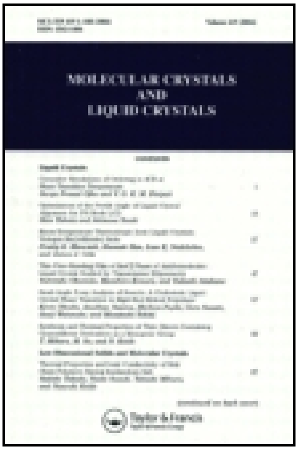

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DETECTION OF HEXAGONAL PHASES BY ESR SPIN LABELING

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Abstract The potential of ESR spin labeling in determining hexagonal amphiphilic phases is assessed. Spin labeled hexagonal phases in which the motional averaging about the cylinder axis is rapid on the ESR time scale can be readily identified by their ESR spectrum. The lineshape is distinctly different from that of smectic lamellar phases. The same ESR lineshape which is characteristic of the hexagonal phase is also obtained with spin labeled rodlike micelles provided that the motional averaging about their cylinder axis is rapid. If, however, this averaging is slow both hexagonal phases and rodlike micelles give ESR spectra which are indistinguishable from smectic phases (disclike micelles).

INTRODUCTION

Many amphiphilic molecules with one or two hydrocarbon chains either pure or mixed with other components form hexagonal phases when dispersed in water. Such phases are known to consist of a two dimensional array of infinite cylinders. In the case of the hexagonal I phase the interior of the cylinders is composed of hydrocarbon chains and the cylinder surface is covered with the polar groups and in contact with water, which also fills the space between the cylinders . The hexagonal II phase is the inverse phase with the cylinders being water channels lined by polar groups and the space between the water channels being occupied by hydrocarbon chains.[1,2]

The ESR spin labeling method has been used extensively in the past to investigate the structure and dynamics of smectic, lamellar phases and a great body of literature has accumulated. See for instance ref. 3. In contrast, there exist only a few examples of the application of this method to structural problems in hexagonal phases[4,5]. Here we apply ESR spin labeling to the question of whether or not the method is capable of differentiating between hexagonal and smectic amphiphilic phases. Related to this question is the problem of micelle shape. Experiment and theory have led to the conclusion that micelles are predominantly disclike or rodlike rather than spherical lipid aggregates. Here the question is whether or not ESR spin labeling can differentiate between these two fundamental micelle shapes.

THEORY

The principle underlying the ESR method is the motional averaging that leads to averaged g-factor $\underline{g}$ and hyperfine splitting $\underline{A}$ tensors. This motional averaging depends on the amphiphilic phase or more precisely on the shape of the lipid aggregate present in this phase. The line shape of the ESR spectrum is sensitive to motional processes provided the motion is comparable to or rapid on the ESR time scale. In principle,morphological information can therefore be deduced from a line shape analysis. By the same token ESR spin labeling should also be useful in the shape analysis of micelles[6]. As in all spin labeling studies the method is based on the assumption that the motion of the spin probe reflects truly the motion of the amphiphile in the micelle[3].

Motional processes contributing to the averaging of the $\underline{g}$ and $\underline{A}$ tensor must be rapid on the ESR time scale,i.e. their frequency ν must be greater than $10^8\ s^{-1}$. Molecular motions such as translational diffusion and rotation of the amphiphile about its molecular long axis are fast on the ESR time scale and hence contribute to the averaging. This is also true for various segmental motions having frequencies $\gg 10^8\ s^{-1}$. In contrast the tumbling rates of the amphiphilic aggregates as a whole are usually slow on the ESR time scale and therefore do not contribute to the averaging[7]. This is also true for micelles except for very small ones with a hydrodynamic radius $r \lesssim$ 2 nm which would have tumbling rates $\nu \gtrsim 10^8\ s^{-1}$.[7]

In order to describe the effect of motional averaging on the $\underline{g}$ and $\underline{A}$ tensors we use the coordinate system and rotation matrices defined in ref. 8. In the nitroxide coordinate system the $\underline{g}$ and $\underline{A}$ tensors are diagonal with principal values g_{xx}, g_{yy}, g_{zz} and A_{xx}, A_{yy}, A_{zz}, respectively. It is convenient to express the two tensors in sample coordinates X_1, Y_1, Z_1 where Z_1 is the bilayer normal in smectic phases or disclike micelles whereas it is the cylinder axis in hexagonal phases or rodlike micelles. For the overall transformation of $\underline{A}$ from nitroxide to $\underline{A}'$ in the sample coordinate system:

$$\underline{A}' = L_1^T \, L_2^T \, \underline{A} \, L_2 \, L_1 \tag{1}$$

where L_2 is the transformation matrix relating nitroxide axes to molecular axes, and L_1 is the transformation matrix relating molecular axes to sample axes, L^T is the transpose of the transformation matrix. Since both nitroxide and molecular axes rotate rapidly in the sample coordinate system the transformation includes the motional averaging, hence

$$\langle \underline{A}' \rangle = \langle L_1^T \, L_2^T \, \underline{A} \, L_2 \, L_1 \rangle \tag{2}$$

Due to the geometry of the 5-doxylstearic acid spin label used here equation (2) simplifies to

$$\langle \underline{A}' \rangle = \langle L_1^T \, \underline{A} \, L_1 \rangle \tag{3}$$

with $L_2 = \underline{1}$. For smectic phases (disclike micelles) where the rapid and anisotropic rotation of the amphiphile about the molecular long axis is the only contribution to the averaging, the motionally averaged $\underline{A}$ tensor derived from eq.(2) is diagonal, and axially symmetric with eigenvalues $\langle A'_{\perp}\rangle^S = 1/2(A_{xx} + A_{yy})$ and $\langle A'_{\parallel}\rangle^S = A_{zz}$. The superscript stands for smectic and the order parameter $S_3=1$. In hexagonal phases (rodlike micelles) with cylindrical symmetry the motion of the amphiphile is also anisotropic; in addition to fast rotation about the molecular long axis there is translational diffusion along the cylinder surface and rotation of aggregates. Provided this process is sufficiently rapid it then corresponds to a rotation of the amphiphile about the cylinder axis leading to further averaging of the $\underline{g}$ and $\underline{A}$ tensors. This yields the motional averaged tensor $\langle \underline{A}'\rangle^H$ (H=hexagonal) which is again diagonal, and axially symmetric with eigenvalues $\langle A'_{\perp}\rangle^H = 1/2(A_{zz} + A'^S_{\perp})$ and $\langle A'_{\parallel}\rangle^H = \langle A'_{\perp}\rangle^S = 1/2(A_{xx}+A_{yy})$ for $S_3=1$.

For the order parameter $S_3<1$ the observed values of the hyperfine splitting tensor are

$$\langle A'_{\parallel}\rangle = (1/3)\mathrm{Tr}\underline{A} + (2/3)S_3(A_{zz} - 1/2(A_{xx}+A_{yy})) \quad (4)$$

$$\langle A'_{\perp}\rangle = (1/3)\mathrm{Tr}\underline{A} - (1/3)S_3(A_{zz} - 1/2(A_{xx}+A_{yy})) \quad (5)$$

where $\mathrm{Tr}\underline{A}$ is the trace of the tensor $\underline{A}$. Both $\langle \underline{A}'\rangle^S$ and $\langle \underline{A}'\rangle^H$ are obtained from these equations if S_3 and S^H (the observed order parameter) are inserted, respectively, where[9]

$$S_3 = -2\,S^H . \quad (6)$$

Replacing A in eqs. 1-5 by g gives the corresponding relations for the $\underline{g}$ tensor.

Spectra were computed for both smectic and hexagonal phases (disclike and rodlike micelles) using the Hamiltonian

$$\hat{\mathcal{H}} = g_{eff}\,\beta\,\vec{H}\,\hat{S} + A_{eff}\,\hat{S}\,\hat{I} \qquad (7)$$

where the first term is the electron Zeeman and the second one the hyperfine interaction energy. $\hat{S}$ and $\hat{I}$ are spin operators; g_{eff} and A_{eff} are the effective g-factor and hyperfine splitting, respectively and as the Hamiltonian (eq.7) they are referred to laboratory coordinates X,Y,Z:

$$<\underline{A}_{X,Y,Z}> = L_0^T <\underline{A}'> L_0 \qquad (8)$$

where L_0 is the rotation matrix relating sample coordinates X_1, Y_1, Z_1 to laboratory coordinates X,Y,Z. Effective values of g-factor and hyperfine splitting are

$$g_{eff} = (<g'_{\parallel}>^2 \cos^2\theta + <g'_{\perp}>^2 \sin^2\theta\,)^{1/2} \qquad (9)$$

$$A_{eff} = (<A'_{\parallel}>^2 \cos^2\theta + <A'_{\perp}>^2 \sin^2\theta\,)^{1/2} \qquad (10)$$

where θ is the angle between the director axis and the magnetic field direction. Solving the Hamiltonian gives

$$H_\theta = \frac{h\,\nu}{g_{eff}\beta} - M_I \frac{A_{eff}}{g_{eff}\,\beta} \qquad (11)$$

where M_I is the nuclear spin quantum number with possible values 1,0,-1. In aqueous dispersions of lipids and at moderate magnetic fields as used in an ESR experiment all orientations of the director axis are equally probable giving rise to a powder spectrum. The spectral intensity $P(H_\theta)$ of such a powder spectrum at the resonance field H_θ

is weighted by a sin θ term[10]

$$P(H_\theta) \propto \frac{\sin\theta}{dH/d\theta} = \left(\frac{h\nu}{\beta}\right)^2 \left(H_\theta^3 \,(g^2 - g^2)\cos\theta\right)^{-1}. \qquad (12)$$

Computed absorption powder spectra for smectic and hexagonal phases are shown in Fig. 1A and 1B, respectively. There are distinct differences: with the former $A_\parallel > A_\perp$ and greater spectral intensity is therefore associated with the inner hyperfine splittings whereas with hexagonal phases the situation is reversed. Furthermore, due to the additional averaging in the latter the spectral anisotropy $|A_\parallel - A_\perp|$ is halved compared to smectic phases.

EXPERIMENTAL

The lyotropic hexagonal and nematic phases were prepared by weighing and mixing the components thoroughly alternately by stirring and centrifuging at room temperature until a homogeneous phase resulted. The amphiphilic phases were subjected to several heating-cooling cycles with the maximum temperature well above their Krafft point. Before measurement they were centrifuged at 5000 rpm to obtain a homogeneous phase.

The composition of the two lyotropic nematic (calamitic) phases was[11,12] (a) 38.1% sodium dodecyl sulphate(SDS),6.8% n-octanol and 55.1% water and (b) 32.8 potassium laurate (KL), 2.8% KCl,64.4% water; that of the discotic nematic phase was

30.9% KL, 1.9% KCl, 6.7% n-octanol and 60.4% H_2O. Unless otherwise stated 5-doxylstearic acid was used as the spin probe. For comparison a spin labeled phospholipid was used (1-palmitoyl-2-(5-doxylstearoyl)-sn-phosphatidylcholine) in some instances.In systems containing octanol the spin probe was added as an octanol solution. In all other cases the spin label was dried on the glass wall and the dried label was equilibrated with the amphiphilic phase. Phospholipid dispersions were prepared by dissolving the lipid(s) and the spin label in organic solvent; the lipid was deposited as a thin film on the glass wall of the vessel by evaporating the solvent on the rotary evaporator and dried.

RESULTS AND DISCUSSION

ESR spectra of spin labeled SDS amphiphilic phases at different detergent concentrations and temperatures are shown in Figs. 2 and 3. At 50 wt% SDS and temperatures between about 40 and 100°C there is greater spectral intensity associated with the outer hyperfine splitting which is characteristic of the hexagonal phase. This is also true for the less concentrated SDS phase (30 wt%)(Fig.3) and 20 wt% SDS dispersion in 1M NaCl (spectra not shown). At temperatures 40°C there are distinct lineshape changes(Fig.3)which can probably be ascribed to reduced motion about the cylinder axis. In the limit of restricted motion about this axis the line shape approaches that characteristic of the smectic phase: under these conditions the only fast motion contributing to the averaging of the g and A tensor and hence determining the line shape is the rotation of the spin probe about its molecular long axis. For comparison the ESR spectra of a 40 wt% dispersion of potassium palmitate in H_2O at different temperatures are shown in Fig.4.Under the conditions of our experiment this system has been reported to form hexagonal phase I [13] and the ESR spectra in Fig. 4 support this conclusion. The temperature dependence of the order parameter S_3 of different amphiphilic systems is depicted in Fig.5. In hexagonal phases of SDS the order parameter is higher and its decrease with temperature (dS_3/dT) smaller the more concentrated the dispersion is. The addition of an excess of counterions produced a significant increase in S_3. The ESR spectra shown in Figs.2-4, which are characteristic of the hexagonal phase, are contrasted by the spectra from equimolar egg phosphatidylethanolamine-cholesterol dispersion in water labeled with 5 doxyl stearic acid.(Fig.6). Over the temperature range 20-90°C the line shape is characteristic of the smectic (lamellar) phase: greater spectral intensity is associated with the inner hyperfine splittings (compare Fig. 1B with Fig.6). ^{31}P NMR measurements indicate that this lipid mixture undergoes a phase change lamellar-to-hexagonal in the temperature range 30-35°C[13]. The tempera-

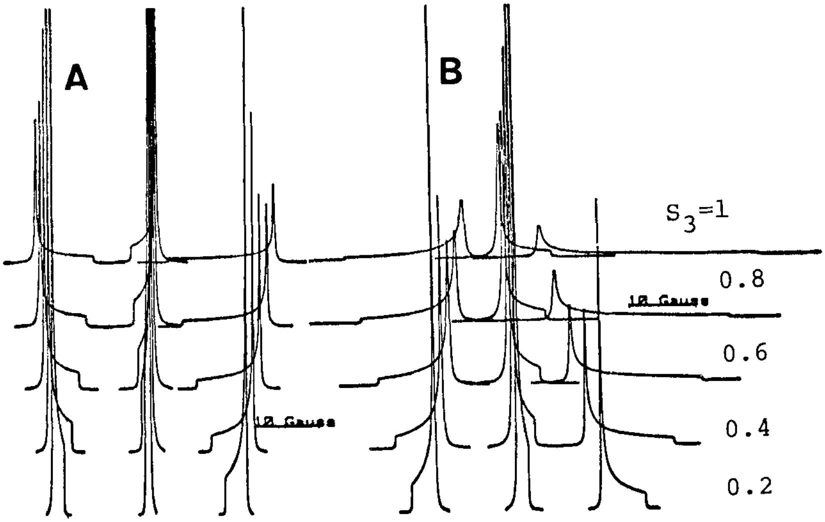

Fig.1: ESR absorption powder spectra were computed for 5-doxylstearic acid incorporated in the hexagonal phase (A) and the smectic(lamellar)(B) phase using eq.1-12. The following parameters were used: S=1/2,I=1,H=3300 G

$$\underline{g} = \begin{bmatrix} 2.0088,0,0 \\ 0,2.0061,0 \\ 0,0,2.0027 \end{bmatrix} \qquad \underline{A} = \begin{bmatrix} 6, & 0, & 0 \\ 0, & 6, & 0 \\ 0, & 0, & 32 \end{bmatrix}$$

and the order parameter S_3 was varied between 1 and 0.2(see numbers top to bottom).

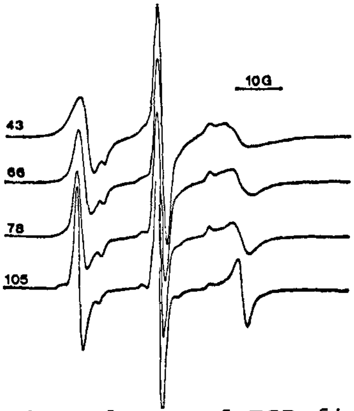

Fig.2: Temperature dependence of ESR first derivative spectra of 5-doxylstearic acid(FASL) incorporated in the lyotropic,hexagonal phase of SDS/water(5:5,by wt).

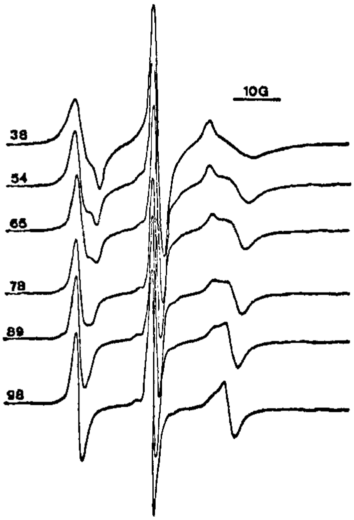

Fig.3: Temperature dependence of ESR first derivative spectra of FASL in the lyotropic,hexagonal phase of SDS/water(3:7,by wt.).

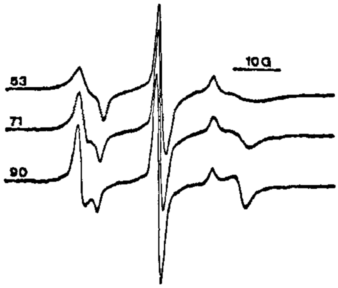

Fig.4: Temperature dependence of ESR first derivative spectra of FASL incorporated in the hexagonal phase of the potassium palmitate/water lyotropic system(4:6,by wt.)

ture dependence of the ESR spectra (Fig.6) shows that this phase transition has practically no effect on the ESR line shape. Our results are in agreement with previous work[14]. From the ESR spectra we conclude that in this lipid system the additional motional averaging about the cylinder axis of the hexagonal phase is slow on the ESR time scale. The only observable change in the ESR spectra (Fig.6) up to ~90°C is a continous decrease of the spectral anisotropy ($A_{\parallel} - A_{\perp}$). At 98°C the rate of the motion about the cylinder axis is still slow but apparently sufficiently increased to give an intermediate spectrum comparable to the slow motion spectrum discussed before (cf Fig.3,38°C,Fig.4,53°C). While Figs. 3 and 4 clearly show the transition from intermediate to rapid motional averaging, such a transition is not apparent in the temperature dependence of the hexagonal phase of egg phosphatidylethanolamine-cholesterol up to 98°C. Similar observations were made with 10 % egg phosphatidylethanolamine dispersions in water. Under the conditions of our experiment this lipid dispersion was shown to undergo a phase transition lamellar-to-hexagonal II and again smectic type ESR spectra were observed both below and above the transition temperature. Only at 100°C spectra indicative of limited averaging about the cylinder axis were obtained. To fully understand these spectra one would have to introduce time dependent

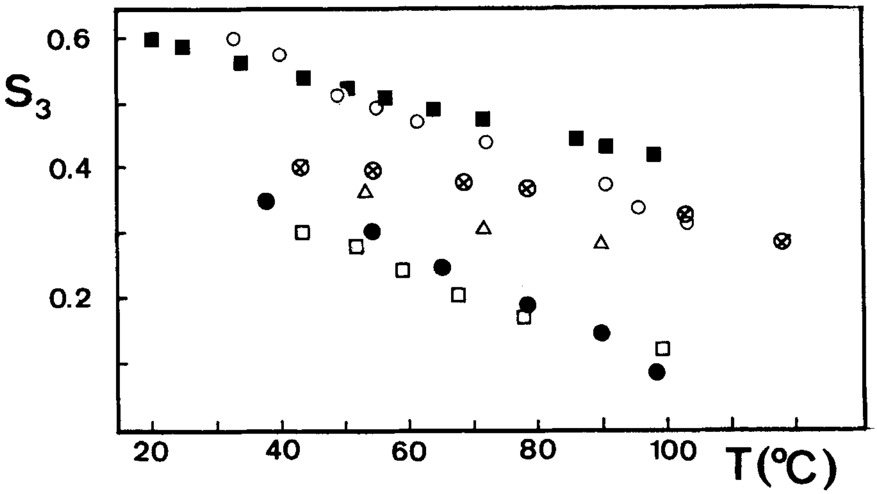

Fig.5: Temperature dependence of the order parameter S_3 for various lyotropic systems:potassium palmitate/water,4:6,(△); SDS/water,5:5,(⊗); SDS/water,3:7,(●); SDS/1M NaCl in water,2:8,(○); of the nematic calamitic phase(38.1%SDS,6.8% octanol and 55.1% water),(□); and the nematic, discotic phase(30.9%potassium laurate,1.9% KCl, 6.7% octanol and 60.4% water),(■). The order parameter is the "lamellar" order parameter,i.e. it is referred to the cylinder surface in case of hexagonal phases:$S_3=-2S^H$,where S^H is the observed order parameter.

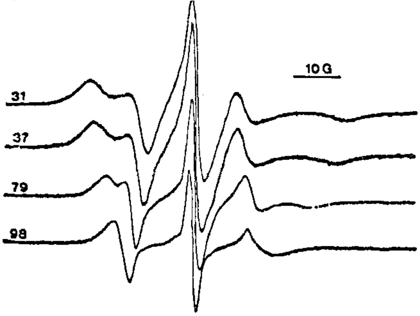

Fig.6: Temperature dependence of ESR first derivative spectra of FASL incorporated in equimolar egg phosphatidylethanolamine/cholesterol dispersion in water. The total lipid concentration was 13.5 wt%.

rotation matrices in eq(3).

For comparison, the temperature dependence of the ESR spectra of two lyotropic nematic phases is shown in Fig.7. The nematic phase investigated in Fig.7A was described to consist of elongated, rodlike aggregates (calamitic phase) while that of Fig.7B was shown to consist of flattened, disclike aggregates (discotic phase). From X-ray and neutron scattering it was concluded[12] that both the disclike (oblate spheroids) and rodlike aggregates (prolate spheroids) are organized with some orientational order but without any translational order. Such lyotropic phases then represent an intermediate state between well-ordered phases such as the smectic or the hexagonal phases and the totally disordered phase of an isotropic micellar solution. They may be viewed as micellar dispersions with some long range order and are therefore relevant to micellar systems in general. The ESR spectra recorded from the calamitic phase are typical for cylindrical symmetry at least at temperatures $\gtrsim$ 50°C. Below this temperature the spectra are indicative of intermediate motional averaging. Similar spectra were obtained when the calamitic phase was labeled with 5-doxylstearic acid and phospholipid spin probe. The ESR spectra obtained from the discotic phase(Fig.7B) are characteristic of the smectic phases. The ESR evidence is consistent with the conclusions drawn from X-ray

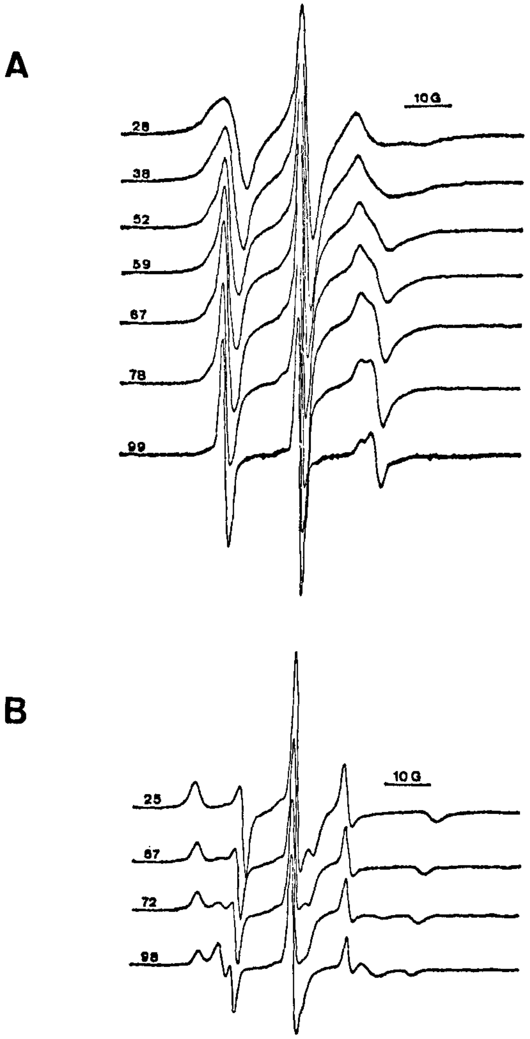

Fig.7: Temperature dependence of ESR first derivative spectra: (A) calamitic phase (38.1%SDS, 6.8%octanol,55.1%water) labeled with 5-doxylphosphatidylcholine and (B) discotic phase(30.9 %KL,1.9%KCl,6.7%octanol,60.4%water) labeled with 5-doxylstearic acid.

and neutron scattering[12] as well as ^{2}H NMR measurements[15]. It also implies that ESR spin labeling can differentiate between disclike and rodlike micelles. However, as with the hexagonal phase,the detection of rodlike micelles by ESR is only possible if the motional averaging about the cylinder axis is rapid on the ESR time scale.

We conclude that ESR spin labeling can be used to detect hexagonal phases provided that motional averaging about the cylinder axis is fast on the ESR time scale. If the motion about this axis is slowed down the spectral line shape approaches that characteristic of smectic(lamellar) phases; in the limit of restricted motion about this axis the spectrum becomes indistinguishable from the smectic type spectrum.

REFERENCES

1.R.M.Williams,D.Chapman,Progr.Chem.FatsOther Lipids,11,1(1970).
2.H.Hauser in"Water,a comprehensive treatise", Ed.F.Franks(Plenum Press,New York,1975),pp.209.
3.J.Seelig in "Spin labeling",Ed.L.J.Berliner (Academic Press,New York,1976),pp.373.
4.J.Seelig,H.Limacher,Mol.Cryst.Liq.Cryst. 25, 105(1974).
5.M.Schara,F.Pušnik,M.Šentjurc,Croat.Chem.Acta, 48,147(1976).
6.D.D.Lasič,H.Hauser,submitted for publication.
7.F.Perrin, J.Physique Radium 5,497(1934).
8.O.H.Griffith,P.C.Jost in"Spin labeling",Ed.L.J. Berliner(Ac.Press,New York,1976),pp.454
9.J.Charvolin,P.Rigny,J.Chem.Phys.58,3999(1973).
10.J.E.Wertz,J.R.Bolton,Electron Spin Resonance Elementary Theory and Practical Applications,

(Mcgraw Hill,New York,1972),pp.156.
11.Y.Hendrikx,J.Charvolin,M.Rawiso,L.Liébert, M.C.Holmes, J.Phys.Chem.87,3991(1983).
12.D.D.Lasič,M.E.Marcondes,L.W.Reeves,M.Szarka, Croat.Chem.Acta 57,129(1984).
13.P.R.Cullis,B.DeKruijff, Biochim.Biophys.Acta, 513,31(1978).
14.M.G.Taylor,I.C.P.Smith,Chem.Phys.Lipids, 28,119(1981).
15.B.J.Forrest,L.W.Reeves,Chem.Rev.81,1(1981).