The Quadrupolar Spectrum of a Spin $I = 1$ in a Lipid Bilayer in the Presence of Paramagnetic Ions

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The NMR signal from selectively deuterated molecules in a lipid bilayer where there are paramagnetic ions present in the aqueous region is influenced by both the nuclear quadrupole interaction and the dipolar interaction between the deuterium nuclei and the surrounding ions. The quadrupolar split powder pattern is no longer symmetric about the center of the spectrum. The spectra which result from the use of the quadrupole echo pulse sequence are quite complex since this sequence does not refocus the dephasing due to the dipolar interaction. A new pulse sequence which refocuses both the dipolar and quadrupolar interactions is suggested. Both $^2$H and $^{14}$N spectra from lipid molecules with the phosphatidylcholine headgroup obtained with the conventional quadrupole echo sequence and the new sequence are compared with predictions of density matrix theory calculations. There is excellent agreement between the experimental and simulated spectra.

INTRODUCTION

The technique of wide-line deuterium nuclear magnetic resonance spectroscopy is now well established as a sensitive, nonperturbative method of probing the conformation and dynamics of lipid molecules in both model and biological membrane systems (1-3). Recently, wide-line $^{14}$N NMR spectroscopy has also been employed in studies of the polar headgroup region of choline-containing lipids such as lecithin (4, 5), sphingomyelin (6), and various lecithin analogs (7). Since the introduction of the quadrupolar echo technique (8) it has been possible to obtain undistorted (2, 3) NMR lineshapes of spin $I = 1$ nuclei such as $^2$H and $^{14}$N which are almost exclusively determined by the interaction between the electric quadrupole moment of the nucleus and the surrounding electric field gradient. In the anisotropic environment of a lipid bilayer, the NMR spectrum is a quadrupolar powder pattern. The frequency separation, $\Delta \nu_Q$, between the peaks in the spectrum is a direct measure of the orientational order.

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parameter for the symmetry axis of the electric field gradient tensor \((J=3)\). The order parameter, \(S\), reflects both the average orientation of the symmetry axis and the extent to which the quadrupolar interaction is averaged by any molecular motion. Order parameter measurements provide valuable insight into the average orientation of the symmetry axis and the degree of orientational disorder induced by molecular motion. Furthermore, changes in \(\Delta \nu_0\) as a result of the interaction of the lipid molecule with metal ions, anesthetics, and membrane bound protein \((9-11)\) have made wide-line NMR of spin \(I = 1\) nuclei an even more valuable tool for the investigation of model membrane systems.

In contrast to the usual situation for \(I = 1\) nuclei where the quadrupolar interaction dominates, the presence of paramagnetic ions may give rise to a dipolar interaction as large as, or even larger than the quadrupolar one. Such a situation was encountered when the interaction of the choline headgroup of lecithin molecules with various metals was investigated in model membrane systems \((9, 12)\). Several of the ions used in this binding study were paramagnetic, belonging to the lanthanide series. The dipole–dipole interaction between the paramagnetic spin and the quadrupolar nucleus results in a powder pattern which is no longer symmetric about the Zeeman frequency. Two examples of such spectra are shown in Fig. 1. These spectra show the differences in spectral shape which occur with the addition of paramagnetic ions.

In the next section of this paper a density matrix calculation is used to obtain expressions for the expected lineshape so that simulations of the experimental spectra

![Fig. 1. Quadrupolar spectra in lipid bilayers in the presence and absence of paramagnetic ions.](image)

(A) \(^{14}\text{N}\) spectrum of egg-PC in a EuCl\(_3\) solution at a lipid/ion ratio of 5.0 at 290 K. (B) \(^{14}\text{N}\) spectrum of egg-PC in water at 290 K. (C) \(^{2}\text{H}\) spectrum of DPPC-\(\gamma_d\) in 10 mM pipes buffer at 310 K. (D) \(^{2}\text{H}\) spectrum of DPPC-\(\gamma_d\) in a 20 mM EuCl\(_3\) solution at 310 K. In the presence of paramagnetic Eu\(^{3+}\) ions the quadrupolar powder patterns are no longer symmetric about the original Zeeman frequency. 
can be carried out. Simulations of the lineshapes obtained by using the quadrupole echo sequence \([(\pi/2),-\tau-(\pi/2),-\tau-\text{echo}]\) are also given. Since this sequence only refocuses the dephasing due to the quadrupolar interaction, severe distortion of the lineshape is predicted when the dipolar interaction is of the same order of magnitude as the quadrupolar interaction. The pulse sequence
\[(\pi/2),-\tau/2-(\pi/2),-\tau/2-(\pi/2),-\tau/2-(\pi/2),-\tau/2-\text{echo}\]
refocuses the dephasing due to both interactions and can be used to obtain undistorted spectra. Experimental results which verify the theoretical predictions are presented using a number of deuterium-labeled and \(^{14}\text{N}\)-containing lipids. All of the lipids used had the same choline headgroup.

\[
\gamma \quad \beta \quad \alpha \\
(\text{CH}_3)_2\text{NCH}_2\text{CH}_2\text{O}^{-}
\]

Samples selectively deuterated at the \(\alpha\), \(\beta\), and \(\gamma\) positions were used but in each case the hydrocarbon chains were different. The lipids were 1,2-dimyristoyl-sn-glycero-3-phosphocholine deuterated in the \(\alpha\) position, 1-palmitoyl-2-oleyl-sn-glycero-3-phosphocholine deuterated in the \(\beta\) position, and 1,2 dipalmitoyl-sn-glycero-3-phosphocholine deuterated in the \(\gamma\) position; these are abbreviated as DMPC-\(\alpha\)d2, POPC-\(\beta\)d2 and DPPC-\(\gamma\)d3. The \(^{14}\text{N}\) measurements were carried out on egg-phosphatidylcholine because a large sample was needed to have sufficient sensitivity to obtain good spectra.

**THEORY**

The total interaction Hamiltonian appropriate to the description of a spin \(I = 1\) in a lipid bilayer in the presence of paramagnetic ions is the following
\[
\mathcal{H} = H_Z + H_Q + H_D + H_{\text{ef}}
\]  
[1]

where
\[
H_Z = \hbar \omega_0 I_z \\
H_Q = (\hbar \omega_Q/3)P_2(\cos \theta)[3I_z^2 - I_x^2 - I_y^2] \\
H_D = \hbar \omega_D P_2(\cos \theta)I_x \\
H_{\text{ef}} = -\hbar \omega_1[\cos (\omega t + \phi)I_x + \sin (\omega t + \phi)I_y].
\]  
[2]

The angle \(\theta\) is between the bilayer normal and the direction of the applied magnetic field while
\[
P_2(\cos \theta) = (3 \cos^2 \theta - 1)/2.
\]  
[3]

The Zeeman frequency, \(\omega_0 = \gamma B_0\). The quadrupolar frequency is
\[
\omega_Q = (3e^2qQ/4\hbar)S
\]  
[4]

where \(e^2qQ\) is the quadrupole coupling constant and \(S\) is the order parameter for the axis of symmetry of the electric field gradient. For the case of \(^2\text{H}\), \(S\) is the order parameter for the appropriate C–D bond direction (1–3). For \(^{14}\text{N}\) in the choline head group, \(S\) is the orientational order parameter for the C\(_\beta\)–N direction (4).
The dipolar frequency, $\omega_D$, is a direct measure of the interaction between the paramagnetic ions dissolved in the aqueous regions between the lipid bilayers and the nuclear spins in the lipid molecules. It is possible to derive an approximate formula for $\omega_D$ by a simple consideration of the dipolar coupling between the resonant nucleus and the paramagnetic spin. The paramagnetic spins give rise to a magnetic susceptibility $\chi$, which is in general anisotropic. The anisotropy, however, is expected to be small since the metal ions are not complexed with surrounding ligands and are in an aqueous environment. In the aqueous region, there are $N$ equivalent paramagnetic ions and each will have a magnetic moment $\mu_s$. Consider a coordinate system where the $z$ axis is along the bilayer normal and the applied magnetic field, $B_0$, in the $xz$ plane. The magnetic moment will have the components $\mu_x = (\chi B_0/N) \sin \theta$ and $\mu_z = (\chi B_0/N) \cos \theta$. The nuclear moment $\mu_1$ is aligned along the field and located at the position $r = (x, y, z)$ with respect to the paramagnetic ion. The dipolar coupling is

$$H_D = \mu_1 \cdot \mu_s / r^3 - 3(\mu_1 \cdot r)(\mu_s \cdot r) / r^5. \quad [5]$$

The motion of the ions in solution and the lipid headgroup makes $r$ time dependent and a time average over these motions must be taken. Furthermore, since the dipolar interaction is a small perturbation on the nuclear Zeeman interaction, only terms in $I_z$ need be retained. Therefore

$$H_D = (\gamma \hbar B_0 \chi / N)[\langle (r^2 - x^2 - y^2 - z^2) / r^5 \rangle$$

$$+ \langle (x^2 - z^2) / r^5 \rangle (3 \cos^2 \theta - 1) - 6 \langle xz / r^5 \rangle \sin \theta \cos \theta] I_z \quad [6]$$

where the angular brackets indicate a time average over the molecular motion. Because of the cylindrical symmetry about the bilayer normal (the $z$ axis) the first and third terms in [6] are zero. The interaction of the nucleus with all $N$ paramagnetic ions in the sample is then

$$H_D = \gamma \hbar B_0 \chi (x^2 - z^2) / r^5 (3 \cos^2 \theta - 1) I_z \quad [7]$$

$$= \hbar \omega_D I_z P_2(\cos \theta). \quad [8]$$

It should be pointed out that $H_D$ is nonzero only because of the anisotropy of the lipid–water dispersion. In an isotropic fluid $H_D = 0$ and much smaller effects such as the contact and pseudocontact interaction (13, 14) observed in high-resolution NMR became apparent.

Finally the last term in [1] is the coupling between the applied radiofrequency field and the nuclear spins. The strength of the applied rf field is

$$\omega_1 = \gamma B_1. \quad [9]$$

The frequency of the field is $\omega$ and the phase is $\phi$.

When each of the interactions $H_Q$ and $H_D$ are treated as first-order perturbations of the Zeeman interaction, the energy levels of the total interaction Hamiltonian in the absence of $H_r$ are given by

$$E_m = -\hbar \nu_0 + (\hbar \nu_Q / 3) P_2(\cos \theta)(3m^2 - 2) + \hbar \nu_D P_2(\cos \theta) \quad [10]$$

where $m = -1, 0, +1$. These levels are shown schematically in Fig. 2. Using the
selection rule $\Delta m = \pm 1$, the allowed transitions between these levels give rise to the angular dependent resonance frequencies

$$\nu_\pm = \nu_0 \pm \nu_Q (1 \mp \delta) P_2(\cos \theta)$$

[11]

where the paramagnetic shift parameter $\delta = \nu_D/\nu_Q$ is a measure of the strength of the dipolar interaction with the paramagnetic ion relative to the quadrupolar interaction. When discussing the observed powder pattern lineshape it is convenient to use the reduced frequencies

$$\epsilon_\pm = (\nu_\pm - \nu_0)/\nu_Q = \pm (1 \mp \delta) P_2(\cos \theta).$$

[12]

As $\theta$ ranges from 0 to 90°, $\epsilon_+$ has values between 1 - $\delta$ and $(1/2)(1 - \delta)$ while $\epsilon_-$ has values between $-1 - \delta$ and $(1/2)(1 + \delta)$. Therefore, the width of the powder pattern corresponding to the $m = 0$ to 1 transition is $3/2(1 + \delta)$ where as the width of the $-1$ to 0 transition is $3/2(1 - \delta)$.

To calculate the observed NMR spectra, expressions for the free induction decay signals which appear in the two channels of the spectrometer quadrature detector ($S_x$ and $S_y$) are necessary. These can be obtained using standard density matrix techniques ($15, 16$). The two signals are given by the relations
The time evolution of the density matrix, $\rho(t)$, in the rotating reference frame is determined by the Liouville equation.

$$
\frac{d\rho(t)}{dt} = (i/\hbar)[\rho(t), H^*].
$$

$H^*$, the Hamiltonian in the frame rotating at the frequency of the applied rf field, is

$$
H^* = -\hbar w D I_z + \frac{\hbar \omega Q}{3}(3I_z^2 - I^2) + \hbar \omega_1 \cos \phi I_1 - \hbar \omega_1 \sin \phi I_y.
$$

Expressions for $\rho(t)$ from [14] must be obtained in two quite different situations. In the first case $\omega_1 \gg \omega_Q, \omega_D$, or $\Delta \omega$. In essence, the rf pulses are considered to be $\delta$ functions and the influence of the quadrupolar or dipolar interactions can be neglected during the pulses. Between and following the rf pulses, the spin system evolves under the influence of $H^*$ where $\omega_1 = 0$. For the purposes of this paper it is sufficient to consider the case where $\Delta \omega$ is nearly zero. In addition, the effects of spin–spin relaxation due to magnetic dipolar interactions between nuclei, the time-dependent quadrupole interaction or static inhomogeneity in the quadrupole interaction will be taken care of by introducing an exponential decay with a time constant $T_2$. Spin–lattice relaxation effects will be completely neglected. The free induction decay signals are given by

$$
S_x(t) = K e^{-t/T_2} \int_{-1}^{1} \cos \left\{ (\omega_D P_2(\mu) + \Delta \omega)t \right\} \cos \left\{ \omega_Q P_2(\mu)t \right\} d\mu
$$

$$
S_y(t) = K e^{-t/T_2} \int_{-1}^{1} \sin \left\{ (\omega_D P_2(\mu) + \Delta \omega)t \right\} \cos \left\{ \omega_Q P_2(\mu)t \right\} d\mu
$$

where $\mu = \cos \theta$ and the integral over $\mu$ creates the observed powder pattern. The spectrum is obtained by taking the complex Fourier transform of $S_x$ and $S_y$.

Examples of powder pattern spectra to be expected for various values of $\omega_Q/\omega_0 = \delta$ are shown in Fig. 3. The spectra for $\omega_D \neq 0$ are no longer symmetric about the Zeeman frequency. Notice that when $\delta = 1/3$ the high-frequency shoulder disappears and when $\delta \rightarrow 1$ the powder pattern corresponding to the $-1$ to $0$ transition collapses to a very narrow line. As well, it should be noted that the $\Delta \nu_Q$, defined as the frequency separation between the peaks corresponding to $90^\circ$ orientations of the bilayer normal, does not vary as $\delta$ changes. The origin of the asymmetry in the spectra lies in the fact that the lineshape is composed of two overlapping powder patterns corresponding to each of the two allowed single-quantum transitions. The interaction with the paramagnetic ions increases the width of one pattern at the expense of the other.

Because of the usual receiver deadtime problems [8], it is impossible to get undistorted spectra from observations of the free induction decay signal. To circumvent this problem an echo sequence is usually employed and the spectrum obtained by Fourier transforming the nuclear signal starting at the top of the echo. The quadrupole echo sequence $(\pi/2)_x - \tau - (\pi/2)_y - \tau - $echo yields the following signals at a time $t$ starting from the top of echo:

$$
S_x(t) = K e^{-t/T_2} \int_{-1}^{1} \cos \left\{ (\omega_D P_2(\mu) + \Delta \omega)t \right\} \cos \left\{ \omega_Q P_2(\mu)t \right\} d\mu
$$

$$
S_y(t) = K e^{-t/T_2} \int_{-1}^{1} \sin \left\{ (\omega_D P_2(\mu) + \Delta \omega)t \right\} \cos \left\{ \omega_Q P_2(\mu)t \right\} d\mu
$$

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Simulations of the quadrupolar spectra for various sizes of the paramagnetic dipole interaction. These spectra are derived from the Fourier transform of the free induction decay signals. In each case $v_0 = 10$ kHz, $T_2 = 10$ msec, and the ratio of $v_D/v_0$ is indicated to the left of each spectrum.

\[
S_2^E(t) = Ke^{-\left(t+\tau\right)/T_2} \int_{-\infty}^{\infty} \cos \left\{ \left(\omega_D P_2(\mu) + \Delta \omega\right) \tau \right\} \\
\times \cos \left\{ \left(\omega_D P_2(\mu) + \Delta \omega \right) \left(t + \tau\right) \right\} \cos \left\{ \omega_Q P_2(\mu \tau) \right\} d\mu
\]

\[
S_2^F(t) = Ke^{-\left(t+\tau\right)/T_2} \int_{-\infty}^{\infty} \cos \left\{ \left(\omega_D P_2(\mu) + \Delta \omega\right) \tau \right\} \\
\times \sin \left\{ \left(\omega_D P_2(\mu) + \Delta \omega \right) \left(t + \tau\right) \right\} \cos \left\{ \omega_Q P_2(\mu \tau) \right\} d\mu. \tag{17}
\]

Simulations of the quadrupolar echo spectra for $\omega_D/\omega_Q = 0.25$ are shown in Fig. 4. The changes in spectral shape with the spacing between the pulses, $\tau$, are very noticeable. The distortion at large $\tau$ values arises because the quadrupolar echo sequence does not refocus the dipolar interaction in the way that it refocuses the quadrupole interaction. It is apparent that there is a dephasing of the nuclear signal which varies across the spectrum.
To refocus both the dipolar and quadrupolar interactions it is necessary to add two additional pulses to the quadrupole echo sequence. The new sequence is

\[(\pi/2)_x - \tau/2 - (\pi)_{y} - \tau/2 - (\pi/2)_{y} - \tau/2 - (\pi)_{y} - \tau/2 - \text{echo}\]

and the signals at a time \(t\) starting from the peak of the echo are

\[
S_x^N(t) = Ke^{-t(t+2\tau)/T_2} \int_{-1}^{1} \cos \{(\omega_D P_2(\mu) + \Delta \omega)t\} \cos \{\omega_Q P_2(\mu)t\} d\mu
\]

\[
S_y^N(t) = Ke^{-t(t+2\tau)/T_2} \int_{-1}^{1} \sin \{(\omega_D P_2(\mu) + \Delta \omega)t\} \cos \{\omega_Q P_2(\mu)t\} d\mu. \tag{18}
\]

These expressions have the same form as those for the free induction decay [16] except that the amplitude is reduced because of the relaxation term \(\exp(-2\tau/T_2)\).

It is possible to understand how this sequence works when it is recognized that the first \((\pi)_{y}\) pulse refocuses the dephasing due to the dipolar interaction at the time that the \((\pi/2)_{y}\) pulse is applied. The \((\pi)_{y}\) pulse, however, does not affect the dephasing
due to the quadrupolar interaction. At the time of the echo, dephasing due to the quadrupole interaction is refocused by the \((\pi/2)\) pulse and the dephasing due to the dipolar interaction which occurs following the \((\pi/2)\) pulse is refocused by the second \((\pi)\) pulse. The result is that effects due to both the quadrupolar and dipolar interactions are refocused and the amplitude of the echo only decays at a rate given by \(T_2\).

### APPARATUS AND SAMPLES

The \(^{14}\text{N}\) NMR spectra were obtained at a frequency of 19.4 MHz using a home-built spectrometer \((17)\) and a Bruker superconducting solenoid. The \(^2\text{H}\) spectra were obtained on a Bruker CXP-300 spectrometer at a frequency of 46.1 MHz. Typical 90° pulse widths were 8 \(\mu\)sec in the case of \(^{14}\text{N}\) and 5 \(\mu\)sec for \(^2\text{H}\) spectra. The spin–lattice relaxation times for all the samples were less than 50 msec so that spectra could be accumulated at a rate of four times a second. For the \(^2\text{H}\) spectra about 5000 scans were averaged while for the \(^{14}\text{N}\) spectra 100,000 scans were required.

The egg-PC was prepared by the method of Singleton \textit{et al.} \((18)\) and its concentration was determined using phosphorus analysis assuming a molecular weight of 770. DMPC-\(\alpha_d\), POPC-\(\beta_d\), and DPPC-\(\gamma_d\) were synthesized using methods described in reference \((19)\). Aliquots of the lipid molecules in CHCl\(_3\) were evaporated to dryness, followed by exposure to high vacuum for at least 24 hr. A egg-PC sample was mixed with a EuCl\(_3\) solution made with deuterium depleted water so that the final water concentration was 50% by weight and the lipid/ion ratio was 5.0. A second egg-PC sample was made with TmCl\(_3\) in a similar way with a lipid/ion ratio of 1.6. For the \(^2\text{H}\) labeled lipids a large excess of 20 \(\text{mM}\) EuCl\(_3\) was mixed with about 40 mg of lipid. The mixture was vortexed and then centrifuged. The lipid dispersion formed a loose pellet at the bottom of the tube and some of the clear supernatant was removed. The final water concentration was about 90% in each case.

### EXPERIMENTAL RESULTS AND DISCUSSION

In Fig. 5I examples of spectra for nuclei located at four different positions in the choline headgroup are shown for the situation where there is EuCl\(_3\) in the aqueous region. Simulations of these spectra are shown in Fig. 5I and the parameters used in the simulations are listed in the figure caption. In general the simple theory given above can be used to obtain a very good fit to the observed lineshapes. The intensity of the shoulder in the spectra arising from those domains in the sample oriented parallel to the bilayer normal is, however, too large in comparison to the peaks arising from the domains perpendicular to the bilayer normal especially in examples B and C. The exact origin of the discrepancy is not known at present. The lineshape of \(I = 1\) spectra in lipid bilayers, however, is not a simple Lorentzian broadened quadrupolar powder pattern as has been assumed even in the absence of paramagnetic ions. There are molecular motions with correlation times of the order of \(1/\Delta \nu_0\) which can cause marked deviations from the simple behaviour expected from the theory given above \((20)\). For the purposes of this paper the fitting procedure is adequate since it has been demonstrated that it is both the quadrupolar and paramagnetic shift interactions which determine the important features of the observed lineshape.

A comparison of the parameters for the three \(^2\text{H}\) spectra is possible since the Eu\(^{3+}\)
Quadrupolar spectrum of spin \( I = 1 \) in bilayer

**Fig. 5.** (I) Examples of quadrupolar spectra in the presence of paramagnetic ions. Spectra for four positions in the choline headgroup are shown. The spectra were obtained using the quadrupole echo sequence with the \( \tau \) values indicated. (A) \(^1\)H spectrum of DMPC-\( \alpha \) in a 20 mM EuCl\(_3\) solution at 310 K with \( \tau = 60 \) \( \mu \)sec. (B) \(^1\)H spectrum of POPC-\( \beta \) in a 20 mM EuCl\(_3\) solution at 310 K with \( \tau = 40 \) \( \mu \)sec. (C) \(^1\)H spectrum of DPPC-\( \gamma \) in a 20 mM EuCl\(_3\) solution at 318 K with \( \tau = 50 \) \( \mu \)sec. (D) \(^14\)N spectrum of egg-PC in a EuCl\(_3\) solution at a lipid/ion ratio of 5.0 at 290 K with \( \tau = 120 \) \( \mu \)sec. (II) Simulations of the spectra using Eq. [17] and the parameters listed below:

<table>
<thead>
<tr>
<th>Quadrupolar splitting (kHz)</th>
<th>Paramagnetic shift (kHz)</th>
<th>( T_2 ) (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.6</td>
<td>0.55</td>
</tr>
<tr>
<td>B</td>
<td>6.25</td>
<td>0.35</td>
</tr>
<tr>
<td>C</td>
<td>1.18</td>
<td>0.37</td>
</tr>
<tr>
<td>D</td>
<td>9.2</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Concentration is the same for each and the lipids only differ in the composition of the fatty acyl chains. The choline headgroup is common to each and the binding of the paramagnetic ion will be similar in each case. The strength of the paramagnetic ion interaction is definitely largest for the \( \alpha \) carbon position as expected since it is closest to the phosphate binding site. The shift parameters for the \( \beta \) and \( \gamma \) positions are about the same indicating that there is significant population of those \( C_\alpha-C_\beta \) gauche conformations which bring the methyl deuterons to a position relatively close to the phosphate group.
The spectra in Fig. 5 show little of the dephasing due to the use of the quadrupolar echo (expected from Fig. 4). The reason, of course, is that $\tau$ is relatively small in each case and the dephasing due to the paramagnetic shift does not become important. If $\tau$ is made larger are the distorted quadrupolar spectra observed experimentally? Figure 6 shows two examples. The extreme example shown in Fig. 4 is not observed because the spectra decay rapidly in amplitude with increasing $\tau$ and it is difficult to obtain good spectra at large values of $\tau$. However, the spectra do adequately show that the dephasing effects due to the use of the quadrupolar echo sequence do in fact occur and the simulations are again quite good.

As an example of a situation where a very severe distortion of the spectrum occurs due to use of the quadrupolar echo, Fig. 7 shows spectra for $^{14}$N in egg-PC with Tm$^{3+}$ ions at a lipid to ion ratio of 1.6. The actual spectra on the left, show the very large changes which take place with increasing $\tau$ value in the case where $\omega_0$ is a significant fraction of $\omega_Q$. Nevertheless, the theory presented above leads to a very good description of these effects as the simulations in Fig. 7 show.

![Fig. 6](image_url)

**Fig. 6.** The influence of increasing the spacing between the two pulses, $\tau$, in the quadrupole echo sequence. (A) Spectra of DMPC-$\alpha_{12}$ in a 20 mM EuCl$_3$ solution at 310 K for the $\tau$ values indicated. (B) Spectra of POPC-$\beta_{12}$ in a 20 mM EuCl$_3$ solution at 310 K for the $\tau$ values indicated. (C) Simulations of the spectra in (A). Parameters used in the simulations were $\nu_0 = 2.6$ kHz, $\nu_D = 0.55$ kHz, and $T_2 = 2$ msec. (D) Simulations of the spectra in B. Parameters used in the simulations were $\nu_0 = 6.25$ kHz, $\nu_D = 0.38$ kHz, and $T_2 = 2$ msec.
The distortion in the quadrupolar echo spectra at large $\tau$ values can be eliminated with the use of the sequence

$$(\pi/2)_x - \tau/2 - (-\pi)_y - \tau/2 - (\pi/2)_y - \tau/2 - (-\pi)_y - \tau/2 - \text{echo}.$$
An experimental demonstration of the use of this sequence is shown in Fig. 8. When \( \omega D \tau < 1 \) as is the case in Figs. 8A and B there is little dephasing due to the paramagnetic shift interaction and the two pulse sequences give virtually the same result. At larger \( \tau \) values the influence of the dephasing is seen in the spectra obtained with the quadrupolar echo sequence (Fig. 8C). The new sequence restores the spectrum back to the shape found at small \( \tau \) values as can be seen in Fig. 8D because it refocuses both the quadrupolar and dipolar interactions.

CONCLUSIONS

When paramagnetic ions are present in an anisotropic fluid the dipolar coupling with spin \( J = 1 \) nuclei may be as large as or even larger than the quadrupolar interaction. The resulting spectra are no longer symmetric about the Zeeman frequency. The strength of the quadrupolar interaction can still be obtained from the separation

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**Fig. 8.** Spectra taken with the quadrupole echo sequence and with the new sequence \((\pi/2)_2-\tau/2-(\pi)_2-\tau/2-(\pi/2)_2-\tau/2-(\pi)_2-\tau/2-\text{echo}\). The sample is DMPC-<code>echo</code> in a 20 mM EuCl<sub>3</sub> solution at 310 K. (A) Spectrum obtained with the quadrupole echo and \( \tau = 60 \mu\text{sec} \). (B) Spectrum obtained with new sequence and \( \tau = 60 \mu\text{sec} \). With \( \tau = 60 \mu\text{sec} \) \( \omega_D \tau < 1 \) and there is little difference in the two spectra since the dephasing due to the paramagnetic shift has not had time to develop. (C) Spectrum with quadrupole echo and \( \tau = 300 \mu\text{sec} \). (D) Spectrum with new sequence and \( \tau = 300 \mu\text{sec} \). Now \( \omega_D \tau \approx 1 \) and significant differences are seen in the two spectra. The new sequence restores the spectral shape to that obtained with small \( \tau \) values.
between the 90° peaks while the positions of the shoulders in the powder pattern determine the size of the dipolar interaction. The echo sequence usually employed for \( I = 1 \) nuclei in lipid bilayer studies causes distortions of the spectral lineshape when the separation between the pulses is of the order of the reciprocal of the dipolar frequency. Special care must be taken if the usual quadrupole echo sequence is used. It is possible to miss the signal completely since there are combinations of \( \Delta \omega \), the offset frequency and \( \tau \), the spacing between the pulses where there is no intensity in the echo signal.

There can be no doubt that the quadrupolar spectra observed in the presence of paramagnetic ions are influenced by the strong dipolar interaction between the ions and the quadrupolar nuclei. If both the quadrupolar and dipolar interactions are to be refocused then one sequence which can be used in place of the usual quadrupolar echo sequence is the following

\[
\frac{\pi}{2}, -\tau/2 - \frac{\pi}{2}, -\tau/2 - \frac{\pi}{2}, -\tau/2 - \frac{\pi}{2}, -\tau/2 - \frac{\pi}{2} - \text{echo.}
\]

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