JOURNAL OF MAGNETIC RESONANCE **43**, 141–153 (1981)

The Polyliquid Crystalline EPR Spectra of Nitroxide Spin Probes and Their Interpretation

CLAUDIO ZANNONI

Istituto di Chimica Organica, Viale Risorgimento 4, Bologna 40136, Italy

GIAN FRANCO PEDULLI

Istituto Chimico dell'Università, Via Ospedale 72, Cagliari 09100, Italy

AND

LANFRANCO MASOTTI AND ALBERTO SPISNI

Istituto di Chimica Biologica, Facoltà di Medicina, Parma 43100, Italy

Received February 6, 1980; revised July 8, 1980

The EPR spectrum of 5-doxyl stearic acid dissolved in the randomly oriented lamellar G phase of sodium decanoate, decanol, and water was recorded at room temperature and the spectral shape was analyzed using relaxation theory. The spectrum was calculated as the superposition of fast-motion spectra coming from differently oriented domains characterized by a linewidth dependent on the angle formed by the director and the magnetic field. It is shown that a quantitative interpretation of the spectral shape requires the introduction of the effect of nonquantization of the nitrogen nuclear spin along the magnetic field, the $m = 0$ line being especially sensitive to the inclusion of this effect. The fourth rank order parameter $\bar{P}_4$ for the spin probe was also obtained and found to be lower than the value predicted by Maier–Saupe theory on the basis of $\bar{P}_2$. A semiquantitative treatment of the polyliquid crystalline spectrum is also given providing simple explanations of the relevant spectral features.

INTRODUCTION

Electron paramagnetic resonance spectra of nitroxide spin probes of convenient structure for interacting with selected regions of phospholipid bilayers have been widely used to elucidate the ordering and motional properties of biological membranes (*1*). In particular these investigations may provide the second and fourth rank order parameters $\bar{P}_2$ and $\bar{P}_4$, and the correlation times for reorientation of the incorporated spin probe. $\bar{P}_2$ can be easily calculated from the separation of the outer peaks once the principal components of the nitrogen hyperfine tensor are known. $\bar{P}_4$ and the correlation times can be obtained from a careful analysis of the lineshape by making use of suitable theories.

The best way of determining the latter parameters is to use monodomain samples

0022–2364/81/040141-13$02.00/0

and to record the full angular dependence of the EPR spectra (*2*). However, with most biological substrates this is impracticable since these systems consist of a distribution of domains, which cannot be oriented macroscopically, each one with the director at a given angle to the magnetic field of the spectrometer. If exchange between domains is slow on the EPR time scale, as is usually the case, the observed spectrum can be considered the sum of the spectra from each orientation (domain). We shall call this a polyliquid crystalline (PLC) spectrum. Actually the distribution of domains is in itself one of the findings which can be extracted from the experiment. It therefore appears important to develop correct theories for interpreting polyliquid crystalline spectra of spin probes incorporated in micellar systems and biological membranes.

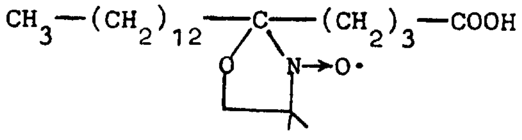
Attempts in this direction have been made by several authors. The simplest approach (*3*,*4*) is to treat the PLC spectrum as a powder spectrum; thus relaxation effects are completely neglected and the linewidth is introduced as an empirical parameter. This amounts, in practice, to renouncing all the dynamic information contained in the spectral lineshape. However, relaxation effects can be taken into account for PLC spectra (*5*) by extending the treatments employed for interpreting angular dependent linewidths in monodomain samples (*6*). While this seems to have been considered earlier (*5*), the analysis contains several unsatisfactory features which will be discussed in the next sections. In particular it has recently been shown that quantitative interpretation of the angular dependence of the linewidth for a nitroxide spin probe requires the introduction of effects of nonquantization of the nuclear spin along the magnetic field (*7*, *8*). Other treatments have been proposed for the important case of slow tumbling, where the electron spin relaxes on a time scale similar to that of reorientation (*9*). This type of treatment is often necessary when dealing with viscous systems.

Here, however, we wish to confine ourselves to fast-motion spectra, where Redfield theory is applicable (*2*, *10*). Within this approximation, nonquantization effects have been considered in the present investigation and a comparison between experimental and computer-simulated spectra is shown. We feel that it is important to understand the influence of the various parameters on the spectra, and to this end we shall also first give a semiquantitative treatment which provides simple explanations of the most relevant spectral features.

EXPERIMENTAL

The liquid crystalline phase used in the present investigation was obtained by mixing 42% by weight of 1-*n*-decyl alcohol, 28% of sodium 1-*n*-decanoate, and 30% of water (*11*). This composition corresponds to a lyotropic liquid crystal having a typical bilayer structure, where the amphiphilic regions are separated by layers of water.

The spin probe is the 5-doxyl stearic acid



$CH_3-(CH_2)_{12}-C-(CH_2)_3-COOH$

which was synthesized from the appropriate ketone using the procedure described by Waggoner *et al.* (*12*). The EPR spectra were recorded on a Varian E-104 spectrometer by placing the doped mesophase in a test tube having an internal diameter of 4 mm to reduce surface effects as much as possible.

GENERAL FEATURES OF THE PLC SPECTRA OF NITROXIDE SPIN PROBES

In a polyliquid crystalline spectrum the intensity of the absorption at a given magnetic field value is proportional to the number of molecules resonating at this field. For a cylindrically symmetric domain at an angle γ with the magnetic field direction, the resonance condition is given at first order by (*6*, *13*)

$$B_m(\gamma) = \frac{\hbar}{\beta g(\gamma)}[\omega_0 - a(\gamma)m], \qquad [1]$$

where $B_m(\gamma)$ is the resonant field, ω_0 the klystron frequency,

$$g(\gamma) = [\tilde{g}_{\parallel}^2 \cos^2\gamma + \tilde{g}_{\perp}^2 \sin^2\gamma]^{1/2}, \qquad [2]$$

and

$$a(\gamma) = [\tilde{g}_{\parallel}^2\tilde{A}_{\parallel}^2 \cos^2\gamma + \tilde{g}_{\perp}^2\tilde{A}_{\perp}^2 \sin^2\gamma]^{1/2}/g(\gamma). \qquad [3]$$

In these expressions m is the nuclear spin quantum number, while $\tilde{g}_{\parallel}$, $\tilde{g}_{\perp}$ and $\tilde{A}_{\parallel}$, $\tilde{A}_{\perp}$ are the partially averaged g and hyperfine tensor components parallel and perpendicular to the director. These are in turn defined in terms of the second rank order parameters $\overline{D^2_{0,p}}$ as

$$\tilde{F}_{\parallel} = f + (2/3)^{1/2}\sum_p (-)^p F^{2,p}\overline{D^2_{0,-p}}, \qquad [4a]$$

$$\tilde{F}_{\perp} = f - 1/6^{1/2}\sum_p (-)^p F^{2,p}\overline{D^2_{0,-p}}, \qquad [4b]$$

where f is the isotropic value and $F^{2,p}$ the molecule fixed components of a particular magnetic interaction ($F = g$ or A). If we work in the principal system of the ordering matrix, as we elect to do here,

$$\tilde{F}_{\parallel} - \tilde{F}_{\perp} = (3/2)^{1/2}\{F^{2,0}\overline{D^2_{0,0}} + 2\,\mathrm{Re}[F^{2,2}\overline{D^2_{0,-2}}]\}, \qquad [5]$$

where the upper bar denotes an equilibrium orientational average,

$$\overline{D^2_{q,-p}} = \frac{\int D^2_{q,-p}(\Omega)e^{-U(\Omega)/kT}d\Omega}{\int e^{-U(\Omega)/kT}d\Omega},$$

and $U(\Omega)$ is the effective anisotropic potential acting on a molecule in terms of the three Euler angles $\Omega \equiv (\alpha\beta\gamma)$ (*14*) giving the orientation of the molecule in the director frame (*15*).

In the important case that the ordering matrix has cylindrical symmetry, or when the magnetic components $g^{2,2}$ and $A^{2,2}$ in the ordering matrix principal system are accidentally zero, one has simply (*10*)

$$\tilde{F}_{\parallel} - \tilde{F}_{\perp} = (3/2)^{1/2} F^{2,0}\overline{D^2_{0,0}}. \qquad [6]$$

On this basis the polyliquid crystalline spectrum is equivalent to a powder spectrum of a radical with axially symmetric magnetic interactions. Thus if the partially averaged $\tilde{A}_{\parallel}$, $\tilde{A}_{\perp}$, and $\tilde{g}_{\parallel}$, $\tilde{g}_{\perp}$ components may be measured from the positions of the spectral peaks, the two order parameters $\overline{D^2_{0,0}}$ and $\overline{D^2_{0,-2}}$ can be determined if we know the principal values of the hyperfine and g tensors.

For a nitroxide spin probe, the X-band EPR spectrum consists of three transitions, two of which are highly anisotropic (for $m = \pm 1$) and one almost isotropic ($m = 0$) because of the relatively small value of $\tilde{g}_{\parallel} - \tilde{g}_{\perp}$. Within each transition the parallel absorption shows a much lower intensity than the perpendicular absorption since each domain has a much larger probability of being found perpendicular to the magnetic field than aligned to it. As a consequence the appearance of a PLC spectrum of an elongated nitroxide, with $\overline{D^2_{0,0}} > 0$, may be remarkably different depending on whether the symmetry axis of the $2p_z$, orbital of the nitrogen atom is preferentially oriented parallel ($\tilde{A}_{\parallel} > \tilde{A}_{\perp}$) or perpendicular ($\tilde{A}_{\parallel} < \tilde{A}_{\perp}$) to the director of the liquid crystal. The former case arises, e.g., with stearic spin probes which show low-intensity peaks due to the parallel absorptions of the $m = \pm 1$ lines at the extremes of the spectrum. The situation is reversed with cholestane probes, where the intense outer peaks are due to the domains perpendicular to the magnetic field. Here we show that in either case the shape of the parallel peaks may easily provide additional information about the spectral parameters, besides that which can be obtained from the line positions.

In a powder spectrum it has been shown that the parallel lines of the first derivative spectrum are essentially absorptionlike lines whose width is given by the intrinsic linewidth (*4*). In a polyliquid crystalline spectrum of a nitroxide spin probe in an isotropic distribution of oriented domains the linewidths are dependent on the nuclear quantum number and on orientation with respect to the magnetic field, and therefore different in principle for the various domains. In this case the spectrum will therefore be a convolution of the shape function for powder samples with a lineshape function (*13*); i.e., taking the center of the spectrum as the origin,

$$S(B) = \sum_{m} \int f_m(\bar{B}) \mathscr{L}_m[B - \bar{B}, W_m(\gamma)]\, d\bar{B}, \qquad [7]$$

where $\mathscr{L}$ is a shape function that here we take to be Lorentzian

$$\mathscr{L}(\omega, W) = W/(W^2 + \omega^2) \qquad [8]$$

of width W, which according to the Redfield relaxation theory is given by (*5–8*)

$$W_m(\gamma) \equiv T_2^{-1}(m,\gamma) = A(\gamma) + B(\gamma)m + C(\gamma)m^2, \qquad [9]$$

and (*13*)

$$f_m(\bar{B}) \propto |\delta \cos \gamma / \delta \bar{B}| \qquad [10]$$

is proportional to the number of molecules resonating at the field $\bar{B}$. The latter is introduced for economy of notation and is defined as

$$\bar{B} \equiv B_m(\gamma) - B_0(\gamma), \qquad [11]$$

with $B_0(\gamma) = \hbar\omega_0/\beta g(\gamma)$.

If we confine ourselves to the $m = \pm 1$ lines and neglect for the moment the $\tilde{g}$-tensor anisotropy, $f_m(\bar{B})$ is, for a nitroxide radical,

$$f_m(\bar{B}) \propto \left|\bar{B}[(\bar{B}^2 - m^2\tilde{A}_\perp^2)m^2(\tilde{A}_\parallel^2 - \tilde{A}_\perp^2)]^{-1/2}\right|. \qquad [12]$$

The range of the powder band shape is

$$-s\tilde{A}_\parallel \leq s\bar{B} \leq -s\tilde{A}_\perp \qquad \text{for} \qquad m = 1 \qquad [13a]$$

and

$$s\tilde{A}_\perp \leq s\bar{B} \leq s\tilde{A}_\parallel \qquad \text{for} \qquad m = -1, \qquad [13b]$$

where $s \equiv \operatorname{sgn}(\tilde{A}_\parallel - \tilde{A}_\perp)$. Here sgn (x) gives the sign of the argument x.

Near $\bar{B} = |\tilde{A}_\parallel|$ the function $f_m(\tilde{B})$ resembles a step function:

$$f_m(-m\tilde{A}_\parallel) = |\tilde{A}_\parallel/(\tilde{A}_\parallel^2 - \tilde{A}_\perp^2)|, \qquad [14a]$$

$$f_m[-m(\tilde{A}_\parallel + s|\delta B|)] = 0. \qquad [14b]$$

Since the presentation of the spectrum is normally a first derivative one with respect to the magnetic field, we differentiate Eq. [7] after making the variable change $U \equiv B - \bar{B}$ and we find

$$S'(B) = \sum_m \int [\partial f_m(B - U)/\partial B]\mathscr{L}[U, W_m(\gamma)]dU. \qquad [15]$$

This allows us to take advantage of the well-known fact that the derivative of a step function is a Dirac delta function. Then, near $\bar{B} = -m\tilde{A}_\parallel$, we obtain

$$\begin{aligned} S'[-m\tilde{A}_\parallel + \delta B] &= |\tilde{A}_\parallel/(\tilde{A}_\parallel^2 - \tilde{A}_\perp^2)| \int ms\delta(B + m\tilde{A}_\parallel - U)\mathscr{L}[U, W_m(0)]dU \\ &= ms|\tilde{A}_\parallel/(\tilde{A}_\parallel^2 - \tilde{A}_\perp^2)|\mathscr{L}[B + m\tilde{A}_\parallel, W_m(0)]. \qquad [16] \end{aligned}$$

In words, when the field is near $|\tilde{A}_\parallel|$, the spectrum consists actually of absorption Lorentzians whose widths are given by

$$W_1 = A + B + C, \qquad [17a]$$

$$W_{-1} = A - B + C. \qquad [17b]$$

This result is particularly interesting for highly ordered probes, where the parallel peaks are normally resolved and may allow an easy calculation of the B coefficient from the difference of their widths. The experimental observation that W_1 and W_{-1} are considerably different emphasizes the importance of relaxation effects. From the value of the B coefficient and the expressions given in Ref. (*15*) the correlation time τ_0 can be estimated without recourse to spectral simulations if an estimate of the fourth rank order parameter $\overline{D_{0,0}^4}$ can be made from theoretical considerations.

QUANTITATIVE ANALYSIS OF THE PLC EPR SPECTRA

We have seen in the previous section that the positions of the spectral peaks in a PLC spectrum may provide the second rank order parameters, while from the widths of the parallel absorption-like lines a rough estimate of the correlation time can be made. However, a detailed analysis of the spectral shape yields more precise information about the dynamics of the reorientation process and the fourth rank order parameter. This can be obtained by comparing the experimental spectra with those simulated by using appropriate theories. Since the PLC spectrum consists of a superposition of spectra coming from all the possible orientations, the angular dependence of the linewidth should be taken explicitly into account. Theories to interpret this angular dependence have been developed by several authors (*6*, *15*, *16*) since the pioneering work of McFarland and McConnell (*7*), while their use to analyze polyliquid crystalline spectra has been reported by Seelig and co-workers (*5*). However, most of these theories assume that both the electron and nuclear spins are quantized along the magnetic field for any angle γ made by the director and the applied field. It has been pointed out that this assumption is not correct when γ is different from 0 or 90° (*8*). Detailed studies of the angular dependence of the linewidth of nitroxide spin probes in monodomain samples have shown that the effect of nonquantization is not negligible and that considerable deviation of the B and C coefficients from predictions of earlier theories are actually observed (*2*). Since allowing for this effect does not require the introduction of any new parameter we think it is worthwhile also to use the improved theory to simulate PLC spectra.

We shall assume, as usual, that the dominant relaxation mechanism determining the linewidth in the EPR spectrum of a nitroxide spin probe is the molecular reorientation coupled with the anisotropy of the g and hyperfine tensors, and that the rate of rotation is fast in the sense that the Redfield theory is applicable (*10*). Under this assumption the width of the three spectral lines originating from a domain at angle γ with the magnetic field direction is given by Eq. [9], where the angular-dependent linewidth coefficients are (*8*)

$$A(\gamma) = \frac{2\omega_0^2}{3g^2} J_0^{gg}(\gamma) + \frac{I(I+1)}{4} J_1^{AA}(\gamma), \qquad [18]$$

$$B(\gamma) = \frac{4\omega_0}{3g} J_0^{gA}(\gamma), \qquad [19]$$

$$C(\gamma) = \frac{2}{3} J_0^{AA}(\gamma) - \frac{1}{4} J_1^{AA}(\gamma), \qquad [20]$$

with

$$J_0^{gg}(\gamma) = \sum_{p,q} |g^{2,p}|^2 \{d_{0,q}^2(\gamma)\}^2 j_{q,-p}, \qquad [21]$$

$$J_0^{gA}(\gamma) = (3/2)^{1/2} \sum_{p,q,r} g^{2,p} A^{2,p*} d_{0,q}^2(\gamma) d_{r,q}^2(\gamma) C(112;\, 0r) d_{0,r}^1(\chi) j_{q,-p}, \qquad [22]$$

$$J_m^{AA}(\gamma) = \frac{1}{C(112;\, m0)^2} \sum_p |A^{2,p}|^2 \sum_q \{\sum_r d_{r,q}^2(\gamma) d_{m,r}^1(\chi) C(112;\, 0r)\}^2 j_{q,-p}. \qquad [23]$$

Here $j_{q,-p}$ are spectral densities, $d_{p,q}^L(\beta)$ reduced Wigner rotation matrices and $C(LL'L'';\, pq)$ Clebsch–Gordan coefficients. The explicit expressions of these coefficients and of the rotation matrices appearing in the above equations are, for convenience, reported in the Appendix. The angle χ represents the deviation

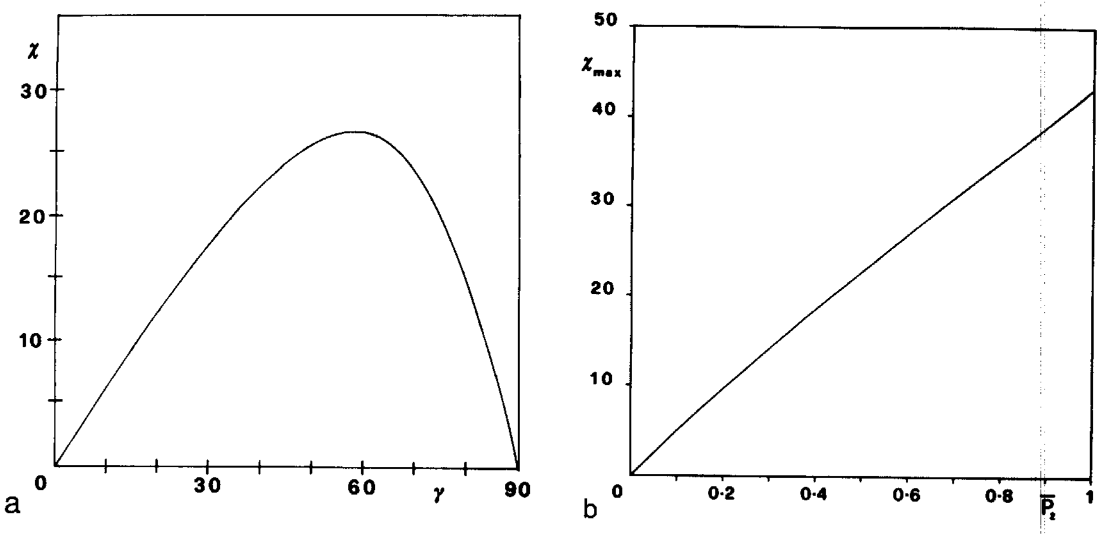


FIG. 1. (a) Computed values of the angle χ between applied field and the nitrogen nuclear spin quantization axis as function of the angle γ between director and magnetic field, for a stearic spin probe with $\bar{P}_2 = 0.60$. (b) Maximum value of the angle χ against the order parameter $\bar{P}_2$ for a stearic spin probe.

between the applied field and the quantization axis of the nuclear spin, I, and is given by

$$\tan \chi = \frac{(\tilde{A}_{\perp} - \tilde{A}_{\parallel}) \sin \gamma \cos \gamma}{\tilde{A}_{\perp} \sin^2 \gamma + \tilde{A}_{\parallel} \cos^2 \gamma} . \qquad [24]$$

This angle may be fairly large for high degrees of order. As an illustration, Fig. 1a shows the dependence of χ on the angle γ between director and magnetic field for an arbitrary chosen value of $\bar{P}_2$, while in Fig. 1b the maximum deviation angle χ_{max} is plotted as a function of the order parameter for a stearic probe.

When γ is equal to 0 or 90°, Eq. [24] shows that χ is zero and therefore the linewidth coefficients coincide with those previously reported (*15*).

The spectral densities $j_{q,-p}$, which are the Fourier transforms of the correlation functions of the $D^2_{q,-p}$ Wigner rotation matrices connecting the molecular and director coordinate systems, are defined as

$$j_{q,-p} = \frac{1}{2} \int_{-\infty}^{\infty} \{\overline{D^2_{q,-p}(0) D^2_{q,-p}(t)^*} - \delta_{0q} \overline{D^2_{0,-p}}\, \overline{D^2_{0,-p}{}^*}\} dt , \qquad [25]$$

where we have implicitly assumed that the Fourier transform is taken at zero frequency. This amounts to neglecting the nonsecular terms, an excellent approximation for correlation times greater than ω_0^{-1}, and to assuming $\omega_n \tau \ll 1$ (*17*).

To calculate these spectral densities we should adopt some kind of model to describe molecular reorientation as Eqs. [18] to [23] above have been derived without making assumptions on the molecular dynamics. The simplest hypothesis is to assume that the normalized correlation functions decay exponentially, as

$$\frac{\overline{D^2_{q,-p}(0) D^2_{q,-p}(t)^*} - \overline{D^2_{q,-p}}\, \overline{D^2_{q,-p}{}^*}}{\overline{D^2_{q,-p}(0) D^2_{q,-p}(0)^*} - \overline{D^2_{q,-p}}\, \overline{D^2_{q,-p}{}^*}} = e^{-t/\tau_p} , \qquad [26]$$

where τ_p is the relaxation time coupled to the $F^{2,p}$ component of a magnetic interaction in the molecule-fixed system. Choosing the long molecular axis as the z axis, it turns out that τ_0 refers to the reorientation of z and τ_2 mainly to reorien-

TABLE 1

THE EXPLICIT EXPRESSIONS FOR THE STRONG COLLISION SPECTRAL DENSITIES j_{mn}

m	n	$j_{mn} = \{\overline{D^2_{mn}D^{2*}_{mn}} - (\overline{D^2_{00}})^2\delta_{0m}\delta_{0n}\}\tau_n$
0	0	$\{1/5 + 2\bar{P}_2/7 + 18\bar{P}_4/35 - \bar{P}_2^2\}\tau_0$
±1	0	$\{1/5 + \bar{P}_2/7 - 12\,\bar{P}_4/35\}\tau_0$
±2	0	$\{1/5 - 2\bar{P}_2/7 + 3\bar{P}_4/35\}\tau_0$
0	±1	$\{1/5 + \bar{P}_2/7 - 12\bar{P}_4/35\}\tau_1$
±1	±1	$\{1/5 + \bar{P}_2/14 + 8\bar{P}_4/35\}\tau_1$
±2	±1	$\{1/5 - \bar{P}_2/7 - 2\bar{P}_4/35\}\tau_1$
0	±2	$\{1/5 - 2\bar{P}_2/7 + 3\bar{P}_4/35\}\tau_2$
±1	±2	$\{1/5 - \bar{P}_2/7 - 2\bar{P}_4/35\}\tau_2$
±2	±2	$\{1/5 + 2\bar{P}_2/7 + \bar{P}_4/70\}\tau_2$

tation about z. Equation [26] is equivalent to the strong collision model (*10*) and to the first term in the series expansion of the correlation function obtained from the diffusion model (*18*), and provides a particularly simple expression for the spectral densities which becomes, for a uniaxial domain,

$$j_{q,-p} = \{\overline{D^2_{q,-p}(0)D^2_{q,-p}(0)^*} - \delta_{0q}\overline{D^2_{0,-p}}\,\overline{D^2_{0,-p}}^*\}\tau_p. \quad [27]$$

Explicit expressions for the $j_{q,-p}$'s are reported in Table 1.

Although the simple assumption we have made about the dynamics is sufficient for most purposes, the treatment of PLC spectra given here is obviously valid for other choices of the spectral densities. For example, one could use the well-known diffusional model. However, this has been derived for rigid molecules and is not necessarily justified for flexible molecules such as the stearic spin probe we have used. Another advantage of the simple expression [27] is that it gives $j_{q,-p}$ in terms of $\bar{P}_2 \equiv \overline{D^2_{00}}$ and $\bar{P}_4 \equiv \overline{D^4_{00}}$, which are then left as parameters to be determined from a fitting of the theoretical spectrum to the experimental one.

SPECTRAL SIMULATIONS

In practice the polyliquid crystalline EPR spectrum of a nitroxide is calculated as the superposition of fast-motion spectra, coming from differently oriented domains, by using the equation

$$S'(B) \propto \sum_m \int_0^{\pi/2} \mathscr{L}'[B - B_m(\gamma), W_m(\gamma)]P(\gamma)d\gamma, \quad [28]$$

where $P(\gamma)$ is the probability distribution of finding the director at an angle γ to the field. It is defined as sin γ for a three-dimensional distribution of domains, as 1 for a two-dimensional distribution, and as a delta function $\delta(\gamma - \gamma_0)$ for a monodomain spectrum. Other distributions, e.g., the Gaussian one proposed by Libertini *et al.* (*3*) can obviously be used. The integration can be limited to the first quadrant as long as $P(\gamma) = P(\pi - \gamma)$.

The effective linewidth $W_m(\gamma)$ employed in the simulations is taken to be the sum of two terms (*2*):

$$W^2_m(\gamma) = T_2^{-2}(m,\gamma) + X^2(\gamma). \quad [29]$$

The first is calculated from relaxation theory, and the second is introduced to take into account the broadening due to unresolved hyperfine structure. Since the latter term can also change with orientation, its angular dependence has been assumed to be of the form (*19*)

$$X(\gamma) = X_0 + X_2 \cos^2 \gamma. \qquad [30]$$

A quadratic relation [29] is different from the linear relation used by other authors (*5*, *9*). We may recall that an additive relation is only valid when the residual linewidth is due to some broadening process giving rise to a Lorentzian lineshape (*20*), while a Gaussian lineshape seems more appropriate for the inhomogeneous broadening from unresolved hyperfine structure (*20*). Of course $X(\gamma)$ does not depend on m and thus it only contributes to the $A(\gamma)$ linewidth coefficient, while $B(\gamma)$ and $C(\gamma)$ are independent of it. On the other hand, the assumption of a linear relation brings out an unjustified extra term dependent on m in the Lorentzian shape function since there the width appears squared.

The molecule-fixed components of the g and hyperfine tensors for the oxazolidine ring are known from solid-state EPR spectra (*21*). Since, however, the magnitude of the tensor elements depends slightly on the environment of the probe and its polarity, the hyperfine components have been scaled to reproduce the measured nitrogen isotropic splitting, while for the g components we have used the values employed by other authors (*5*); the corrected parameters in the coordinate system assumed to diagonalize the ordering matrix are:

$$\begin{array}{ll} a = 14.9\ \mathrm{G}, & g = 2.00586, \\ A^{2,0} = (3/2)^{1/2}(A_{zz} - a) = 21.53\ \mathrm{G}, & g^{2,0} = (3/2)^{1/2}(g_{zz} - g) = -0.00387, \\ A^{2,\pm 2} = (1/2)(A_{xx} - A_{yy}) = 0, & g^{2,\pm 2} = (1/2)(g_{xx} - g_{yy}) = 0.00128. \end{array}$$

It should be pointed out that for the 5-doxyl stearic probe the z axis of the ordering matrix system is assumed to be coincident with the long molecular axis and therefore with the symmetry axis of the $2p_z$ orbital of the nitrogen atom. We take also the x axis of our coordinate system along the N–O bond. The terms $A^{2,\pm 1}$ and $g^{2,\pm 1}$ vanish for this type of radical since both g and hyperfine tensors share the same principal-axis system.

The EPR spectrum of the 5-doxyl stearic acid recorded at 22°C in the lamellar G phase of sodium decanoate, decanol and water, is shown in Fig. 2a. Spectra were slightly contaminated by the isotropic lines from a small amount of the spin probe not incorporated in the hydrophobic region of the liquid crystal and a reasonable reconstruction of the polyliquid crystalline spectrum is reported as a dotted line. The best agreement between simulated and experimental spectrum has been sought by introducing as input data the irreducible components of the magnetic interactions given previously, the parameters $\overline{P_2}$ and $\overline{P_{2,2}} \equiv \mathrm{Re}\, \overline{D^2_{0,-2}}$ determined from the positions of the spectral lines, and by optimizing the values of τ_0, $\overline{P_4}$, X_0, and X_2. For the present, probe τ_2 in practice has no effect on the spectral shape because of the cylindrical symmetry of the hyperfine tensor about the long axis of the molecule. Therefore its value has been fixed as 10^{-11} sec rad^{-1} and left unchanged during the optimization of the other parameters. We also did not find it

FIG. 2. Room temperature (a) EPR spectrum of the 5-doxyl stearic acid in the polyliquid crystalline phase of 42% decanol, 28% sodium decanoate, and 30% water, and spectra simulated using the following parameters: $\bar{P}_2 = 0.60$, $\tau_0 = 1.35 \times 10^{-9}$ sec rad^{-1}, $\tau_2 = 1 \times 10^{-11}$ sec rad^{-1}, $X_0 = 0.6$ G, $X_2 = 0.2$ G, $\bar{P}_4 = 0.11$ (b) $\bar{P}_4 = 0.25$ (c), $\bar{P}_4 = 0.11$ (d). Spectrum (d) was simulated without including nonquantization effects. In spectrum (a), which is contaminated by a small amount of spin probe not incorporated in the liquid crystal, the dotted line represents a reconstruction of the PLC spectrum.

essential to introduce deviation from effective cylindrical symmetry of the ordering matrix (i.e., $\overline{P_{2,2}}$ was found to be zero). Such a deviation was introduced by Schindler and Seelig (*5*) in their study of 4-doxyl stearic acid in the same mesophase. They also assumed that the orientational distribution function could be factorized as a product of three Maier–Saupe distributions for the three molecule-fixed axes (*22*). However, this can be misleading in view of the symmetry requirements for a uniaxial mesophase made up of noncylindrically symmetric particles. Indeed it was previously found (*23*) that this distribution might be, for a uniaxial phase made up of biaxial particles,

$$P(\alpha\beta\gamma) = P(\beta\gamma) = \exp[-U(\beta\gamma)/kT] \Big/ \int \exp[-U(\beta\gamma)/kT]\,d\gamma \,\sin\beta\, d\beta \qquad [31]$$

with

$$U(\beta\gamma)/kT = -\{a d^2_{0,0}(\beta) + b d^2_{0,2}(\beta) \cos 2\gamma\}. \qquad [32]$$

In the particularly simple case in which anisotropic intermolecular interactions are dominated by dispersion forces we have

$$a = -\alpha(\overline{P_2} + 2\lambda \overline{P_{2,2}}) \qquad [33]$$

and

$$b = 2\lambda a, \qquad [34]$$

where α is an average interaction energy and λ gives the deviation from cylindrical symmetry. For a cylindrical probe $\lambda = 0$ and then we recover the Maier–Saupe distribution. It should be noted that in the distribution given by Eq. [31] it is not possible to disentangle the dependence on the two order parameters $\overline{P_2}$ and $\overline{P_{2,2}}$. Moreover the temperature dependence of $\overline{P_{2,2}}$ is completely different from that of $\overline{P_2}$; in particular, to preserve the uniaxial symmetry of the mesophase $\overline{P_{2,2}}$ must become zero when the order is complete, i.e., $\overline{P_2} = 1$. The assumed uniaxial symmetry is, on the other hand, consistent with the general appearance of the spectrum. Indeed if the mesophase were biaxial we would expect the perpendicular lines of the spectrum to be at least partially resolved into two components.

In the optimization process, $\overline{P_4}$ was at first set equal to the value calculated from the Maier–Saupe theory after determining $\overline{P_2}$, i.e., $\overline{P_4} = 0.25$. The same procedure has been followed by Schindler and Seelig (*5*), who claim that the $\overline{P_4}$ parameter is not accessible experimentally. The simulation obtained within this assumption (see Fig. 2c) was unsatisfactory mainly in the high-field region of the spectrum, which appears broader than that in the experimental spectrum. In a second stage we relaxed this assumption and treated $\overline{P_4}$ as a parameter to be determined from the spectral shape. As is shown in Fig. 2b, the agreement with experiment is markedly improved by employing the much lower value of 0.11. A similar value was also found in an investigation on the angular dependence of monodomain spectra of this probe dissolved in the same mesophase (*2*). The deviation of $\overline{P_4}$ from predictions based on Maier–Saupe theory is not totally unexpected since we deal with nonrigid molecules. In fact, in the previous work (*2*) only the rigid cholestane probe was found to yield values of $\overline{P_4}$ in agreement with Maier–Saupe theory.

Finally we checked the importance of introducing the nonquantization of the nuclear spin along the magnetic field direction. To this purpose we simulated the experimental spectrum by neglecting this effect, all the other parameters being the same, and the resulting spectrum is shown in Fig. 2d. Although the main spectral features are obviously the same, particularly since the linewidths at 0 and 90° are not affected, the detailed shape is remarkably different and in worse agreement with experiment. This is particularly evident in the central line, where the closeness of the parallel and perpendicular absorptions makes the contribution of the intermediate orientations more important than in the case of the $m = \pm 1$ lines.

CONCLUSIONS

The present results show that the interpretation of the EPR spectra of nitroxide spin probes incorporated in nonuniformly aligned systems can be put on a reasonably rigorous basis. This is achieved by calculating the angular dependent contribution to the linewidth. We have shown that the spectra can be interpreted by assuming a simple function. This has the advantage of making explicit the dependence of the spectral shape on $\overline{P_4}$, which therefore becomes a parameter to be optimized. With a flexible spin probe, $\overline{P_4}$ is lower than the value predicted by the Maier–Saupe theory on the basis of $\overline{P_2}$ determined from the line positions. The present simulations emphasize also the importance of taking into account nonquantization effects, since the agreement with the experimental spectra can be improved without the addition of other parameters. The detailed shape of the $m = 0$ line is especially sensitive to the inclusion of this effect.

Good simulations of EPR spectra of doxyl stearic probes have been obtained previously by other authors (*5*) by neglecting nonquantization effects and using the $\overline{P_4}$ value calculated from the Maier–Saupe distribution. We suspect, however, that the good fitting is essentially due to the assumption of an angular dependent contribution to the intrinsic linewidth larger than reasonable. In fact this contribution should originate from unresolved hyperfine structure which is angularly dependent because of the anisotropy of the coupling tensors. However, as has been pointed out by Derbyshire (*24*), the anisotropy of β-proton splittings is usually small (typically less than 10% of the isotropic coupling) because of the greatly reduced value of $\langle r^{-3}\rangle$, r being the distance between a given nucleus and the unpaired electron, compared, for example, with an α proton. This should be even more true for doxyl stearic probes, where only γ or δ protons may be coupled with the unpaired electron.

Moreover it should be pointed out that for the lines due to the orientations of the director parallel to the magnetic field, whose widths can be easily measured also from the PLC spectrum as 1.56 G ($m = 1$) and 1.80 G ($m = -1$), the intrinsic contribution, in the present simulations, amounts to only 26 and 20%, respectively, of the total linewidth. On the other hand the use of the X_0 (0.65 G) and X_2 (0.55 G) values employed by Schindler and Seelig together with a linear instead of a quadratic relation for Eq. [30] would lead to an intrinsic contribution accounting for 77 and 67% of the width of the same lines. This seems too much, in the light of the previous study on the angular dependence of the linewidth of monodomain spectra of the same probe (*2*), which showed that relaxation effects are responsible for the largest part of the total width.

APPENDIX

We give here explicit expressions for the Clebsch–Gordan coefficients and Wigner functions appearing in Eqs. [21] to [23].

The Clebsch–Gordan coefficients may be calculated using the following formulas:

$$C(220;\, q\; -q) = (-)^q/5^{1/2}, \tag{A1}$$

$$C(222;\, q\; -q) = (-)^q(q^2 - 2)/(14)^{1/2}, \tag{A2}$$

$$C(224;\, q\; -q) = 24/\{(70)^{1/2}(2 + q)!(2 - q)!\}, \tag{A3}$$

$$C(22L;\, q\; -q) = C(22L;\, -qq), \tag{A4}$$

$$C(112;\, q\; -q) = (1/2)^{|q|}/(2/3)^{1/2}, \tag{A5}$$

$$C(112;\, 0 \pm 1) = 1/2^{1/2}, \tag{A6}$$

$$C(112;\, qp) = C(112;\, pq). \tag{A7}$$

The reduced Wigner functions $d^L_{mn}(\beta)$ of rank $L = 1, 2$ employed in this paper can be written most simply in terms of $c \equiv \cos\beta/2$ and $s \equiv \sin\beta/2$. They are [25]:

$$d^1_{1,1} = d^1_{-1,-1} = c^2, \tag{A8}$$

$$d^1_{1,0} = -d^1_{-1,0} = -d^1_{0,1} = d^1_{0,-1} = -2^{1/2}sc, \tag{A9}$$

$$d^1_{1,-1} = d^1_{-1,1} = s^2, \tag{A10}$$

$$d^1_{00} = 2c^2 - 1, \tag{A11}$$

$$d^2_{2,2} = d^2_{-2,-2} = c^4, \qquad [A12]$$

$$d^2_{2,1} = -d^2_{-2,-1} = -d^2_{1,2} = d^2_{-1,-2} = -2c^3s, \qquad [A13]$$

$$d^2_{2,0} = d^2_{-2,0} = d^2_{0,2} = d^2_{0,-2} = 6^{1/2}(c^2 - c^4), \qquad [A14]$$

$$d^2_{2,-1} = -d^2_{-2,1} = -d^2_{-1,2} = d^2_{1,-2} = -2s^3c, \qquad [A15]$$

$$d^2_{2,-2} = d^2_{-2,2} = s^4, \qquad [A16]$$

$$d^2_{1,1} = d^2_{-1,-1} = -3c^2 + 4c^4, \qquad [A17]$$

$$d^2_{1,0} = -d^2_{-1,0} = -d^2_{0,1} = d^2_{0,-1} = 6^{1/2}(cs - 2c^3s), \qquad [A18]$$

$$d^2_{1,-1} = d^2_{-1,1} = 3s^2 - 4s^4, \qquad [A19]$$

$$d^2_{00} = 6c^4 - 6c^2 + 1. \qquad [A20]$$

ACKNOWLEDGMENT

Financial support to this work from CNR (Rome) is gratefully acknowledged.

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