ANGLE RESOLVED FLUORESCENCE DEPOLARIZATION EXPERIMENTS ON SMECTIC MESOPHASES

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ABSTRACT
   Second and fourth rank order parameters ($<P_2>$ and $<P_4>$) have been
determined for some smectics from measurements of the angle-resolved
fluorescence depolarization profile of a probe embedded in the systems.
It is shown that the degree of order can be separated from the preferential
molecular orientation when both $<P_2>$ and $<P_4>$ are known. The degree of
order could be described by an extended mean field potential.

INTRODUCTION
   The study of the molecular physics of liquid crystals has been an active
field of research for many years. An important characteristic of these
systems is that the elongated constituent molecules are preferentially
aligned along a local axis. Much attention has been paid to experimental
studies of the orientational ordering and its description in terms of inter-
molecular interactions (ref. 1,2).
   We shall here be concerned with the biologically important smectic
mesophase (fig. 1) in which the molecules are arranged in stacked layers and
lie with their long axes preferentially aligned, mostly along the normal to
the plane of the layer. This molecular arrangement can be considered as the
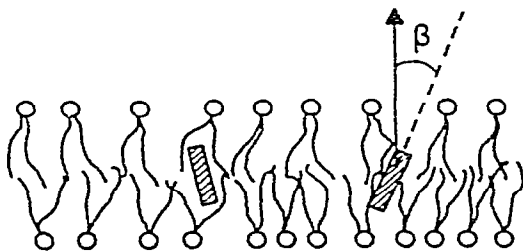basic structure of biological membranes.

Fig. 1   Schematic view of smectic mesophase.
         Rectangles denote probe molecules. β is the angle between the
         symmetry-axis of the smectic and that of the probe.

The thermodynamic properties of such an anisotropic system will be
determined to a large extent by the molecular orientational distribution
function (ref. 3,4). This distribution function can be characterized by a
set of order parameters $<P_L>$, which are the ensemble-averages of Legendre
polynomials or order L; L = 2,4 ... (ref. 3). The experimental determination
of $<P_L>$ provides a test of the pseudo-potential used in the description of
the intermolecular interactions. Up to now, however, only a limited test of
the theoretical description has been possible, as most experimental techniques
yielded values for $<P_2>$ only. In a number of recent papers from our
laboratory (ref. 5-7) we have shown that both $<P_2>$ and $<P_4>$ can be determined
from measurements of the angle-resolved steady-state fluorescence depolarization
(AFD) profile of a probe incorporated into a macroscopically ordered smectic
membrane system. We have found that for some membrane systems knowledge of
$<P_4>$ is indeed essential for an adequate description of the orientational
order. Here we present some preliminary results and consider the form of the
pseudo-potential needed. The results suggest that fourth-rank interactions
play an important role in these systems.

EXPERIMENTAL
    Macroscopically oriented multibilayers were prepared from dimyristoyl-
lecithin (DML) or dipalmitoyllecithin (DPL) + $\sim$20% water, as previously
described (ref. 5,7). As a fluorescent probe we have used 1,6 diphenyl
1,3,5 hexatriene (DPH) in 250-fold molar dilution. For our purposes this probe
can be viewed as a rod-like molecule, situated in the interior of the membrane
system (see fig. 1).
    AFD experiments were carried out on a home-built fluorimeter. The method is
described in detail elsewhere (ref. 7). In brief, in AFD experiments, the
polarization of the fluorescence is measured as a function of two angles which
describe, respectively, the direction of the incoming and outgoing beam
relative to the membrane symmetry axis. A fit of the experimental AFD profile
to the theoretical predictions yields $<P_2>$ and $<P_4>$; furthermore, the
rotational correlation time for molecular reorientation can be determined.
We shall not consider this last parameter here.

RESULTS AND DISCUSSION
    Values of $<P_2>$ and $<P_4>$ determined for the smectic phases of DML-water and
DPL-water mixtures are shown in Table 1. The hydrocarbon chains in these
systems undergo a crystalline $\rightarrow$ liquid crystalline phase transition at
$T_c = 24^{\circ}C$ for DML and $T_c = 42^{\circ}C$ for DPL. The experimental results can be
compared to theoretical predictions by expressing the order parameters as a
function of the reduced temperature T/T_c.

TABLE 1. Order parameters of DML and DPL

System	Temp(^{O}C)	$<P_2>^d$	$<P_4>^d$	B_0	$<P_2'>^c$	$<P_4'>^c$
DML[a]	9	.68	.23	20	.83	.48
	17	.78	.57	0	.78	.57
	23	.64	.44	0	.64	.44
	40	.42	.25	0	.42	.25
	54	.40	.21	0	.40	.21
DPL[b]	9	.65	.16	25	.89	.65
	23	.60	.12	25	.82	.49
	56	.46	.24	0	.46	.24

a transition temperature 24°C
b transition temperature 42°C
c calculated according to eq.(1)
d absolute error ~ .05
e tilt angle (cf. eq.(2))

This has been done in fig. 2. It can be seen that the experimental $<P_2>$ values, as quoted in Table 1, in no way fit to the theor- ical curve a, which represents the prediction of simple Maier-Saupe (MS) theory (ref. 1): the experimental $<P_2>$ are much too large, and, moreover, $<P_2>$ is not a smoothly decreasing
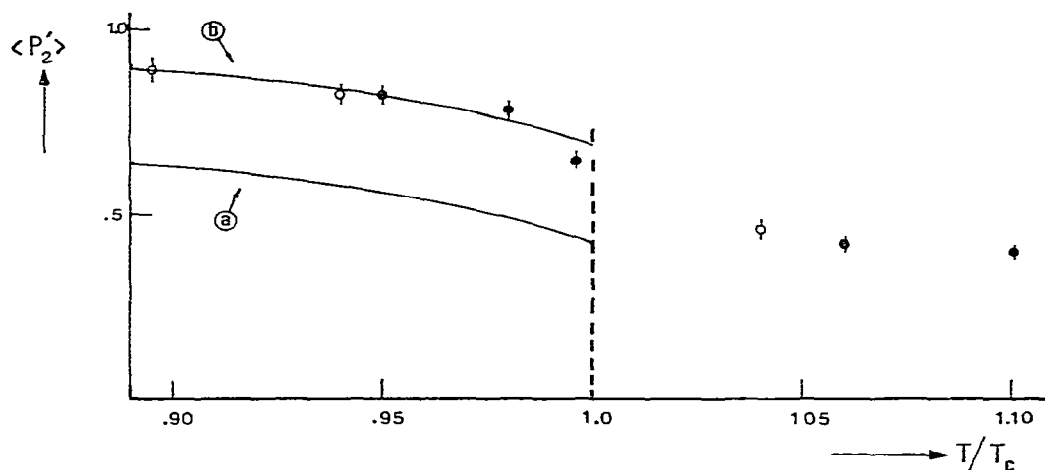

Fig. 2 P_2' as a function of the reduced temperature
         Φ DML; $\blacklozenge$ DPL
         a: MS theory; b: HJL theory (see text)

function of T/T_c. Furthermore, the relation between $<P_2>$ and $<P_4>$ cannot be described in terms of the MS orientational pseudo-potential.

The discrepancy can be partially resolved by noting that experimentally only macroscopically-averaged order parameters can be obtained (ref. 6). Thus the values of $<P_2>$ and $<P_4>$ describe not only the degree of molecular alignment about the local axis of orientation within each layer, but also the distribution of the local axes relative to the macroscopic axis of the sample. These two effects can be deconvolved if both $<P_2>$ *and* $<P_2>$ are known. On using the closure relation for Wigner rotation matrices (ref. 3), it can be shown that

$$<P_L>_{obs} = P_L(\cos\beta_0)<P_L'> \, , \qquad\qquad (L = 2,4) \qquad\qquad (1)$$

where $<P_L'>$ is the local order parameter within each layer and β_0 the tilt of the local axis of orientation relative to the normal to the plane of the layers. This deconvolution is realized by reconstructing the macroscopic orientational distribution function on the basis of an information-theoretic approach (ref. 6,7) and identifying β_0 with the angle for which the distribution function is a maximum. The tilt angles, shown in Table 1, are in excellent agreement with those determined from X-ray diffraction experiments (ref. 8). The deconvolved values of $<P_2'>$ (see Table I) have been plotted in fig. 2. It can be seen that $<P_2'>$ reasonably follows a curve (line 2b), which is shifted from the MS prediction (curve 2a). This finding points to the applicability of a more sophisticated version of MS-theory, ie. the Humphries-James-Luckhurst (HJL) theory (ref. 2). In contrast to MS theory, HJL theory assumes the intermolecular potential $U(\beta)$ responsible for the ordering behaviour to be explicitly dependent on the function P_4:

$$U(\beta) \sim \{<P_2>P_2(\cos\beta) + \lambda<P_4>P_4(\cos\beta)\} \qquad\qquad\qquad (2)$$

where λ is a measure of the relative importance of the fourth-rank term; if $\lambda = 0$, eq. (2) reduces to the MS potential (for a definition of β, see fig. 1). Curve 2b corresponds to an estimated $\lambda \cong .7$; apparently the fourth rank term appreciably contributes to the potential in eq.(2). Consequently, knowledge of $<P_4>$ is indeed important for an adequate description of $U(\beta)$.
For $<P_4'>$ the maximum deviation between experiment and HJL theory is found to be 20% and thus considerably larger than that for $<P_2'>$. However, in view of the fact that MS theory predicts $<P_4'>$ to vary from $\sim .3$ to $\sim .15$ over the considered temperature range, whereas curve b in fig. 2 is consistent with a $<P_4'>$ range of $\sim .6 - .4$, we believe that for $<P_4'>$ as well as HJL theory provides a better description as compared with MS theory.

One may wonder why we try here to apply HJL theory, and not a theory which

accounts for the specific interactions in lipid systems, such as published by Marcelja (ref. 4). The reason for this can be found in the fact that we are essentially studying the ordering behaviour of a nonpolar rigid probe, situated in the membrane interior. For such a probe, the intermolecular potential is not expected to be strongly dependent on lateral pressure effects and on lipid chain conformation dependent interactions, and we are mainly left with a Van der Waals type interaction which is just described by the potential of eq. (2). Some evidence for this view can also be obtained from the theoretical results obtained by Marcelja (ref. 4): below the phase transition the *average* order (which is approximately the order measured by DPH) is shown to be substantially less affected by lateral pressure effects than above the phase transition. This is in line with the experimental observation that contrary to nematics lipid membrane systems exhibit some orientational order above the phase transition (cf. data points above T_c in fig. 2). Thus in this temperature regime HJL theory cannot be applied.

CONCLUSION

Although no firm conclusions can be drawn from the limited data presented here, we can make the following preliminary observations:

(1) Knowledge of $<P_2>$ and $<P_4>$ is necessary to separate order and tilt effects.

(2) Below the phase transition the results for DPL and DML can be described in terms of a single pseudo-potential when expressed as a function of the reduced temperature. This indicates that as for homologous series of nematogens, the pseudo-potential is system independent. We note, however, that systems containing cholesterol or unsaturated lipids (ref. 7,9) do not fit into curve 2a,   pointing to the fact that for these systems the potential governing the ordering behaviour is different from that considered here.

Further experiments are now in progress with the aim of substantiating these observations.

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