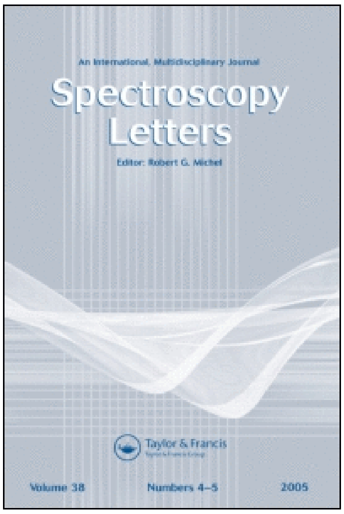

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Direct and Reverse Problems in Linear Dichroism Studies

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DIRECT AND REVERSE PROBLEMS IN LINEAR DICHROISM STUDIES

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ABSTRACT

A new approach is proposed for linear dichroism studies of particles (macromolecules) of unknown shape oriented in stretched or compressed polymer matrices. If properties of the matrix are known, and the measurements are taken at known deformations of the sample, then comparison of experimental results with various theoretical models for orientation can reveal the shape of studied particles. As an example, it is shown that linear dichroism measurements allow to distingiush between rod-shaped and disc-shaped particles.

INTRODUCTION

Linear dichroism (LD) measurements applied to pigment molecules oriented in stretched polymer matrices can yield information about the angles α between the transition dipoles and the molecular axes[1]. The case when the molecular shape and an adequate model for orientation are known will be referred here as a DIRECT problem in LD studies. The most principal drawback here is lack of a general theoretical model for orientation[2,3,4]. The picture appears even more obscure in the case of objects which structure is not known a priori such as pigment-protein complexes and reaction centers (RC) isolated from photosynthetic organisms[5]. The first quantitative analysis of LD spectra of oriented RC[6] included an oversimplified Fraser's model for orientation[7] and an assumption of perfect orientation of RC in dried gelatin films[6].

Here we propose a new way for LD studies of objects with unknown structure. Its main features are:

1) one should use a polymer matrix with known properties. For example, it should be amorphous, continuous, and uniform;

2) LD measurements should be taken at various known degrees of deformation of the polymer sample;

3) then, one can construct various theoretical models for orientation which refer to several particular cases of molecular shape of the studied objects;

4) comparison of theoretical calculations with experimental results can reveal which of the models is valid, and hence one can draw conclusions about molecular shape of the objects under study. Moreover,

the angles α can be calculated with the help of the valid model.

We shall refer the approach (1-4) as a REVERSE problem in LD studies. In this paper we outline its solution for a particular case of rod-shaped and disc-shaped particles.

THEORY

Let us consider orientation of rigid axial symmetric particles: with axes $a \gg b = c$ (rod-shaped) and with $a \ll b = c$ (disc-shaped). Let the particles be embedded into a three-dimensional network of amorphous polymer with characteristic mesh size $d \ll (a,b,c)$. This requirement can be most readily valid if oriented particles are macromolecules or macromolecular complexes. We assume that the polymer network does not bind chemically to the particles under study, and that 'molecular friction' between the particle surface and the polymer can be neglected. Then orientation of the particles would be completely determined by deformation of the polymer sample. Consider uniaxial deformation:

$$L_x = \frac{L_{xo}}{\sqrt{n}} ; \quad L_y = \frac{L_{yo}}{\sqrt{n}} ; \quad L_z = L_{zo} \cdot n \qquad (1)$$

L_{xo}, L_{yo}, L_{zo} and L_x, L_y, L_z being the dimensions of a rectangular sample before and after deformation, respectively; n being the parameter of deformation:

$n > 1$ for stretching and $n < 1$ for compression. Initially ($n=1$) the particles are oriented randomly. In accordance with earlier works[8,9] we assume that upon deformation rod-shaped particles would rotate so that the ratio of projections of the symmetry axis (the longest axis) of a given particle upon the axes of the laboratory framework would change exactly as the ratio of the corresponding sample dimensions (1). For disc-shaped particles, the same equation would hold for the ratio of projections of the 'molecular diameter' that lies in the same plane with the laboratory axis z and molecular symmetry axis a[10]. The direction of a given transition dipole $\vec{M}$ may be described with a single angle α between $\vec{M}$ and the symmetry axis a. LD is characterized by dichroic value

$$P = \frac{A_z - A_x}{A_z + A_x} \qquad (2)$$

A_x and A_z being optical densities measured with linearly polarized light passing the sample along its y axis, with the electrical vector parallel either to the x or to the z axis of the laboratory framework.

A usual averaging over Euler's angles[1] gives:

$$P_k(\alpha,u) = \frac{(3\cos^2\alpha - 1)(3F_k(u) - 1)}{3 - \cos^2\alpha + F_k(u)(3\cos^2\alpha - 1)} \qquad (3)$$

$$F_1(u) = \frac{u^3}{u^3-1} \cdot \left(1 - \frac{\tan^{-1}\sqrt{u^3-1}}{\sqrt{u^3-1}}\right) \qquad (4)$$

$$F_2(u)= \frac{1}{u^3-1} \cdot (-1+ \sqrt{\frac{u^3}{u^3-1}} \cdot \log(\sqrt{u^3} + \sqrt{u^3-1}) \qquad (5)$$

$$\text{where } u=n \quad \text{if} \quad n>1 \qquad (6)$$

$$u=n^{-1} \text{ if } \quad n<1 \qquad (7)$$

Recently we have shown[10] that for $n>1$ the solution F_1 describes orientation of rod-shaped particles (the result (3,4,6) coincides with that published by Tanizaki[9]), while F_2 refers to disc-shaped particles[10]. If $n<1$ (7) the solutions "interchange": F_1 is for discs, and F_2 is for rods. The reason for this "interchange" can be most easily understood in the limiting case $u \rightarrow \infty$ ($n \rightarrow \infty$ and $n \rightarrow 0$). In the "perfectly stretched" sample ($n \rightarrow \infty$) the symmetry axes of all rod-shaped particles are parallel to the z axis, while the symmetry axes of all disc-shaped particles are perpendicular to z and isotropically distributed in the (x,y) plane. In other words, the orientation of rods is more strictly limited in this case. The dichroic values vary from -1 to $+1$ for rods and from -1 to $+\frac{1}{3}$ for discs. In the "perfectly compressed" sample ($n \rightarrow 0$) the symmetry axes of all disc-shaped particles are parallel to the z axis, and all discs are in the (x,y) plane. All rods are also lying in the (x,y) plane, and so their symmetry axes are randomly distributed in this plane. Hence, this case provides a more strictly limited orientation for disc-shaped particles. The dichroic values vary from -1 to $+\frac{1}{3}$ for rods and from -1 to +1 for discs. At any finite u, the intrevals of P corresponding to discs and rods also do not overlap completely. For example if $n > 1$, then

$$-1 < P^{disc}_{min} < P^{rod}_{min} < 0 < P^{disc}_{max} < P^{rod}_{max} < +1 \tag{8}$$

If the dichroic value P falls into the interval

$$P^{disc}_{min} < P < P^{rod}_{min} \tag{9}$$

then the objects under study are obviously oriented as disc-shaped particles. If P falls into the interval

$$P^{disc}_{max} < P < P^{rod}_{max} \tag{10}$$

then the studied objects behave as rods.

If the measured P is near zero

$$P^{rod}_{min} < P < P^{disc}_{max} \tag{11}$$

then the two orientation models are indistinguishable. If $n < 1$, the inequalities similar to (8-10) can help to choose an adequate orientation model (FIG.1). Then the angles α can be calculated from equation (3).

Evidently, the equations (3-7) and all procedures discussed above hold only for non-overlapping absorption bands. In the opposite case, extrapolation of spectra to perfect orientation would be more fruitful. Let the spectra measured at a given u equal to:

$$A_x(\lambda,u) = A_y(\lambda,u) = \sum A_i(\lambda)\cdot M^2_{ix}(\alpha,u) \tag{12}$$

$$A_z(\lambda,u) = \sum A_i(\lambda)\cdot M^2_{iz}(\alpha,u) \tag{13}$$

where $2\cdot M^2_{ix} + M^2_{iz} = 1$ for any (α,u) (14)

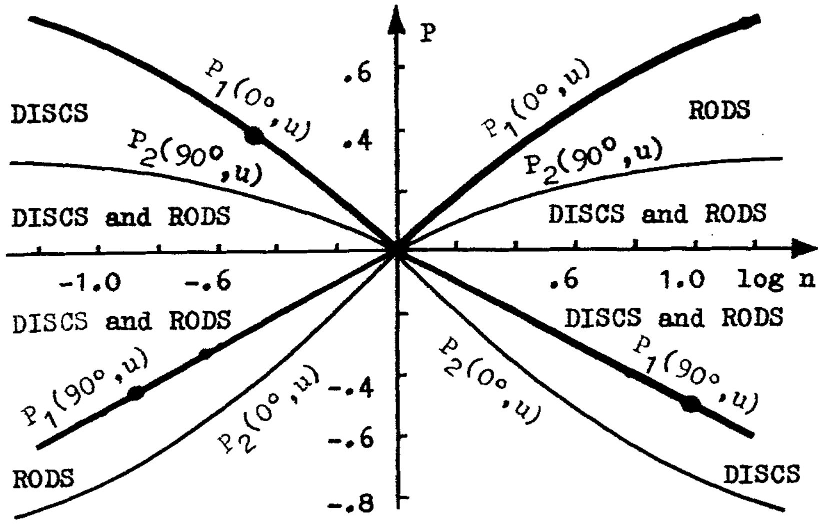

FIG. 1.

$$M^2_{iz}(\alpha,u)=\frac{1}{2}((1-\cos^2\alpha_i)-(1-3\cos^2\alpha_i)\cdot F_k(u)) \qquad (15)$$

and $A_i(\lambda)$ includes molar extinction, concentration, bandshape as a function of wavelength λ (sample thickness assumed to equal unit)[10,11]. If the function $F_k(u)$ is known, and its limit value $F_k(\infty)$ can be calculated, then absorption spectra $A_x(\lambda,\infty)$ and $A_z(\lambda,\infty)$ which correspond to perfect orientation are easily calculated by a linear transformation valid for any number of overlapping components. This approach was used by several authors[12,13,14] and in our earlier work[11] as a part of solution of a DIRECT problem in LD. We shall emphasize its applicability for the REVERSE problem solution.

For the orientation models discussed here, $F_1(\infty)=1$, $F_2(\infty)=0$, and one obtains for F_1:

$$A_z^{(1)}(\lambda,\infty)=\frac{2A_z(\lambda,u)-(1-F_1(u))\cdot S(\lambda,u)}{3F_1(u)-1} \tag{16}$$

$$A_x^{(1)}(\lambda,\infty)=\frac{F_1(u)\cdot S(\lambda,u)-A_z(\lambda,u)}{3F_1(u)-1} \tag{17}$$

For F_2:

$$A_z^{(2)}(\lambda,\infty)=\frac{A_z(\lambda,u)-F_2(u)\cdot S(\lambda,u)}{1-3F_2(u)} \tag{18}$$

$$A_x^{(2)}(\lambda,\infty)=\frac{(1-2F_2(u))\cdot S(\lambda,u)-A_z(\lambda,u)}{1-3F_2(u)} \tag{19}$$

where $S(\lambda,u)=2A_x(\lambda,u)+A_z(\lambda,u)$ (20)

Provided several pairs of absorption spectra $A_x(\lambda,u)$, $A_z(\lambda,u)$ measured at various known values of deformation parameter u, one can calculate pairs of extrapolated spectra using either (16-17) or (18-19). If the equations are chosen correctly, then all pairs of spectra $A_x(\lambda,\infty)$ and $A_z(\lambda,\infty)$ calculated from different values of u would coincide. If the equations correspond to an invalid orientation model, then the spectra calculated from different u would diverge. In the case of small LD values, this divergence can be also small, in accordance with ambiguity of the REVERSE problem for near-zero dichroic values (11).

DISCUSSION

The orientation model with $F_1(u)$ distribution function (3,4,6) is identical with Tanizaki's model[9] which appeared to be unsatisfactory in several applications (see e.g. discussion in[10]). We believe that theory-to-experiment contradictions in this case were caused by violation of the assumptions intrinsic for the model[9]. Namely, the polymer matrix should behave as a continuous and amorphous medium, and its stretching should be uniaxial. Recently we have published an example of solution of a REVERSE problem in LD studies of RC from photosynthetic bacteria *Rhodopseudomonas sphaeroides* R-26 oriented in polyacrylamide gel. The experiment carried out with precautions necessary to fulfil the assumptions revealed applicability of the model[9] and showed that RC were oriented as rod-shaped particles. The angles α_i were calculated for principal absorption bands with an accuracy of ±2-3°[10].

The REVERSE problem applied to asymmetric particles should involve asymmetric deformation of the polymer sample and LD measurements in two mutually perpendicular planes, e.g. (x,z) and (y,z)[15,16].

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