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Spectroscopic Investigation of the Anisotropy of a Liquid Crystal Matrix Doped with Photosynthetic Pigments: Effect of Pigment Concentration on the Degree of Macroscopic Order

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ABSTRACT

The macroscopic order parameters of the homogeneously aligned liquid crystal (MBBA) with dissolved photosynthetic pigments (chlorophyll a, *chlorophyll* b *and β-carotene) were determined using polarized absorption, emission and Raman scattering methods.*

Assuming the cylindrical symmetry of the pigment orientation around the liquid crystal director, the experimental and calculated values of the order parameter were correlated by using the distribution function of the nematics. It was shown that the order parameter of the matrix is dramatically destroyed by a high chlorophyll concentration (3×10^{-3} M) whereas over a wide range of concentration the carotene did not change the matrix order.

1 INTRODUCTION

Studies of the various types of model systems simulating the porphyrin pigment orientation in lamellar structures of the photosynthetic organism have been made by a number of investigators in order to explain the mechanism of energy transfer and charge separation in photosynthetic membranes.[1] Anisotropic media with chlorophyll simulating the pigment orientation in a lipid matrix of the photosynthetic membrane may provide some information either about the electronic transition moment responsible

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for the molecular absorption and emission anisotropy, or about the various structural factors governing the orientation.

In a previous report[2] we have shown [on the basis of preliminary results of chlorophyll *a* (chl *a*) spectroscopy] that pigment molecules can have influence on a high initial order of a liquid crystal matrix. In this study, the anisotropic properties of the liquid crystal (LC) model of photosynthetic membranes are discussed in detail. The order parameters, both the LC matrix and guest pigment molecules in the LC matrix as a function of different parameters, have been measured by means of two types of porphyrin pigments as well as carotene.

2 MATERIALS AND METHODS

Commercial liquid crystal (MBBA) from Riedel de Haen AG and β-carotene from Fluka were used without further purification. The chlorophyll pigments were extracted from leaves using the method proposed by Keiji and Nagao.[3] The purity of the pigments was found to be good from the absorption spectra. The liquid crystalline sample for the measurements was prepared in a specially constructed cell made of two glass plates covered with SiO evaporated at an angle of $\frac{2}{3}\pi$ to the normal to the plates. The thickness of the cell was controlled by the mylar spacer. The LC samples investigated behave as uniaxial crystals with the optical axis parallel to the glass plates.

Absorption spectra were measured with an M40 spectrophotometer (C. Zeiss) equipped with two polaroids. The polarized components of the

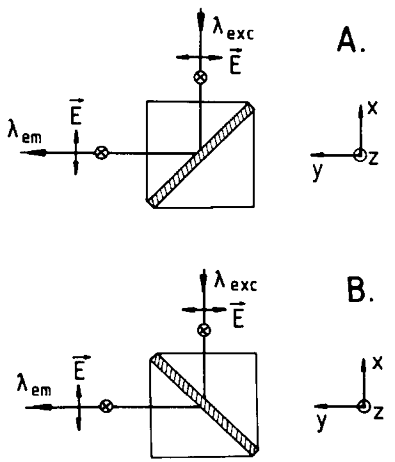

Fig. 1. Prismatic LC cell for measurement of the anisotropy of emission. Arrows indicate the direction of excited and emitted light. E vector indicates the polarization of the light. (A) emitted light collected from the surface; (B) emitted light collected through the sample.

fluorescence spectra were carried out by means of $\pi/2$ geometry (Fig. 1) using a home-made photon-counting fluorimeter.

The Raman measurements were performed using a back-scattering arrangement. The exciting radiation of the Ar^+ laser was attenuated as much as possible down to several milliwatts in order to reduce the local heating effect. The refractive index of the sample was determined by the total reflection method.

2.1 Macroscopic order of the guest dye molecules

Anisotropy of both the LC molecules and guest molecules is described simply in terms of a degree of order parameter (S) or more precisely by the distribution function [$f(\beta\theta\phi)$] which can be reproduced by a series expression involving the Legendre polynomials $P_1(\cos\beta)$.[4] The following are the first few terms of a polynomial expression:

$$\langle P_0\rangle = 1 \tag{1}$$

$$\langle P_2\rangle = \tfrac{1}{2}\langle(3\cos^2\beta - 1)\rangle \tag{2}$$

$$\langle P_4\rangle = \tfrac{1}{8}\langle 35\cos^4\beta - 30\cos^2\beta + 3\rangle \tag{3}$$

where β is an angle between the long molecular axis and the LC director.

The second term ($\langle P_2\rangle$) is used as a definition of the order parameter. In the case when the angle between the LC director (d) and the direction of the electronic absorption transition moment equals zero, the order parameter S_{exp} is given by:

$$S_{exp} = (A_{\parallel} - A_{\perp})/(A_{\parallel} + 2A_{\perp}) \tag{4}$$

where $A_{\parallel}$ and $A_{\perp}$ denote parallel and perpendicular components of absorption, referred to the electric vector of the light and the LC director.

If the angle between the absorption transition moment and the LC director (d) does not equal zero then eqn (5) holds:

$$S_{exp} = S(1 - \tfrac{3}{2}\sin^2\alpha) \tag{5}$$

in which the order parameter S is equal to $\langle P_2\rangle$.

In order to discuss the distribution function on the basis of the measurements of absorption and emission polarized components it is necessary to consider two types of coordinate systems i.e.

(1) The laboratory frame (XYZ) fixed with respect to the LC and reflecting the macroscopic symmetry of the system; the Z-axis is parallel to the average director (d).

(2) The molecular frame (abc) and ($a'b'c'$) fixed with respect to the molecule of the LC considering the molecular transition moments of the pigments.

Let us consider two transition moments P and Q (Fig. 2) which are defined in the molecular coordinate systems (abc) and ($a'b'c'$), respectively. The components of those vectors in each of the two molecular coordinate systems are related to their components in the laboratory system by the transition matrices T and T':[6]

$$P_{XYZ} = TP_{abc} \qquad Q_{XYZ} = T'Q_{a'b'c'} \tag{6}$$

where

$$T = \begin{vmatrix} \cos\phi\cos\theta - \cos\beta\sin\phi\sin\theta & -\cos\phi\sin\theta - \cos\beta\cos\phi\sin\theta & \sin\beta\sin\theta \\ \sin\phi\cos\theta - \cos\beta\cos\phi\sin\theta & -\sin\phi\sin\theta + \cos\beta\cos\phi\cos\theta & -\sin\beta\cos\theta \\ \sin\phi\sin\theta & \sin\beta\cos\theta & \cos\beta \end{vmatrix}$$

and T' is the same as T but with θ replaced by $(\theta + \delta)$.

Having the polarized components of absorption (A_z, A_Y) and emission ($F_{ZZ}, F_{ZY}, F_{XZ}, F_{XY}$), we can define the dichroic ratio D and the fluorescence polarization ratios R_Z, R_X by the equations:

$$D = \frac{A_Z}{A_Y} = \left[\int_0^{\pi}\int_0^{2\pi}\int_0^{2\pi} g(\beta\theta\phi)P_Z^2 \, d\phi \, d\theta \, d\beta\right] \times \left[\int_0^{\pi}\int_0^{2\pi}\int_0^{2\pi} g(\beta\theta\phi)P_Y^2 \, d\phi \, d\theta \, d\beta\right] \tag{7}$$

$$R = \frac{F_{ZZ}}{F_{ZY}} = \left[\int_0^{\pi}\int_0^{2\pi}\int_0^{2\pi} g(\beta\theta\phi)P_Z^2 Q_Z^2 \, d\phi \, d\theta \, d\beta\right] \times \left[\int_0^{\pi}\int_0^{2\pi}\int_0^{2\pi} g(\beta\theta\phi)P_Y^2 Q_Z^2 \, d\phi \, d\theta \, d\beta\right] \tag{8}$$

$$R = \frac{F_{XZ}}{F_{XY}} = \left[\int\int\int g(\beta\phi\theta)P_Z^2 Q_X^2 \, d\phi \, d\theta \, d\beta\right] \times \left[\int\int\int g(\beta\phi\theta)P_Y^2 Q_X^2 \, d\phi \, d\theta \, d\beta\right] \tag{9}$$

where A_Z, A_Y and F_{XZ}, F_{ZZ}, F_{XY}, F_{ZY}, are the polarized components of absorption and emission, respectively.

The three parameters in question can be calculated either on the basis of the experimental data (A_Z, A_Y, F_{XZ}, F_{XY}, F_{ZZ}, F_{ZY}) or in terms of the distribution function $g(\beta\phi\theta)$. The latter approach is adopted for the purpose of this present paper.

On the basis of Nishijima's method[7] the above dichroic ratios can be calculated. In the case under consideration, the distribution function $g(\beta)$

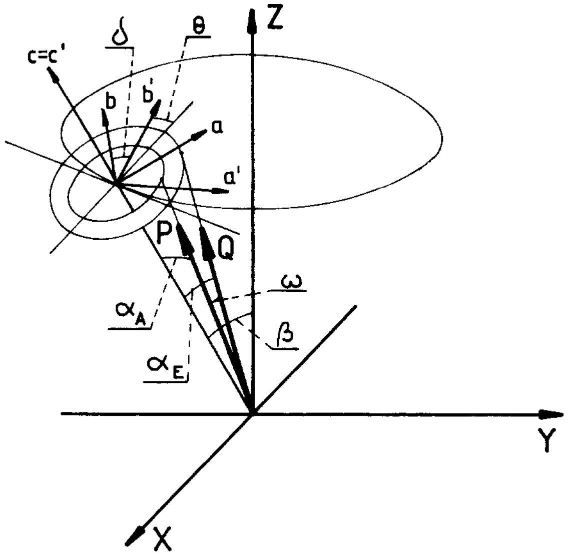

Fig. 2. Coordinate system describing the molecular orientation in an oriented matrix. *XYZ* is the laboratory coordinate system and *abc* and *a′b′c′* are the molecular coordinate systems incorporated in the absorption and emission transition moments, respectively.

depends only on the angle β (Fig. 2). Assuming that the dye molecule used has only one absorption and one emission transition moment we have obtained for the dichroic ratios:

$$D=\left[\int_0^{\pi} g(\beta)G_Z\,\mathrm{d}\beta\right]\left[\int_0^{\pi} g(\beta)G_Y\,\mathrm{d}\beta\right] \qquad (10)$$

$$R_Z=\left[\int_0^{\pi} g(\beta)G_{ZZ}\,\mathrm{d}\beta\right]\left[\int_0^{\pi} g(\beta)G_{ZY}\,\mathrm{d}\beta\right] \qquad (11)$$

$$R_X=\left[\int_0^{\pi} g(\beta)G_{XZ}\,\mathrm{d}\beta\right]\left[\int_0^{\pi} g(\beta)G_{XY}\,\mathrm{d}\beta\right] \qquad (12)$$

where G are the functions of the angle α_A, α_E between the transition moments and the director (d) and the angle δ between transition moments.

They were obtained by replacing P and Q in eqns (7)–(9) by their corresponding expressions from eqn (6) and integrating over θ and ϕ.[7]

We chose the distribution function proposed by De Vries *et al.*:[8]

$$g(\beta)=A\sin\beta\exp(b\cos^2\beta) \qquad (13)$$

where A is a normalization constant, $b=cS/TV$, S is the order parameter, T is temperature, V is molecular volume and c is a constant.

Substituting this function into eqns (10–12) the following forms of integrals appear:

$$I=\int_{0}^{\pi}\exp\left[b\cos^{2}\beta\right]\sin^{m}\beta\cos^{n}\beta\,\mathrm{d}\beta \tag{14}$$

where m, $n=0$–5.

The final results are as follows:

$$D=\frac{4\cos\alpha_{A}I_{2}+2\sin\alpha_{A}I_{3}}{2\cos\alpha_{A}I_{3}+\sin\alpha_{A}(I_{1}+I_{2})} \tag{15}$$

$$R=\frac{6A^{2}I_{4}+4(C^{2}+D^{2}+AD)I_{6}}{4C^{2}I_{5}+(3A^{2}+8B^{2}+16AB)I_{6}+4C^{2}I_{2}+4D^{2}I_{4}+AI_{3}} \tag{16}$$

$$R=\frac{4A^{2}I_{3}+16C^{2}I_{4}+16D^{2}(I_{2}+I_{5})+(12A^{2}+32B^{2}-64AD)I_{6}}{[12(C^{2}+D^{2})+16AD]I_{3}-8B^{2}I_{4}+A^{2}(2I_{2}+3I_{5}+3I_{1})+4(C^{2}+D^{2}+4AB)I_{6}} \tag{17}$$

where:

$$I_{1}=2[1+b/(3\times 1!)+b/(5\times 2!)+b/(7\times 3!)+\cdots]$$

$$I_{2}=\frac{\exp(b)}{b}-\frac{I_{1}}{2b}$$

$$I_{3}=I_{1}+I_{2}$$

$$I_{4}=I_{1}-2I_{2}+I_{5}$$

$$I_{5}=\frac{\exp(b)}{b}-\frac{3\exp(b)}{2b}-\frac{3I_{1}}{46}$$

$$I_{6}=I_{2}-I_{5}$$

$$A=\sin\alpha_{A}\sin\alpha_{E},\quad B=\cos\alpha_{A}\cos\alpha_{E},\quad C=\sin\alpha_{A}\cos\alpha_{E},\quad D=\cos\alpha_{A}\sin\alpha_{E}$$

Knowing the values of angle α_A we can obtain, by comparison of the calculated dichroic ratios with the experimental ones, the parameter b and the angle α_E. The degree of correspondence between the experimental and calculated data can be obtained from eqn (18).

$$\sigma=[D_{C}-D_{E})/(D_{E})]+[(R_{ZC}-R_{ZE})/(R_{ZE})]+[(\mathrm{R}_{XC}-R_{XE})/(R_{XE})] \tag{18}$$

where D_C, R_{ZC}, R_{XC} denote the calculated dichroic ratios and D_E, R_{ZE}, R_{XE} denote the experimental dichroic ratios.

2.2 Macroscopic order of the LC matrix

The macroscopic order of the LC matrix has been evaluated on the basis of the Raman scattering method. The light beam is incident along the X-axis.

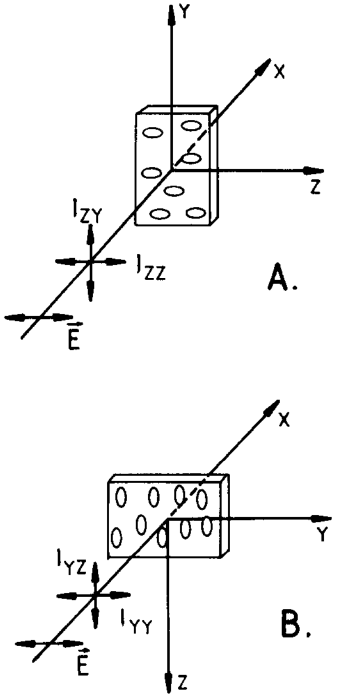

Fig. 3. Experimental geometries for measurements of the scattering anisotropies R_1 (A) and R_2 (B). Arrows indicate the polarization of the scattered light.

The YZ-plane is the plane of the sample with the order direction parallel to the Z-axis. The polarized Raman spectra were performed in the back-scattering geometry, where the electric vector **E** of the incident light was taken either as the Z-direction or the Y-direction after $\pi/2$ rotation of the sample in the YZ-plane I_{ZZ}, I_{ZY}, I_{YY}, I_{YZ} are the appropriate scattering intensities. On the basis of Raman line intensities a scattering anisotropy ratio can be calculated:[9]

$$R_1 = \frac{cI_{ZZ} - I_{ZY}}{cI_{ZZ} + 2I_{ZY}} \tag{19}$$

for the excitation with laser light polarized parallel to the Z-axis (Fig. 3), and

$$R_2 = \frac{I_{YY} - cI_{YZ}}{I_{YY} + 2cI_{YZ}} \tag{20}$$

for the excitation with light polarized parallel to the Y-axis (Fig. 3), where $c = [(n_g - n_e)/(n_g - n_o)]^2$, n_g is the refractive index of the glass plates and, respectively, n_0 and n_e are the ordinary and extraordinary refractive index of MBBA. As has been shown in a previous report,[9] knowing the values of the

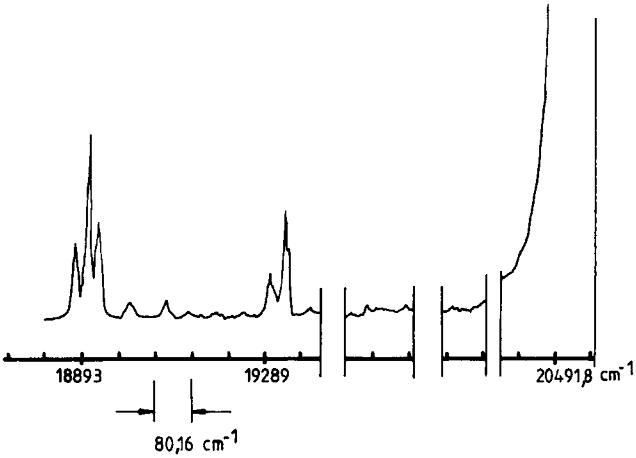

Fig. 4. The Raman spectrum of MBBA in the bulk sample.

scattering anisotropy ratios R_1 and R_2 we can estimate the $\langle P_2 \rangle$ and $\langle P_4 \rangle$ parameters:

$$\langle P_2 \rangle = \frac{2 + 7R_1 - 14R_2 + 5R_1R_2}{23 - 14R_1 + R_2 - 10R_1R_2} \tag{21}$$

$$\langle P_4 \rangle = \frac{-12 + 21R_1 + 21R_2 - 30R_1R_2}{23 - 14R_1 + R_2 - 10R_1R_2} \tag{22}$$

These parameters were calculated by means of the intensities of scattered light at 1597 cm^{-1} (Fig. 4).

3 RESULTS AND DISCUSSION

Figures 5A and 5B show the changes of $\langle P_2 \rangle$, obtained with Raman scattering for the LC matrix (MBBA), as a function of chl *a* concentration, *c*, and the thickness of the sample, *d*, respectively. These figures show that the values of $\langle P_2 \rangle$ are very sensitive to the concentration change of chl *a*. An especially large decrease of $\langle P_2 \rangle$ values is observed for the guest concentrations above 3×10^{-3}M. Similar results which describe the order parameter of guest molecules (measured by polarized absorption) are shown in Figs 6A and 6B.

If we assume that the results which are shown in Fig. 5 describe the macroscopic order of the LC matrix and curves 1, 2 in Fig. 6, indicate the order of pigment molecules in this matrix, one can see how large a difference in degree of orientation between chl *a* and MBBA matrix exists. This

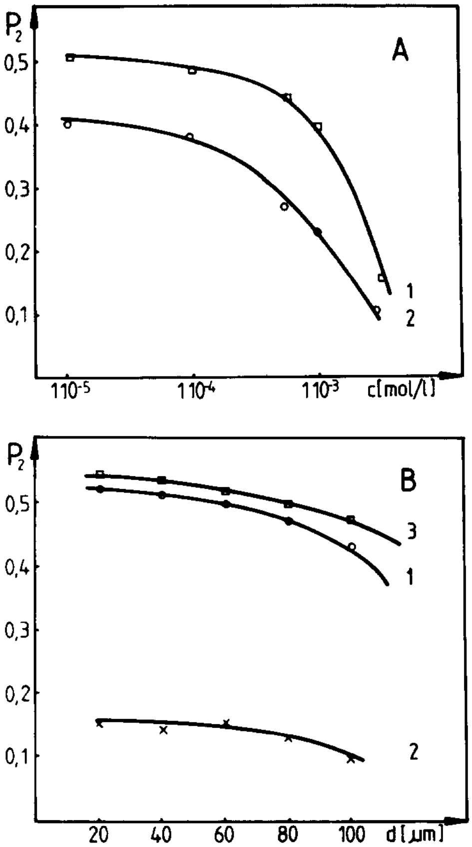

Fig. 5. Experimental values of the order parameters $\langle P_2 \rangle$ obtained from the Raman scattering of MBBA: (A) versus chl *a* concentration with (1) 40 μm and (2) 100 μm sample thickness; (B) versus the sample thickness for different chl *a* concentrations, (1) 3×10^{-3} M, (2) 5×10^{-5} M, (3) neat MBBA.

difference in the values of the degree of orientation can be explained in two ways:

(1) either a small percentage of chl *a* molecules are oriented giving such a low *S* value; or

(2) the appropriate molecular transition moment (in absorption) is ordered in some non-zero angle with respect to the LC director.

Taking into account the second possibility, and assuming that at a low concentration of chl *a* (5×10^{-5}M) the degree of pigment molecule order should be of the same order as the degree of the LC matrix of the given thickness of sample (Fig. 5, curve 3), an average value of angle can be calculated [eqn (5)]. Having this angle, the degree of order *S*, for the next concentration of chlorophyll, is estimated from eqn (5) (Fig. 6, curves 1, 2).

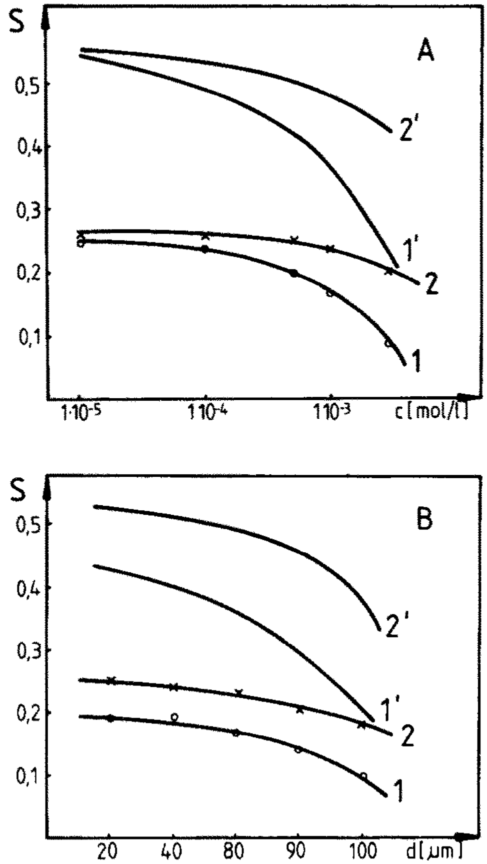

Fig. 6. Experimental (1, 2) and calculated (1′, 2′) values of the order parameters S obtained from the absorption measurement of chl *a* in MBBA: (A) versus chl *a* concentration with (1, 1′) 40 μm and (2, 2′) 100 μm cell thickness; (B) versus cell thickness for different chl *a* concentrations, (1, 1′) 3×10^{-3}M, (2, 2′) 1×10^{-4}M.

A low value of pigment order is confirmed by an anisotropy of emission (AE) defined by:

$$\mathrm{AE} = (F_{\parallel} - F_{\perp})/(F_{\parallel} + 2F_{\perp}) \qquad (23)$$

where $F_{\parallel}$ and $F_{\perp}$ denote the appropriate polarized components of emitted light (see Section 2).

The experimental values of AE are shown in Fig. 7 (Fig. 7A, curves 1–4; Fig. 7B, curves 1, 2). Lower values of AE measured through the sample (curve 2) confirm our previous suggestion[2] that the macroscopic order is higher for the surface layers of the sample than for a central volume. Using the experimental values of polarized fluorescence intensities, and the angle α_A from absorption measurements, we have estimated, using eqns (15), (16)

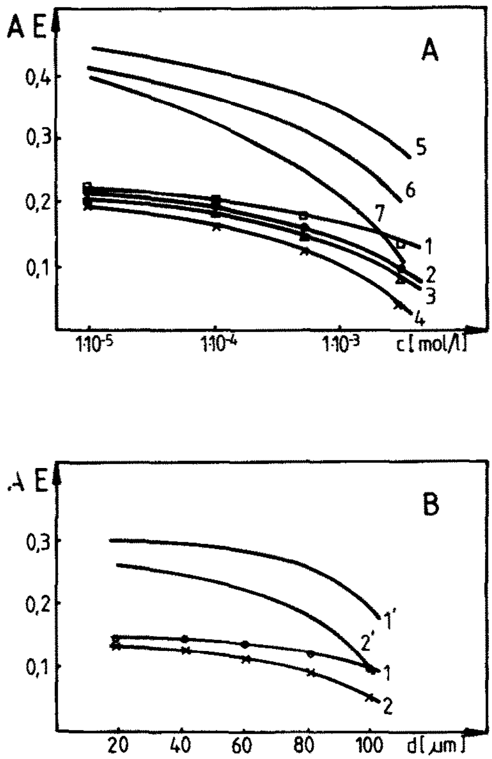

Fig. 7. Anisotropy of emission AE and $\langle P_2 \rangle$ of chl *a* in MBBA: (A) versus pigment concentration *c* for 100 μm cell thickness (1, 2) $\lambda = 436$ nm from the cell surface and through the cell, respectively; (3, 4) $\lambda = 633$ nm from the cell surface and through the cell, respectively; (5, 6, 7) $\langle P_2 \rangle$ calculated on the basis of the distribution function $g(\beta)$ to the curves 1, 2, 3, respectively; (B) versus cell thickness for $\lambda = 436$ *nm* and $c = 3 \times 10^{-3}$ M (1, 2) AE; (1', 2') $\langle P_2 \rangle$ from the cell surface and through the cell, respectively.

and (17), the angle α_E and constant *b*, and finally $\langle P_2 \rangle$ and $\langle P_4 \rangle$. In Fig. 7 one can see these parameters (Fig. 7A, curves 5–7; Fig. 7B, curves 1, 2).

Similar results have been obtained for chl *b* (see Table 1).

It is interesting to compare the behaviour of chlorophylls and carotene in MBBA. In Fig. 8, the values of *S* and $\langle P_2 \rangle$ (using the Raman method) are shown as a function of the dye concentration (Fig. 8A) and the thickness of the sample (Fig. 8B), respectively.

From these results, it follows that carotene does not make any changes in the initial order of neat LC matrix. If that MBBA matrix consists of chlorophyll and carotene, we observe that $\langle P_2 \rangle$ of such a sample (which was estimated for the LC matrix by the Raman method) increases when the carotene concentration increases. Meanwhile the *S* values (which were obtained using absorption spectroscopy of chlorophyll) remained unchanged (Fig. 9).

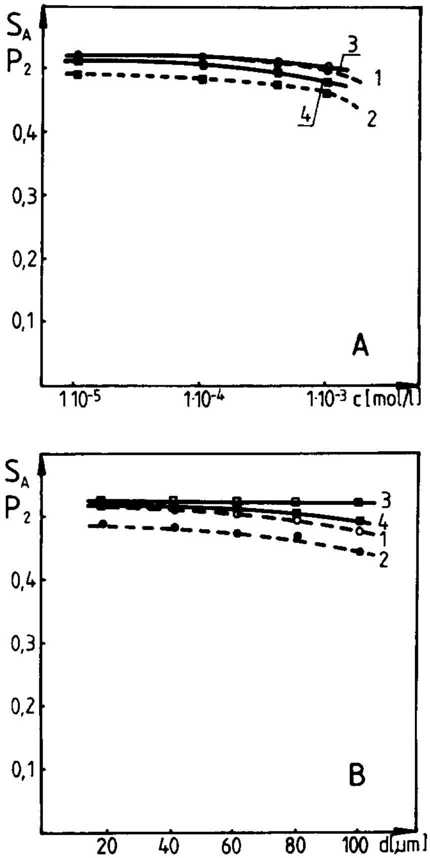

Fig. 8. Order parameter $\langle P_2 \rangle$ of MBBA obtained from the Raman spectra and S of β-carotene from absorption spectra: (A) versus carotene concentration, (1) $\langle P_2 \rangle$, cell thickness $d = 40\,\mu\text{m}$; (2) $\langle P_2 \rangle$, $d = 100\,\mu\text{m}$; (3) S, $d = 40\,\mu\text{m}$; (4) S, $d = 100\,\mu\text{m}$; (B) versus the cell thickness for different carotene concentrations, (1) $\langle P_2 \rangle$, $c = 1 \times 10^{-4}\,\text{M}$; (2) $\langle P_2 \rangle$, $c = 3 \times 10^{-3}\,\text{M}$; (3) S, $c = 1 \times 10^{-4}\,\text{M}$; (4) S, $c = 3 \times 10^{-3}\,\text{M}$.

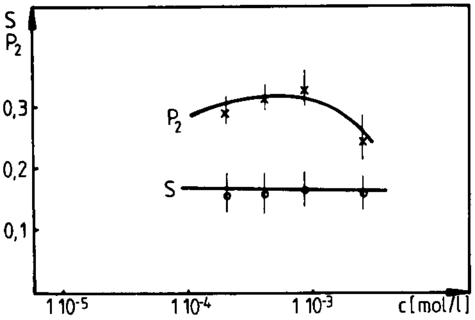

Fig. 9. Experimental values of the order parameters S (for chl a absorbance) and $\langle P_2 \rangle$ (from the Raman spectra of MBBA) versus β-carotene concentration; chl a concentration $c = 3 \times 10^{-3}\,\text{M}$ and cell thickness $d = 40\,\mu\text{m}$.

TABLE 1
Order Parameter of MBBA Doped with Chlorophylls Using the Raman Method and the Angle Between the LC Director and the Transition Moments of Absorption and Emission

Pigment	$\langle P_2 \rangle_{max}$	$\langle P_2 \rangle_{min}$	α_A (*deg.*)	α_E (*deg.*)
chl *a*	0·50	0·175	36	54
chl *b*	0·51	0·180	34	50

Such a result indicates that in the case of a pigment mixture (chl + carotene) the carotene improves the order of the LC matrix disordered by the chlorophyll molecules.

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