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EVIDENCE FOR SHEAR-INDUCED BIAXIALITY IN L_α LECITHINS CLOSE TO SOLID SURFACES

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Résumé. — L'étude optique d'échantillons de lécithine orientés en monodomaine par cisaillement et cycles thermiques montre l'existence d'une biaxialité en phase L_α . Le plan des 2 axes optiques principaux de cette structure biaxe est parallèle à la direction du cisaillement initial. Comme il est bien connu que les phases lamellaires L_α sont uniaxes en volume, nous attribuons cette biaxialité à un effet de surface. Cette hypothèse est justifiée par des mesures préliminaires de biaxialité et de biréfringence. La profondeur de pénétration est de l'ordre du micron.

Abstract. — Optical studies of shear-oriented water-lecithin multilayers were performed in the L_α phase of egg and di-palmitoyl lecithins. The samples appeared biaxial with the plane of the two principal optic axes parallel to the direction of shear. Since L_α phases are known to be uniaxial in the bulk, we attribute this biaxiality to a surface effect. This is consistent with preliminary quantitative measurements of the biaxiality and of the birefringence. The penetration length is estimated to be $\approx 1 \mu\text{m}$.

It is now well established that hydrated lecithins can form lamellar (smectic) phases in well defined ranges of temperature and water content. The different lamellar phases are generally characterized by the order and structure of the alkyl chains inside each layer [1].

Above their melting temperature, the hydrocarbon chains are fluid and disordered, being on the average perpendicular to the plane of the lamellae (phase L_α). Probably all lecithins have a lamellar L_α phase, in the presence of water and at sufficiently high temperature.

Below their melting temperature, the chains are rigid and can adopt a variety of conformations depending on temperature and water content. Several types of lamellar phases have been observed. In phases L_β and $L_{\beta'}$, the chains in each lamella are packed with rotational disorder into a two-dimensional hexagonal lattice. They can lie perpendicular (L_β) or be tilted ($L_{\beta'}$) with respect to the plane of the lamellae. In phase L_δ , the chains are probably coiled into helices and packed with rotational disorder in a two-dimensional square lattice.

The structure of the hydrophilic layer is less well-known. It is generally assumed that the polar heads (the phosphatidyl choline group of lecithin) constitute two dimensional disordered arrays with no periodic organization, in contact with water. However there is some experimental evidence [2-3] for head group orientation at low water content.

Most systematic determinations of the phase diagram for the water-lecithin systems have been made by X-rays on powder samples [1]. More recently the preparation of large monodomains of lecithin multilayers has allowed precise optical measurements [2, 3]. The L_α , L_β and $L_{\beta'}$ phases are uniaxial and are characterized by two refractive indices n_z and n_x , where the z -axis is normal to the bilayers ($x-y$) planes. A optically-biaxial phase (phase C) has also been observed at low temperature and water content. In that case, there is also a refractive index anisotropy $n_x \neq n_y$ in the plane of the bilayers.

In this letter, we describe a previously unreported effect on oriented lecithin multilayers in the L_α phase. It is shown that

1) shearing the samples between glass plates makes a macroscopic biaxiality appear ;

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2) the induced biaxiality is frozen in such samples, remaining observable for months after removal of the shear

3) this is a surface effect probably confined to thicknesses $\sim 1 \mu\text{m}$ near the glass plates.

1. Experimental results. — We studied two different lecithins : 1) natural lecithin (EL) extracted from egg yolk by the method of Singleton and thoroughly purified; 2) synthetic di-palmitoyl lecithin (DPL) purchased from Fluka chemicals. Both were found at least 99 % pure by thin layer chromatography (TLC). After the experiments (thermal treatment, storage : see below) a number of samples was checked by TLC for lecithin degradation. The TLC plates showed no remarkable difference from those obtained before the experiments.

The lecithins were hydrated with $\sim 8\%$ by weight of tridistilled water and allowed to reach equilibrium during 2-3 days at room temperature. The phase diagram of EL is relatively simple and well known [1]. At our water concentration a single L_α phase exists from room temperature to the highest temperature compatible with sample degradation ($\sim 120^\circ\text{C}$) [1]. Differential scanning calorimetry showed no thermal anomaly in the range $\sim 0-120^\circ\text{C}$. The phase diagram of DPL is more complicated [1, 2]. Observations under the microscope in polarized light showed a reproducible and reversible phase transition at $\sim 70^\circ\text{C}$. The high temperature phase was fluid and optically uniaxial. For $T \lesssim 70^\circ\text{C}$ our DPL samples became polycrystalline with a waxy appearance and were very viscous or much like a solid. Our observations are in fair agreement with the DPL phase diagrams reported in the literature [1, 3], that give a L_α phase for $T \geq 70^\circ\text{C}$ at a water content $\sim 8\%$.

Samples were prepared by introducing a small amount of the lipid-water mixture between two clamped glass slides separated by a mylar spacer (10-100 m). The sample was then sheared, at room temperature for EL and at $\sim 95^\circ\text{C}$ for DPL (i.e. well into the L_α phase). Shear rate was up to 10^3 s^{-1} for the thinner samples, but was not measured accurately. Several of the EL samples were hermetically sealed after the shear in order to avoid changes in the water content and to allow prolonged storage. The samples were observed by transmission polarizing microscopy. (Leitz Orthoplan Pol.)

After shearing, polydomain samples were obtained with homeotropically oriented areas, appearing black between crossed polars, and separated by bright defect lines. The homeotropic regions are aligned with the plane of the lamellae parallel to the glass slides. The defect lines are very similar to the oily streaks described by Lehmann and Friedel [4] and are, on the average, aligned in the shear direction. Conoscopic observations on these partially aligned L_α samples showed unambiguously a pattern which, although blurred, is

characteristic of *biaxial* materials, and not of *uniaxial* materials.

In order to improve the alignment, the samples were then annealed at 95°C until the defects almost completely disappeared. The annealing time increased with the sample thickness, and was typically in the range 5 minutes (for the thinnest samples) to a few hours (for the thickest ones). Let us note here, in view of the discussion, that the defects were seen to disappear without migrating. Large ($\sim \text{several mm}^2$) homeotropic regions were then formed in which no visible defects could be observed, even at the highest microscope magnification ($625\times$). Under these conditions, the conoscopic pattern was still biaxial. We observed two hyperbolic brushes (see Fig. 1) which, on rotation of the sample by 45° , degenerate into a cross. A further rotation of 45° caused the hyperbola to reappear in the two other quadrants. This behaviour is typical of biaxial crystals.

The observation of a centred conoscopic pattern is a clear indication that the acute bisectrix of the two

FIG. 1. — Biaxial conoscopic pattern of a sheared sample of egg lecithin with 8 % water. Room temperature, after annealing (see text). Thickness $26 \mu\text{m}$. Optics : objective UMK32 ; $1.25\times$ magnification lens ; $10\times$ ocular : a) extinction position ; b) 45° position. The angle between the optical axes is $2\beta = 18^\circ$. The retardation between ordinary and extraordinary ray, measured with a tilting compensator, is 3.8 nm .

optical axes is normal to the glass slides. From the observation of the orientation of the hyperbolic brushes, it is also possible to conclude that the plane defined by the 2 optical axes also contains the direction of shear. No bands of equal retardation (isochromates) were ever observed with our rather low numerical aperture (0.40) optical system and small sample thicknesses.

Quantitative measurements were made in the 45° position (corresponding to the maximum separation of the hyperbola arms).

1) The distance D between the foci of the hyperbola was measured with the ocular micrometer. D is related to the angle 2β between the optical axes by [5]

$$\sin \beta = \frac{1}{\bar{n}} \sin (\text{arc tg } kD)$$

where k is an instrument constant determined by the optics and $\bar{n}$ is the average refractive index of lecithin. $\bar{n} \simeq 1.47$ from ref. [6] on unoriented samples.

2) The optical phase retardation Δ between the ordinary and extraordinary rays propagating in the z -direction (direct transmitted beam) was measured with a Nikitin-Berek tilting compensator. Δ is related to the biaxiality $b = n_y - n_x$ and to the sample thickness L by [5]

$$\Delta = \bar{b}L$$

where $\bar{b}$ is taken for a homogeneous sample.

Table I gives a set of experimental results from hydrated EL samples with different thicknesses. Typical measuring accuracy was $\sim 20\%$ for Δ , less than 5% for L , and $\sim 8\%$ for D . However, the main source of scattering in the data was the difficulty of controlling the amount of shear imposed on the sample during the alignment procedure. It was indeed observed qualitatively that both D and Δ measurements varied with the shear rate. Even so, these preliminary measurements will be sufficient for our purposes here.

TABLE I

L : sample thickness. β_{exp} : angle between optical axes and normal to layers. Δ_{exp} : retardation between ordinary and extraordinary ray in the direction normal to layers. $\bar{b}$: average biaxiality calculated from equation (1). Egg lecithin samples between float glass slides. Room temperature, after annealing. All the samples were subjected to a moderate shear rate (see text).

L [μm]	β_{exp} [$^\circ$]	Δ_{exp} [nm]	$\bar{b} \times 10^4$
18	6.3	1.1	0.6
20	11.8	6.0	3.0
22	7.9	3.0	1.4
22	8.3	4.0	1.8
26	9.1	3.8	1.5
50	6.3	6.0	1.2
50	3.4	1.4	0.3

As we have already briefly mentioned, the orientation of the 2 optical axes is unambiguously related to the direction of shear. To ascertain this important point, 3 different shearing experiments were performed. First the same sample was successively sheared in two directions at right angles to one another: the plane of optical axes then rotated so as always to be parallel with the shear direction. Secondly, the two glass slides containing the hydrated lecithin were simultaneously rotated in opposite directions; the shear occurred then in concentric circles. In that case the optical axes plane was everywhere tangent to the circles and the biaxiality increased with the distance from the rotation axis. Last, a small amount of lecithin was suddenly squeezed between two glass slides, so as to produce a radical shear: the optical axes plane was radially oriented.

In order to gain information on the role of the glass substrate in the biaxiality, various surface treatments were successively tried: cleaning with ethanol, acetone, and/or hot sulfochromic acid, covering the glass with a semi-transparent conducting layer (by evaporation of gold, or sputtering of indium oxide), treating the glass with surfactants (hexadecyl trimethyl ammonium bromide CTAB [7], N-N-dimethyl-N-octadecyl 3 aminopropyltrimethoxy-silyl chloride DMOAP [8]) which are known to orient the hydrocarbon chains and make the surface more hydrophobic. Surfaces of various origins were also tested: float glass, ordinary borosilicate glass, fused silica and also plexiglass. All surfaces were checked to be optically isotropic. In all these cases, the biaxiality was observed to appear when the water-lecithin mixture was sheared between the glass slides. The conoscopic pattern was always qualitatively the same, with the notable exception that with plexiglass slides it was sometimes not centred, the tilt angle of the acute bisectrix being several degrees.

A few experiments were performed on EL with a higher water content (up to $\sim 30\%$). The effect of a shear was qualitatively the same.

Last, we have already mentioned that the biaxiality was not affected by prolonged annealing at high temperatures. In the EL samples that were hermetically sealed the biaxiality was also unchanged after storage for several months at room temperature. The only method which we have found to suppress the biaxiality was addition of NaCl salt to the water of hydration. High salt content (≥ 1 M) was observed to be effective in eliminating the biaxiality during the annealing while lower salt concentration ~ 0.1 M had little effect.

2. **Discussion.** — 1) The biaxiality is clearly induced by the shear. The plane formed by the two optical axes contains the shear direction. The biaxiality increases with the amount of shear.

2) It does not seem likely that this biaxiality could be due to some ordered array of defects too small to be observable under the microscope. The only defects of

small Burgers vector which are known to exist in lecithins are screw dislocations [9, 10]. It is quite difficult to imagine a realistic array of such defects which could possibly give a biaxial conoscopy pattern.

The existence of stress birefringence within the lecithin samples to explain the observed biaxiality can also be ruled out. Indeed all the visible defects present in the sample before the annealing are seen to disappear *in situ* in a finite time while the biaxiality stays unchanged. Flow in a direction perpendicular to the layers is certainly involved in removing some of these defects. Since this kind of flow is associated with the highest viscosity coefficient and therefore to the longest relaxation time [11] we may assume that there is no remaining mechanical stress associated with visible defects after the annealing is complete.

3) Surface rather than volume effects are involved in the problem. Indeed, our experimental determinations of β and b , if the biaxiality b is supposed uniform $b = \bar{b}$ throughout the sample, lead to a value for the uniaxiality u incompatible with literature data.

The relationship between u , $\bar{b}$ and β is written [5] :

$$\sin^2 \beta = \frac{\bar{b}}{u}. \quad (2)$$

Figure 2 gives a plot of $\sin^2 \beta_{\text{exp}}$ as a function of $\bar{b}$. A least squares fit of experimental data with eq. (2) yields an average value of the uniaxiality

$$u = 0.7 \times 10^{-2} \pm 15\%.$$

It is too small by a factor of five when compared with the literature value of a few 10^{-2} [3]. Moreover, should our determination of u be correct, it would make impossible the observation of well contrasted isogyres as in figure 1 even with 50 μm thick samples.

On the other hand, if we put the literature value for u in eq. (2) and calculate the corresponding β with our values of $\bar{b}$, β is found smaller by a factor of 2 than our experimental determination.

FIG. 2. — Values of $\sin^2 \beta_{\text{exp}}$ versus average biaxiality $\bar{b}$, as calculated from data of table I. Error bars correspond to measurement uncertainties (uncontrolled shear has not been included). The full line corresponds to a least squares fit of eq. (2) to our data. Its slope gives $\bar{u} = 0.7 \times 10^{-2}$. As discussed in the text, this value is not compatible with a volume effect for the biaxiality.

4) In the absence of a detailed model, it is only possible to give a crude estimation of the penetration length of this surface effect. Using our experimental determination of Δ and taking from the literature [3] a reasonable value of a few 10^{-3} for the biaxiality of the ordered biaxial phase of DPL, we find that the surface layer thickness is $\sim \Delta/b \sim 1 \mu\text{m}$. This is in agreement with recent observations of Proust [12] on thin films of thermotropic liquid crystals. It also supports recent evidence [12-14] and speculations [15] on the increased ordering of thermotropic liquid crystals at solid or air interfaces.

5) It is probable that while the order exists because of the shear, it is actually stabilized by some interaction with the solid surfaces. The observation that the order subsists on a large variety of surfaces (hydrophobic or hydrophilic, conducting) lets us suppose that this interaction is quite general, may belong-range van der Waals forces [16].

6) Last, the increased ordering should be attributed to the polar heads rather than to the alkyl chains, as already formulated by Powers and Clark [3]. It is generally stated that the alkyl chains of egg lecithin, because of their heterogeneity, do not tilt but always remain perpendicular to the smectic layers [17]. Moreover, we have observed that the addition of sufficient amounts of ionizable salts to the lecithin-water mixture can suppress the biaxiality. The role of the ions is probably to screen the electric charges on the polar heads.

3. **Conclusion.** — The shear induced biaxiality is due to a structural rearrangement on the molecular scale close to the solid surfaces.

We estimate that the biaxial order propagates from the solid interface into the lecithin multilayers system over rather long distances $\simeq 1 \mu\text{m}$. Our results on lyotropic liquid crystals can be related to a more general class of surface phenomena showing that a liquid at an interface is more ordered than in the bulk [18].

Besides their physical interest, these results may be of importance in biological areas such as cell fusion, cell membrane permeation, cell and tissue hibernation.

Further experiments studying more specifically the structure of very thin samples of hydrated lecithin are currently under way.

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