwell field vectors
e	charge on electron
f	frequency of modulation
f_1, f_2	input signals
g_1, g_2	output signals
$\boldsymbol{H}$	infinitesimal Mueller matrix
I_{dc}, I_f, I_{2f}	mean intensity, fundamental intensity, overtone intensity
$\boldsymbol{I}$	identity matrix
J_0, J_1, J_2	Bessel functions
$\boldsymbol{J}$	general Jones matrix
K	$3(2303)hc/8\pi^3Na = 0.918 \times 10^{-38}$
$\boldsymbol{K}(Z)$	molecular orientation tensor relative to laboratory Z axis
k	unit vector in direction of propagation
L, L', C	complex retardances
L, M	superscripts for laboratory and molecule reference frames
LB, LB′, CB	refractive retardances
LD, LD′, CD	absorptive retardances
M	modulator
M_{ij}	component of Mueller matrix
$\mathbf{M}_{mo}$	magnetic moment of o → m transition
m	mass of electron
m	Jones polarization vector
O_p	general operator
$\boldsymbol{O}$	transform matrix from molecule to lab. frame
P, A	polarizer, analyzer
P_{45}; P_0	polarizer set at 45°; at 0°
p	electronic momentum operator
R_{om}	rotatory strength
$\boldsymbol{R}_{om}$	rotatory strength tensor
$\boldsymbol{R}$	retardance of infinitesimal layer
r	electronic position operator
$\boldsymbol{S}$, $\boldsymbol{S}_{IJ}$	orientation matrix and order parameters
s_i, i = 0–3	component of Stokes vector
$\mathbf{s}_I$, $\mathbf{s}_F$	incoming and outgoing Stokes vectors
T	retardance scalar, a complex quantity
TB, TD	real and complex parts of T
T_{1g}, T_{2g}	symmetry representations in group O_h
T	generalized retardance vector
$\boldsymbol{T}$	arbitrary 2nd rank optical tensor
T_v	projection of $\boldsymbol{T}$ on vector **v**, $\mathbf{v}\cdot\boldsymbol{T}\cdot\mathbf{v}$
$\boldsymbol{U}$	matrix for the rotation of a spinor
U, V, W, X	useful optical parameters
$\boldsymbol{U}$	cross product operator
v	polarization vector
X, Y, Z	coordinate axes in laboratory frame
x, y, z	coordinate axes in a molecular frame
Z	pathlength of light in sample
α	modulator–polarizer orientation, or azimuth of principal axis of polarization
β	ellipticity of polarized light
δ_0	amplitude of time-dependent retardance
ϵ	molecular extinction coefficient
$\Delta\epsilon_{\mp}$	circular dichroism, $\epsilon_l - \epsilon_r$
$\Delta\epsilon_{x-y}$	$\epsilon_x - \epsilon_y$
θ	orientation of strain axis; principal axis for LB
λ_0	vacuum wavelength of light
μ_{om}	electric dipole moment of o → m transition
$\rho(\lambda)$	band shape function, absorbance
$\sigma(\lambda)$	band shape function circular dichroism
σ_0	two-dimensional unit matrix
σ_1, σ_2, σ_3	Pauli spin matrices
χ	principal axis for LD; complex phase, $\chi = \chi' - i\chi''$
χ'	relative phase
§	time-dependent retardance
†	conjugate transpose

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$$e^{\mathbf{A}}\mathrm{v} = (I + \mathbf{A} + (\mathbf{A}^2/2) + \ldots)(c_1\mu_1 + c_2\mu_2) = (1 + a_1 + (a_1^2/2) + \ldots)c_1\mu_1 + (1 + a_2 + (a_2^2/2) + \ldots)c_2\mu_2 = c_1e^{a_1}\mu_1 + c_2e^{a_2}\mu_2$$

The formula may be generalized to n dimensions.
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