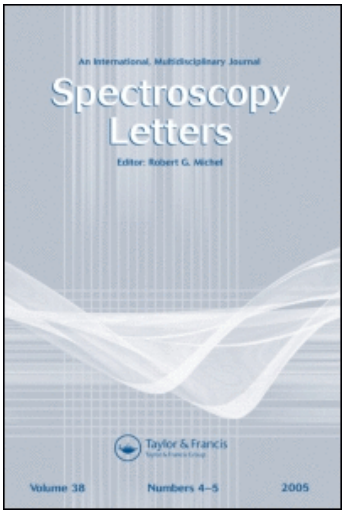


Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

general Aspects on Linear Dichroism Spectroscopy and its Application

Bengt Nordén[a]
[a] Inorganic Chemistry 1, University of Lund, Chemical Center, Lund, Sweden

To cite this Article Nordén, Bengt(1977) 'general Aspects on Linear Dichroism Spectroscopy and its Application', Spectroscopy Letters, 10: 6, 381 — 400
To link to this Article: DOI: 10.1080/00387017708065494
URL: http://dx.doi.org/10.1080/00387017708065494

SPECTROSCOPY LETTERS, 10(6), 381-400 (1977)

GENERAL ASPECTS ON LINEAR DICHROISM SPECTROSCOPY AND ITS APPLICATION

Bengt Nordén

Inorganic Chemistry 1, University of Lund, Chemical Center, P.O.B. 740, S-220 07 Lund, Sweden.

A possible applicatory scope of linear dichroism in solution chemistry is outlined together with some recent measuring and interpretation methods. The possibility to obtain structural information is stressed. The applications are illustrated by experimental results.

INTRODUCTION

By "Linear Dichroism Spectroscopy" I postulate the existence of a spectroscopic method which has a fairly general degree of applicability and for which there is a certain need also in the presence of other "spectroscopies". With "method" we mean that we have technique to prepare linearly dichroic samples and to accurately detect their linear dichroism (LD), and that we have theory to interpret these experiments properly. Concerning "need", we state that study of oriented molecules provides important information at a number of structural levels (generally twice the usual number of experimental parameters may be obtained from an oriented system) and LD applies well at comparatively low concentrations in liquid or solid solutions. It may be noted that besides from LD such information can also be derived from other spectra of the solute, such as polarized Raman and fluorescence.

We define $LD \equiv A_X - A_Y \equiv A_{||} - A_{\perp}$, $A_X = \log_{10}(I_{0||}/I_{||}) =$ the decadic (UV, visible or IR) absorption of plane polarized light, the electric vector oscillating along the laboratory-

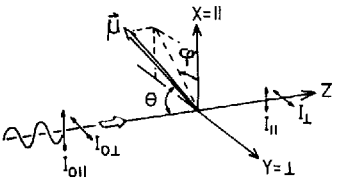
fixed X-axis, A_Y = ... In an isotropic sample (cubic crystal, random solution) LD ≡ 0 since the absorbing units (chromophores) are statistically randomly oriented and any force field is in average isotropic. However, weak LD may arise, if we apply an external disturbance (mechanical force, hydrodynamical gradient, electric field). With the sensitive methods of today very small effects may be detected. On the other hand, in a strongly anisotropic system (a crystal with complete alignment of anisotropic chromophoric units or a polaroid film) we might find that $A_X = 0$ but $A_Y >> 0$, in which case our sample behaves as a polarizer.

RELATIONS BETWEEN LD AND MOLECULAR PARAMETERS

In general it is sufficient to consider electric dipole excitation and if we ignore certain secondary effects (chromophore perturbation, local field effects and macroscopic artifacts such as absorption statistics), LD due to a given transition a → b is in a simple way related to the average orientation of the responsible transition moment $\vec{\mu}$: [1]

$$A_X - A_Y = C\, d\, \frac{4\pi^2 \lambda 6.02 \cdot 10^{23}}{2303\, hc} \cdot \frac{\rho_{ab}(\lambda)}{n} \left(\langle \vec{\mu}_X \rangle^2 - \langle \vec{\mu}_Y \rangle^2 \right) \qquad (1)$$

Here, λ = radiation wavelength, C = solute concentration in $mol \cdot lit^{-1}$, d = optical path in cm, n = refractive index, $\rho_{ab}(\lambda)$ = absorption band shape function, normalized so $\int \rho_{ab}(\lambda) d\lambda = 1$. Averages are over the distribution function appropriate to the method of orientation. Without any assumptions about molecular distribution we thus have for any system



$$\frac{LD_{ab}}{A_{random,ab}} = 3 \langle \sin^2 \theta_{ab} \rangle \langle \cos 2\phi_{ab} \rangle \qquad (2)$$

(θ_{ab} is the angle the transition moment makes with the direction of propagation of light, ϕ is the standard meridinal angle

relative the X axis). If the bands are not separated we have to sum contributions from various transitions a → b.

In a crystal, in an electric field, in a hydrodynamical field and in a stretched polymer matrix, we know the molecular orientation distribution with in this order decreasing degree of accuracy. Instead of basing the evaluation on ambiguous assumptions it is therefore often wise to be satisfied with the average orientation of $\vec{\mu}$ according to eq. (2). It can then be used in discussions about possible molecular orientation and about the intrinsic orientation of $\vec{\mu}$ within the molecular frame.

The first attempts to introduce an orientation factor in stretched sheet experiments, and to estimate an independent orientation of $\vec{\mu}$ in the molecule, were probably made by Fraser, Beer and Higgs. [2-4] They assumed the molecule was of rotation-ellipsoidal shape and that $\vec{\mu}$ was polarized at the angle α to the long axis. Uniaxial distribution within the matrix was also assumed. Then one easily shows [5]

$$\frac{A_{||}}{A_{\perp}} = \frac{f \cos^2\alpha + (1-f)/3}{\frac{1}{2}\sin^2\alpha + (1-f)3} \qquad (3)$$

f may be hypothetically imagined as the fraction of fully oriented material if the remainder were completely random.

It was perhaps not fully realized then, however, that in general no such explicite information about how the molecules are distributed is obtainable, neither from f nor from any other order parameter, unless we add further requirements (e.g. based on the type of interaction) in terms of a distribution function. We can thus strictly often only rule out certain distributions (random e.g. if $f \neq 0$).

Almost simultaneously Tanizaki et al. [7] developed a similar quantitative treatment. By assuming the orientation distribution be simply governed by the flow condition of the solvent (each solute molecule rigidly following "its own solid angle" in a sphere which was deformed to a spheroid) they obtained an analytical expression for the orientation factor f as a function of the degree of stretch performed on the

polymer solvent. The fact that the alignment is obtained equally well when the solute is introduced afterwards as when before stretching, seems to contradict such a mechanical orientation model. However, if the model satisfactorily describes the orientation of the polymer itself, it might apply also to the solute (after some scaling) since the orientation of the latter is obviously due to some interactions with the polymer.

The first thorough analysis of stretched sheet polarized spectra was reported by Eggers, Thulstrup and Michl. [6] They showed, without assumptions about molecule distribution, that for planar π-chromophore molecules (neglect of out-of-plane polarization) of symmetry C_{2v} or D_{2h}, component spectra proportional to those polarized along the in-plane symmetry axes x and y, are simply obtained as linear combinations of the recorded spectra $A_{||}(\lambda)$ and $A_{\perp}(\lambda)$:

$$A_x \propto A_{||} - d^{o}_{||} A_{\perp}$$

$$A_y \propto A_{\perp} - d^{o}_{\perp} A_{||} \qquad (4)$$

The coefficients $d^{o}_{||}$, $d^{o}_{\perp}$ were either obtained from regions of pure x and y polarizations or by a stepwise trial and error procedure. For many purposes the simple model of Fraser and Beer is sufficient and has been successfully employed by Yogev and coworkers. [8] However, its limitations may lead to erroneous conclusions in cases of two inequivalent competing molecular orientation axes. Thus as we shall see the rectangular naphthalene molecule does not behave like a rod but besides the tendency of the long-axis to orient itself parallel to the orienting field, there is a certain tendency to align the short in-plane axis. We have shown that the case of two equivalent orientation axis, i.e. a disc-like molecule, can be accurately described by a modified Fraser-Beer-Higgs equation. [9]

Saupe showed that the average values of the diagonal components of any second-rank tensor in the laboratory system XYZ, can be related to its components T_{ij} in the molecular framework, xyz: [10]

$$< T_{XX} > = (\Sigma_i T_{ii} + 2 \Sigma_{i,j} S_{ij}T_{ij})/3$$

$$< T_{yy} > = < T_{ZZ} > = (\Sigma_i T_{ii} - \Sigma_{i,j} S_{ij}T_{ij})/3$$

where $S_{ij} = < 3 \cos\theta_i \cos\theta_j - \delta_{ij} >/2$, i and j = x,y or z (5)

The X axis is the uniaxial orientation axis ("director"), θ_i the angle between the molecule axis $\underline{i}$ and the X axis. S_{ij} is the orientation tensor. For molecules of D_{2h}, C_{2v} or higher symmetries all cross terms $S_{ij}T_{ij}$, i ≠ j are dropped [11] and we get the following simple expressions of polarized absorption (to accentuate this step and the requirements involved we now call $S_{ii} = f_i$ i.e. the order parameter symbol of Fraser and Beer):[12]

$$A_X(\lambda) = \frac{1}{3} \Sigma_i A_i(\lambda) + \frac{2}{3} \Sigma f_i A_i(\lambda)$$

$$A_Y(\lambda) = \frac{1}{3} \Sigma_i A_i(\lambda) - \frac{1}{3} \Sigma f_i A_i(\lambda)$$

$$\Sigma f_i = 0, \quad i = x,y,z \qquad (6)$$

At complete orientation of the i-th molecule axis parallel to the X axis, $f_i = 1$; at complete perpendicular orientation, $f_i = -0.5$. Obviously we have two independent orientation parameters and as was recently pointed out by Thulstrup and Michl [13] this description is easily translated to their model and their orientation factors K_1 and K_2. With aromatic molecules one assumes the out-of-plane component of the diagonal absorption tensor (A_x, A_y, A_z) be zero. The f_i:s are then obtained either by fitting A_x, A_y (A_z=0) to the experimentally observed A_X and A_Y or by solving eqs. (6) at two wavelengths, one where A_x is known to be zero, one where $A_y = 0$. Both procedures rest on assumptions (or knowledge) about purity of polarization of certain bands. The application of eq. (6) for LD is described in references 12, 14 and 15.

MEASURING TECHNIQUES

If the LD(λ) spectrum is fine-structured or of weak intensity it is often more convenient to obtain it differentially than from separate recordings of $A_{||}(\lambda)$ and $A_{\perp}(\lambda)$. The observation of strong influence by linear dichroism in combination with linear birefringence, on the apparent circular dichroism (CD) detected by the Legrand-Grosjean method, led to the idea of the LD converted CD spectrometer.[14,17] FIG 1 illustrates the function of a CD spectrometer: Monochromatic linearly polarized light is in the Pochels cell, P, subjected to a sinusoidally varying birefringence, which when being λ/4 yields circularly polarized light impinging on the sample, S. If S exhibits CD the light intensity at the photomultiplier, PM, will contain an AC component of the same frequency as the vibration in P. This AC component is by lock-in electronics selected and amplified to the wanted CD.

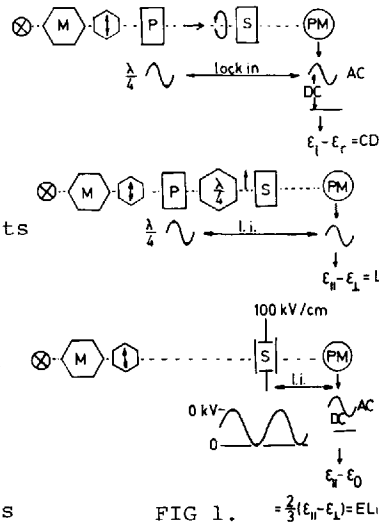


FIG 1. Converting CD spectrometer to LD and ELD.

If a static achromatic λ/4 device is inserted after the Pockels cell it is shown [14] that LD is instead obtained with high accuracy (certain corrections may be necessary depending on optics and LD magnitude).[14]

The sample may also be subjected to a static electric field to study the resulting electric linear dichroism, ELD. However, we found,[39] ELD be more sensitively detected by disconnecting the Pockels cell, converting its excitation voltage to an AC + DC high tension (oscillating between zero and a suitable positive value) and applying it on the sample. Provided the molecular rotational diffusion is fast in comparison with the period of the AC voltage, the selected AC component from the PM current will then yield the ELD.

SAMPLES OF APPLICATIONS

We shall not consider polarized studies on crystals or similar methods which have been employed for a long time to

obtain transitional assignments, but shall instead deal with various applications of LD on quasi-oriented liquid systems. We start by summarizing the available methods of producing the necessary molecular alignment (TABLE 1).

We illustrate the conclusional potential of the method by some experiments from our laboratory.

1. 1,2-dithiane

We want to assign the lowest transition (290 nm). Upon irradiating a random solution in a rigid hydrocarbon matrix with linearly polarized light (λ > 300 nm) a, by means of the above mentioned method, easily detectable LD arises even after a photodissociation of only a few % (dotted curve FIG 2). The

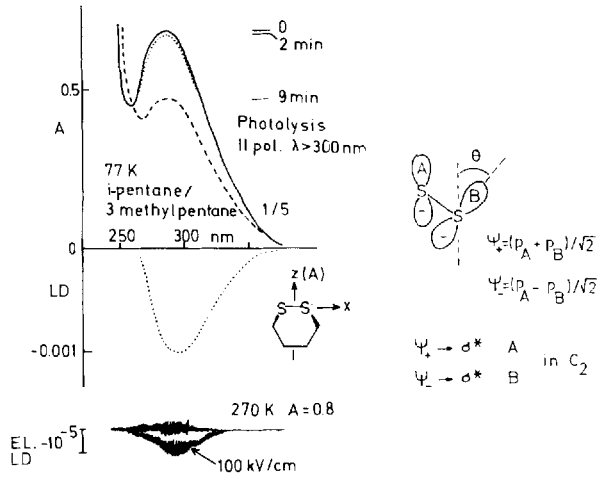


FIG 2.

LD maximum coincides with the absorption maxim showing that the band is not of composite polarization, hence it is most probably due to a single transition. This may, however, be of either A or B symmetry. The one with B symmetry (a promotion from the antibonding $p\pi$ orbital on the disulfide group to the antibonding σ orbital) is the more probable. A negative ELD of the liquid solution suggests that the transition is polarized perpendicular to the permanent dipolemoment (which by symmetry is along the z-axis) i.e. the transition has B symmetry.

TABLE 1

Method of alignement	Ref.	Advantages	Problems
Electric field (10^7 V m^{-1})	18	uniaxial orientation, any path-length possible	very small orientations, Stark-perturbations
Magnetic field (1 T)	19	uniaxial orientation, any path-length	requires large particles or crystalline domains
Liquid crystals			
a. nematic phases	20	a-b. easy to handle	a. requires electric, magnetic or shear field
b. lamellar phases, monolayers	21, 22	b. orients uniaxially between glassplates	b. requires evaluation under inclined incidence

Micellar solutions ($CTAB/H_2O$)	23	easy to align macroscopically by flow	poor microscopic orientation, Wiener LD
Stretched matrix (PE, PVC, PVA)	6,24	easy to handle	perhaps not uniaxial orientation, solubility problems due to short path-length
and stroked thin film	25-26		
Hydrodynamic gradient	27,28 34	can be switched off to yield base-line	requires macro-molecules
Photoorientation with linearly polarized light in rigid or viscous matrix a. photodissociation b. photoinduced rota-tion	29	transient trapped species may be studied	yields only relative polarizations

The photodissociation orientation method requires that no disturbing products are formed (with dithiane they are uncoloured and prevented to react secondarily by the rigid trapping). An interesting alternative method is photorotation i.e. rotational diffusion selected by energy dissipation of the polarized excitation. [29] Only molecules which have their transition moments perpendicular to the electric vector of the incident radiation will be saved from photorotation so their orientation defines the orientation direction of the photostationarily oriented solution. When the light is switched off the relaxation back to random orientation yields the molecular diffusion constant, D_1: $LD(t) = LD(t=0) \exp(-6D_1 t)$. We have in preliminary studies found that large water polymers in glycerol at 240 K may be photooriented by polarized UV scattering.

2. Naphthalene

LD spectra of naphthalene in stretched polyethylene (PE) and isotactic polypropylene (PP) films are shown in FIG 3. Resolution according to eq. (6) into independent molecular absorption components A_x, A_y led to essentially the same result in the PE and PP cases [12] so the differences in LD are not due to solvent (matrix) effects on the chromophore but to different orientational distributions (the order parameter f_y is negative in PE but positive in PP). Besides the components of the extinction tensor, LD thus may provide important information about the nature of interaction in the matrix. Let us propose two mechanisms of orientation in amorphous media:

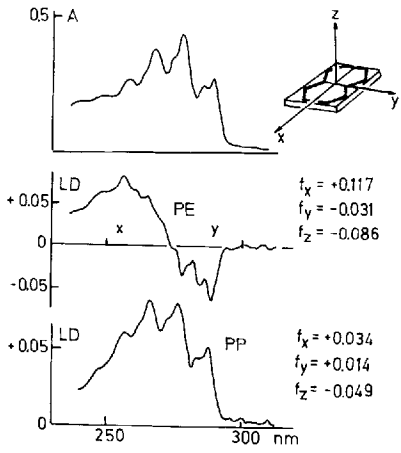


FIG 3.
Naphthalene in polyethylene and polypropylene

A. Dispersion effects. The molecule orients to optimize its overlap with the matrix molecule-chains. The axis of maximum polarizability aligns parallel to the polymer chains.

B. Sterical effects. The molecule orients to fit into the available space (vacuum) between the polymers. Spacefilling and repulsion. The molecular shape is the primary factor, long-axis is orientation axis.

In the present example we might understand the different orientations in PE and PP as the effect of a smaller contribution from mechanism B in PP.

3. Benzoic acid

LD spectrum of C_6H_5COOH in stretched polyethylene in FIG 4. The fact that the z-axis is 20 times more efficiently oriented than in nitrobenzene is an effect of the rod-shaped dimer (benzoic acid is probably to 100 % dimeric in PE). The negative, and hence x-polarized, fine-structured system at 280 nm is assigned to the ^1L_b transition of benzene and the positive (z-polarized) 230 nm band can be attributed to an intramolecular charge transfer from the benzene nucleus to the carboxyl group (in monomeric benzene claimed to be polarized at an angle of 6.5° to the 1-1' axis).[30] Also this case illustrates the need of more than one order parameter: Though the molecule appears

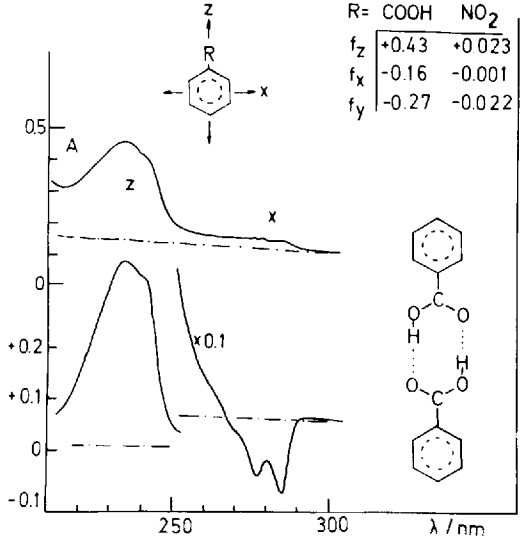


FIG 4

Benzoic acid in stretched polyethylene

be an extreme rod, f_x and f_y are far from equal (for the ideal rod $f_x = f_y$ and we have eq. 3). This difference is as expected more pronounced in the monomeric nitrobenzene. It may be noted that in stretched polyvinylalcohol no vibrational structure can be distinquished in the ^1L_b band due to interfering hydrogen bonding with the OH-groups of the matrix. The orientation is then also considerably smaller as expected when hydrogen bondings to the matrix compete with the dimerization. Such behaviour has been observed with other benzoic acids too. [31]

4. Poly-γ-benzyl-L-glutamate (PBG)

Robinson [32] was the first to report the liquid crystalline nature of polypeplides. Iizuka [33] proposed that above a critical concentration rod-like molecular dusters (10^5 polypeptides) are formed which may be easily oriented by electric, magnetic or shearing fields. We have been able to confirm the conclusions about strong orientation in the aggregated solutions but also to obtain the LD spectrum of a very dilute PBG solution, which (except for being several magnitudes smaller) has a very similar shape (dotted curve FIG 5.). From the positive LD we conclude an average orientation of the phenyl plane at an angle smaller than 54.7° (<θ> = 52° was

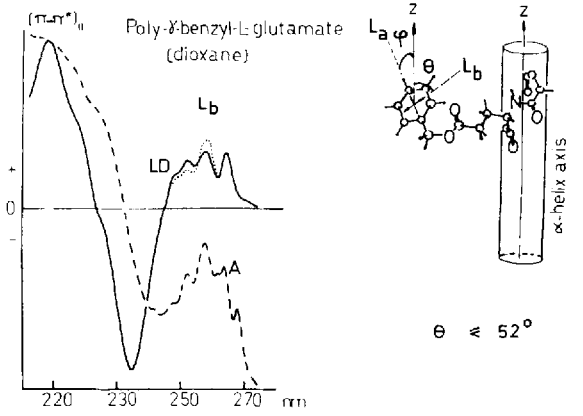


FIG 5.

estimated from LD/A = +0.038 at 260 nm at a fiber orientation f = 0.144 obtained from polarized IR; however, the purity of the L_b band is generally uncertain , so we may only conclude $\langle\theta\rangle \leq 52^{\circ}$). This "conformational analysis" is necessarily very qualitative since an apparent $\langle\theta\rangle$ may origin from rigid fixation at that angle as well as it may be the average of a wide distribution perhaps only little deviating from complete freedom. For example, let us assume $\phi = 90^{\circ}$ and that phenyl group rotates freely around the 1-1' bond. Then an LD equal to that of $\langle\theta\rangle = 45^{\circ}$ is expected. A fact which indicates that the phenyl group is practically free is that LD/A at a fixed orientation was independent of PBG concentration.

We now show how LD may be employed to detect weak non-random interactions in solution. In FIG 6. anthracene added to a flow-oriented very dilute PBG/$CHCl_3$ solution gives rise to negative LD at its short-axis polarized long-wavelength band. The LD curve crosses the zero-line indicating that the long-axis polarized components at shorter wavelength appear with positive LD. This indicates 1. that anthracene binds to PBG and 2. in such a way that its long-axis is in average parallel to the polypeplide fibre axis. In the corresponding experiment with liquid crystalline PBG, both long and short axis polarized bands appear with negative LD suggesting that here the anthracene plane prefers to be perpendicular to the fibre axis. The association is easy to understand in terms of van

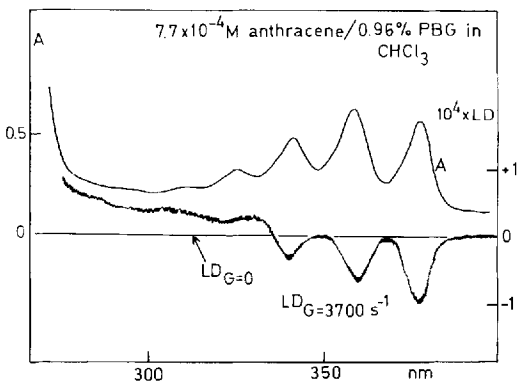


FIG 6.

der Waal interaction with the aromatic residues of PBG. The weak effect shows that the orientation and/or the association is very weak.

5. Complexes with DNA in aqueous solution

We have employed LD to study complex formation between DNA and various cationic ligands: ethidium and other dyes, inert metal complexes, but also neutral complexes (Cu en(acac)$_2$) and molecules (pyrene). [34] A Couette flow cell with radial light path was used. Theories considering refractive phenomena (birefringence) as functions of alignment, chain fluctuation and perturbation (elongation) in flowing liquids apply also for LD. If perturbations and fluctuations can be ignored, the Peterlin-Stuart distribution of rigid ellipsoids may be adopted. We can then relate the observed LD to the true direction of the responsible transition moment (by its angle, θ_o, to the major axis of the ellipsoid): [27]

$$\frac{LD_G}{A_{G=0}} = F\,\frac{3}{2}(3 < \cos^2\theta_o > -1) \qquad (7)$$

The orientation factor, F, is obtainable as a function of the reduced gradient G/D and the axial ratio a/b of the ellipsoid. Unfortunately, with flexible macromolecules, like DNA, the conformation will depend on the gradient so an external orientation factor of the transition moment can no longer be separated. It can however be shown that also with models assuming chains of stiff links the LD expression factorizes into an optical factor representing the intrinsic linear dichroism of a chain segment (base pair) and an orientation and perturbation factor. [28] The latter seems in practice impossible to evaluate analytically with sufficient accuracy at finite gradients, but we postulate it be obtainable empirically from a transition of known θ_o. We then have a formula coinciding with eq. (7) in which we can thus "calibrate" F.

We illustrate this application by the flow LD spectrum in FIG 7. referring to a complex between DNA and a triphenyl methane dye (Mehyl Green). Two band polarizations are known in the dye: z at 422 nm and x at 648 nm.

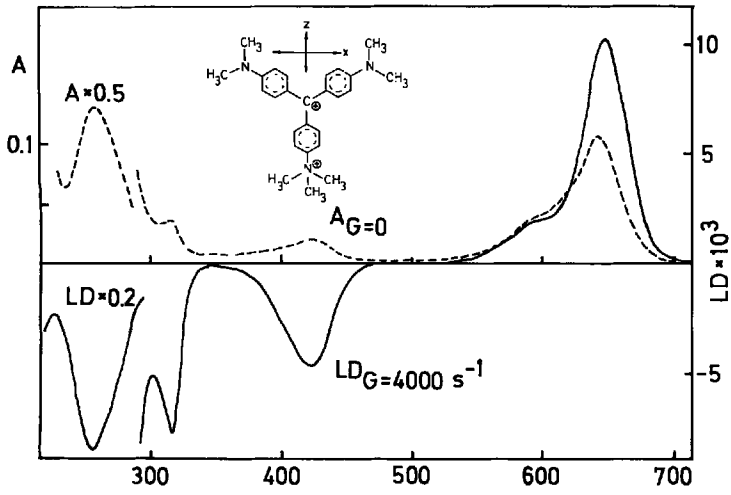


FIG 7.

To solve the problem of how these axes are oriented in the complex:

1. The stability constants are determined and LD/A of the specific complex is calculated (essentially the spectrum of FIG 7).
2. The dye is transparent at 257 nm so the LD/A = -0.16 at this wavelength exclusively represents the average base-pair orientation. Adopting the Watson-Crick model, i.e. $\theta_o = 90^o$ we get F = 0.107 from eq. (7).
3. From LD/A = -0.257 and +0.104 at 422 and 648 nm, respectively, we calculate from eq. (7), $\langle\theta_z\rangle \approx 90^o$ and $\langle\theta_x\rangle \approx 42^o$.

As will be described elsewhere this leads to a distinct picture of the structure of this and other triphenylmethane-DNA complexes.

OTHER APPLICATIONS

TABLE 2 summarizes what we consider a potential application field of LD spectroscopy. It has been natural to use mainly examples from our laboratory and studies by others may probably in several cases apply as well.

Several workers have been interested in the special case of a disc-like molecule, since we pointed out that LD may

TABLE 2

Applicatory field of linear dichroism spectroscopy

To study	To obtain	Ref.
	stretched films	
LD(λ) and A(λ) spectra + estimated order parameters	spectroscopic band assignments (excited states), degree of overlapping	6,12
LD(λ)/A(λ) variations	hidden bands	35,36
disc-like molecules, negative LD	out-of plane polarized transitions ($n \rightarrow \pi^*$)	15,37,38
molecular orientation	matrix-solute and solute-solute interactions solute conformation	15
migration of orientable matter from random solution into oriented matrix	linear diffusion	39,40
	flowing solution	
oriented polyelectrolyte + small ligand	complex equilibrium analysis, band assignment on ligand, structure of complex	34
macromolecules, varying gradient	conformational equilibria, structure of intrinsic chromophore, structural persistence, rotational diffusion	27
viscoelastic solutions, micellar solutions, varying gradient	microscopic (structural) explanations of rheologic phenomena	40,42
chloroplasts	internal distribution of chlorophyll, characterization	39
	electric fields (ELD method)	
oriented molecules (polar or polarizable)	spectroscopic assignments (excited states, polarizabilities)	39
random rigid system	perturbation of transition moment (model for effects in solvent fields)	39

(continued...)

(Table 2 continued)

To study	To obtain	Ref.
lamellar liquid crystals (inclined incidence LD method)		
molecule orientation	molecule location and interaction, membrane model systems.	39
porphyrin pigment orientations	model system of photosynthesis, excitation energy trapping	22,26 41
field perturbations of chromophoric probe ions	possible way of estimating reaction field strenghts outside polyelectrolytes and counterion locations	39,40

then sensitively report out-of-plane polarization. [9] According to eq. (6)

$$\frac{LD}{A} \approx \frac{6(f_x\varepsilon_x + f_y\varepsilon_y + f_z\varepsilon_z)}{2(\varepsilon_x+\varepsilon_y+\varepsilon_z) + f_x\varepsilon_x + f_y\varepsilon_y + f_z\varepsilon_z} \qquad (8)$$

For an ideal disc-like molecule two orientation axes, say x and y, are equivalent so $f_x = f_y = -f_z/2$. Sometimes (e.g. in a D_{4h} molecule) even the tranistions in the plane are degenerate, $\varepsilon_x = \varepsilon_y$. In absence of ε_z, LD/A is constant and equal to $6f_x/(2+f_x)$, so a dip in LD/A at a certain wavelength can be taken as evidence for a z-polarized transition at that position. However, the assumption $f_x = f_y$ is probably not often justified and it is recommendable to check the error limits carefully. For instance, if one can only tell that f_x and f_y must be positive, we need at least a negative LD peak to be sure of the z-polarization. This matter was recently discussed in the case of tetraphenyl porphin. [15]

The fact that LD contains much more structural information than e.g. viscosity suggests an interesting way to investigate the microscopic background of various rheologic phenomena. Thus we have observed that certain viscoelastic CTAB solutions become oriented above a certain critical flow gradient. Depending on the time of action of the gradient different

exponential relaxations may be thereafter observed at stopped-flow. In concentrated solutions of the dye pseudoisocyanine we observed a flow-induced aggregation below a certain gradient (average size of the aggregates was determined from LD relaxation at stopped-flow), while at higher gradients the larger aggregates were broken down. [42]

Finally some words about the inclined incidence LD method: Thin layer specimens e.g. a lamellar liquid crystal with uniaxial alignment perpendicular to layer plane, are not straightforwardly studied, since they appear isotropic at normal incidence. However, at inclined incidence (angle ω between sample plane and propagation direction) LD is observed, partly due to polarized reflection [$LD_r(\omega)$], and partly due to the non-random distribution of some absorbing transition moment $\vec{\mu}_{ab}$ (θ = angle between $\vec{\mu}_{ab}$ and normal to the sample plane), $LD_R(\omega)$ depends on the inclination angle and on the refractive indices at the various macroscopic boundaries (e.g., air-quartz, quartz-sample) [43] and may generally be extrapolated from LD spectrum of the transparent region. The average orientation of $\vec{\mu}$ may then be obtained from a simple expression: [21]

$$LD(\omega)/S = LD_R(\omega) + \frac{3}{2}(3\langle\cos^2\omega\rangle-1)\cos^2\omega[1-(\cos\omega/n)^2]^{-\frac{1}{2}} A_r \qquad (9)$$

(S is a known correction factor for light pencil conicity). Experiments with uniaxial samples of known struture have confirmed the accuracy of eq. (9), [21] which we hope to be able to apply in studies on cell membranes to reveal orientations of chromophoric groups or solubilized molecules.

ACKNOWLEDGEMENT

The studies referred to in this survey are financially supported by the Swedish Natural Science Research Council and are proceeding in cooperation with my co-workers, Åke Davidsson, Ivan Jonas, Lennart B.Å. Johansson, Göran Lindblom and Folke Tjerneld.

REFERENCES

1. J.A. Schellman, Chem. Rev., 75 (1975).
2. R.D.B. Fraser, J. Chem. Phys., 21, 1511 (1953).
3. M. Beer, Proc. Roy. Soc., A236, 136 (1956).
4. P.W. Higgs, Proc. Roy. Soc., A220, 472 (1953).
5. B. Nordén, Technical note on polarized spectroscopy, Lund 1968.
6. J. Eggers and E.W. Thulstrup, Chem. Phys. Lett., 1, 690 (1968) and E.W. Thulstrup, J. Michl and J. Eggers, J. Phys. Chem., 74, 3868 and 3878 (1970).
7. Y. Tanizaki, Bull. Chem. Soc. Jap., 32, 75 and 1362 (1959).
8. A. Yogev, L. Margulies, B. Strasberger and Y. Mazur, J. Phys. Chem., 78, 1400 (1974).
9. B. Nordén, Chem. Scr., 1, 145 (1971).
10. A. Saupe, Mol. Cryst., 1, 527 (1966).
11. G.P. Ceasar and H.P. Gray, J. Am. Chem. Soc., 91, 191 (1969) and G.P. Ceasar, Private communication.
12. Å. Davidsson and B. Nordén, Chem. Phys. Lett., 28, 221 (1974).
13. J. Michl, E.W. Thulstrup and J.H. Eggers, Ber. Bunsenges. Physik. Chem., 78, 575 (1974).
14. Å. Davidsson and B. Nordén, Chem. Scr., 4, 49 (1976).
15. B. Nordén and Å. Davidsson, Chem. Phys. Lett., 37, 433 (1976).
16. B. Nordén, Acta. Chem. Scand., 26, 1763 (1972).
17. B. Nordén and Å. Davidsson, Acta. Chem. Scand., 26, 842 (1972) and Tetrahedron Letters, 30, 3093 (1972).
18. T. Troxell and H.A. Scheraga, Macromolecules, 4, 519 (1971). and I. Tinoko, J. Am. Chem. Soc., 81, 1540 (1959).
19. N.E. Geacintov, F. Van Nostrand, J.F. Beker and J.B. Tinkel, Biochim. Biophys. Acta. 267, 65 (1972).
20. A. Saupe and W. Maier, Z. Naturforshg., 16a, 816 (1961) and E. Sackman and H. Möhwald, J. Chem. Phys., 58, 5407 (1973).
21. B. Nordén and G. Lindblom, Biophys. Chem., To be published.
22. A.G. Tweet, G.L. Gaines and W.D. Bellamy, J. Chem. Phys., 41, 1008 (1964).
23. B. Nordén, Å. Davidsson and L.B.Å. Johansson, Nature, 261, 400 (1976) and L.B.Å. Johansson, G. Lindblom and B. Nordén, Chem. Phys. Lett., 39 128 (1976).
24. B. Nordén, Chem. Scr., 7, 167 (1975).

25. K. Rosenheck and S. Somer, J. Chem. Phys., 46, 532 (1967) and J. Brahms, J. Pilet, H. Damany and V. Chandrasekharan, Proc. Natn. Acad. Sci. U.S.A., 60, 1130 (1968).
26. S. Surma and D. Frackowiak, Photosynthetica, 4, 202 (1970) and J. Breton, M. Michel-Villaz and G. Paillotin, Biochim. Biophys. Acta, 314, 42 (1973).
27. A. Wada, Biopolymers, 2, 361 (1964) and in Applied Spectroscopy Reviews, 6, 1 (Ed. E. Brame, M. Dekker, 1973).
28. H.J. Hofrichter and J.A. Schellman, Conformation of Biological Molecules and Polymers, 787 (The Jerusalem Symposia on Quantum Chemistry and Biochemistry, V, Jerusalem 1973) and W. Wilson and J.A. Schellman, Manuscript in preparation.
29. A. Albrecht, J. Chem. Phys., 27, 1413, A. Teitel, Naturwissenschaften, 44, 370 (1957) and B. Nordén, To be published.
30. H. Hosoya, J. Tanaka and S. Nagakuva, J. Mol. Spectr., 8, 257 (1962).
31. A. Yogev, L. Margulies and Y. Mazur, J. Am. Chem. Soc., 92, 6059 (1970).
32. C. Robinson, Trans. Faraday Soc., 52, 571 (1956).
33. E. Iizuka, Polymer J., 7, 650 (1975).
34. B. Nordén and F. Tjerneld, Biophys. Chem., 4, 191 (1976) and 5, 0000 (1977) and FEBS Lett., 67 368 (1976).
35. B. Nordén, Chem. Scr., 7, 226 (1975).
36. Å. Davidsson and B. Nordén, Chem. Phys. Lett., 28, 39 (1974).
37. B. Nordén, Chem. Phys. Lett., 23, 200 (1973).
38. A. Yogev, L. Margulies, B. Strasberger and Y. Mazur.
39. See other lecture of this Nobel Workshop.
40. B. Nordén, To be published.
41. J. Breton, E. Roux and J. Whitmarsh, Biochem. Biophys. Res. Comm., 64, 1274 (1975).
42. B. Nordén, J. Phys. Chem. In press.
43. Å. Davidsson and B. Nordén, Chem. Phys., 8 223 (1975).