DEUTERIUM NUCLEAR MAGNETIC RESONANCE STUDY OF WATER IN MODEL AND BIOLOGICAL MEMBRANE SYSTEMS

by

C. M. WEI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

in

THE DEPARTMENT OF PHYSICS

in

THE FACULTY OF GRADUATE STUDIES

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

October, 1979

C. M. Wei, 1979
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of

Physics

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date Oct. 12, 79
Abstract

A deuteron magnetic resonance study of water has been carried out in the lamellar phase of the egg yolk lecithin-water and outer membrane of E. Coli-water systems in excess water (abbreviated to EYL/EXCD$_2$O and EC/EXCD$_2$O, respectively) and egg yolk lecithin-water in 22% (by weight) water (EYL/22%WD$_2$O). Spectra of these systems were taken as a function of temperature, and their moments were calculated. Analysis of the integrated signal intensities revealed that the excess free (bulk) water in the EC/EXCD$_2$O and EYL/EXCD$_2$O froze at -1°C and -2°C, respectively (pure D$_2$O freezes at 4°C). The water from bilayers in the two excess water systems was frozen immediately after it was squeezed out, whereas the water squeezed out from bilayers in the EYL/22%WD$_2$O remained unfrozen down to -10°C. All the squeezed out water in the EYL/22%WD$_2$O was frozen at -15°C. The bound water in that system was unfreezable in the region under study. The amount of water frozen out in the EC/EXCD$_2$O at -2°C and in the EYL/EXCD$_2$O at -3°C was found to be approximately 85% of the total water content of the systems. The water frozen out in the EYL/22%WD$_2$O at -15°C was determined to be 50% of the total water in that system. A minimum in the second moment vs temperature of the EYL/22%WD$_2$O was observed and ascribed to the presence of isotropic free water squeezed out from the bilayers. Proton magnetic resonance results showed that there was no lipid phase transition in the EYL/22%WD$_2$O in the region from -10°C to 48°C.
Table of Contents

Abstract ii
List of Figures vi
Acknowledgements ix

Chapter

1 Introduction

1.1. Functions of Biological Membranes 1
1.2. Structure of Biological Membranes 1
1.3. Model Membranes 5
1.4. Membrane Fluidity and Transport Carriers 5
1.5. Involvement of Lipid Component of Membrane in Phase Transition 6
1.6. Biological Significance of Phase Transition in Membrane System 7
1.7. Study of Membrane System by NMR Technique 8
1.8. Water in Membrane System 9
1.9. Motivation for the Research Project 11

2 NMR Theory For Nuclei In Lyotropic Amphiphilic Liquid Crystal/Water System

2.1. Basic Principles of Nuclear Magnetic Resonance 12
2.2. Quadrupole Interactions and The First Order Perturbation 12
2.3. Deuterium Magnetic Resonance 15
   a) Deuteron on the Hydrocarbon Chain 15
   b) DMR on $\text{D}_2\text{O}$ 21
2.4. Proton Magnetic Resonance 22
   a) A Two-Spin System 23
b) PMR on a protiated Lipid System

3 Experimental

3.1. The Materials
3.2. Samples Preparation
3.3. NMR Apparatus
3.4. NMR Measurements

4 The Results

4.1. DMR Results
   a) Quadrupole Splittings
   b) Evaluations of the DMR spectra in terms of their moments

4.2. PMR Results

4.3. Sources of Error

5 Discussion And Conclusion

5.1. DMR Spectra of $D_2O$
   a) Spectra of $D_2O$ in the EYL/EXCD$_2$O and EC/EXCD$_2$O systems
   b) Spectra of $D_2O$ in the EYL/22%WD$_2$O system

5.2. The Moments of the DMR Spectra
   a) The integrated signal intensities ($M_o$) of the DMR spectra of $D_2O$ in the EYL/EXCD$_2$O and EC/EXCD$_2$O systems
   b) The integrated intensity of the DMR spectra of $D_2O$ in the EYL/22%WD$_2$O system
   c) The first and the second moments of the DMR spectra of $D_2O$ in the EYL/EXCD$_2$O and EC/EXCD$_2$O systems
   d) The first and second moments of the DMR spectra of $D_2O$ in the EYL/22%WD$_2$O
   e) The temperature dependence of the $\Delta_2$ and the $M_4/M_2^2$ for the EYL/22%WD$_2$O, EYL/EXCD$_2$O and EC/EXCD$_2$O

Page

25
27
27
27
28
30
30
31
31
33
33
39
42
44
44
55
60
5.3. Comparison With Other Work

5.4. PMR Results For The EYL/22%W\textsubscript{2}O

Appendix A Moments Of Nuclear Magnetic Resonance Spectra

Appendix B Contributions To The Second Moment

References
<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td>7</td>
<td>37</td>
</tr>
<tr>
<td>8</td>
<td>38</td>
</tr>
<tr>
<td>9</td>
<td>41</td>
</tr>
<tr>
<td>10</td>
<td>43</td>
</tr>
<tr>
<td>11</td>
<td>45</td>
</tr>
<tr>
<td>12</td>
<td>50</td>
</tr>
<tr>
<td>13</td>
<td>51</td>
</tr>
</tbody>
</table>

A schematic representation of dipolmitoyl-3-sn-phosphatidylcholine lipid molecule.

A schematic representation of lamellar and vesicular structures in a lipid-water system.

A schematic representation of the geometry and the coordinate system in a lipid bilayer.

Theoretical powder pattern for a deuterium nucleus.

Energy levels and the corresponding spectrum of a two-spin system oriented at an angle $\theta$.

Logarithmic PMR lineshape as derived by Bloom et al.

DMR spectra of $D_2O$ in the E. Coli/EXCD$_2$O obtained at (a) $20^\circ$C, (b) $4^\circ$C, (c) $-1^\circ$C, (d) $-2^\circ$C.

DMR spectra of $D_2O$ in the EYL/EXCD$_2$O obtained at (a) $18^\circ$C, (b) $4^\circ$C, (c) $-2^\circ$C, (d) $-3^\circ$C.

DMR spectra of $D_2O$ in the EYL/22%WD$_2$O obtained at (a) $25^\circ$C, (b) $4^\circ$C, (c) $-3^\circ$C, (d) $-4^\circ$C, (e) $-5^\circ$C, (f) $-10^\circ$C, (g) $-15^\circ$C, (h) $-20^\circ$C, (i) $-25^\circ$C.

Temperature dependence of the integrated signal intensity, $M_0$, of DMR of $D_2O$ in the EYL/EXCD$_2$O and the E. Coli/EXCD$_2$O.

Temperature dependence of the integrated signal intensity, $M_0$, of DMR of $D_2O$ in the EYL/22%WD$_2$O.

Temperature dependence of the first moment $M_1$ of the DMR spectrum of $D_2O$ in the EYL/EXCD$_2$O and E. Coli/EXCD$_2$O.

Temperature dependence of the second moment $M_2$ of the DMR spectrum of $D_2O$ in the EYL/EXCD$_2$O and E. Coli/EXCD$_2$O.
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Second moments of the DMR spectra of D$_2$O in the EYL/EXCD$_2$O plotted against that in the EYL/22%WD$_2$O.</td>
<td>52</td>
</tr>
<tr>
<td>15</td>
<td>Temperature dependence of the line width $\Delta \nu$ of the DMR spectrum of D$_2$O in the E. Coli/EXCD$_2$O and EYL/EXCD$_2$O.</td>
<td>53</td>
</tr>
<tr>
<td>16</td>
<td>Temperature dependence of the fourth moment $M_4$ of the DMR spectrum of D$_2$O in the EYL/EXCD$_2$O and E. Coli/EXCD$_2$O.</td>
<td>54</td>
</tr>
<tr>
<td>17</td>
<td>Temperature dependence of the first and the fourth moments, $M_1$, $M_4$, of the DMR spectrum of D$_2$O in the EYL/22%WD$_2$O.</td>
<td>58</td>
</tr>
<tr>
<td>18</td>
<td>Temperature dependence of the second moment $M_2$ of the DMR spectrum of D$_2$O in the EYL/22%WD$_2$O.</td>
<td>59</td>
</tr>
<tr>
<td>19</td>
<td>Temperature dependence of the ratio $M_4/M_2^2$ and the relative mean square deviation $\Delta_2$ of the DMR spectrum of D$_2$O in the E. Coli/EXCD$_2$O.</td>
<td>61</td>
</tr>
<tr>
<td>20</td>
<td>Temperature dependence of the ratio $M_4/M_2^2$ and the relative mean square deviation $\Delta_2$ of the DMR spectrum of D$_2$O in the EYL/EXCD$_2$O.</td>
<td>62</td>
</tr>
<tr>
<td>21</td>
<td>Temperature dependence of the relative mean square deviation $\Delta_2$ and the ratio $M_4/M_2^2$ of the DMR spectrum of D$_2$O in the EYL/22%WD$_2$O.</td>
<td>63</td>
</tr>
<tr>
<td>22</td>
<td>Temperature dependence of the quadrupole splitting $\Delta \nu$ which is measured directly from the DMR spectrum of D$_2$O in the EYL/22%WD$_2$O.</td>
<td>65</td>
</tr>
<tr>
<td>23</td>
<td>Temperature dependence of the ratio $M_4/M_2^2$ and the second moment of the PMR spectrum of the EYL/22%WD$_2$O.</td>
<td>68</td>
</tr>
</tbody>
</table>
Temperature dependence of the second moment of PMR spectrum of (a) the outer membrane of E. Coli in the EC/EXCD₂O, (b) the EYL in the EYL/EXCD₂O, (c) temperature dependence of \( M_4/M_2^2 \) of the PMR spectrum of the EYL in the EYL/EXCD₂O.

(a) Second moments vs temperature of the DMR spectra of outer membranes of E. Coli grown on a medium containing perdeuterated palmitic acid and oleic acid as well as perdeuterated palmitic acid. (b) The parameter \( \Delta \nu \) vs temperature of the DMR spectra of the outer membranes of E. Coli grown on a medium containing oleic acid as well as perdeuterated palmitic acid.
Acknowledgements

First and foremost, I wish to acknowledge the patience and enthusiasm of my supervisor, Dr. Myer Bloom, who has greatly enriched my masters experience. I have benefited greatly from his instruction and the many discussions we have had.

I am very grateful to Dr. Alex Mackay and Dr. James H. Davis for the technical consultations and help in the experiments and for the many fruitful discussions and useful suggestions. In this regard the continued support of Alex in many other respects is greatly appreciated.

Finally, I thank my wife, May San, for typing the thesis and for her patience and continuing encouragement and moral support.
Chapter 1

Introduction

1.1. Functions of Biological Membranes

Every living cell is enclosed by a membrane that serves not only as a sturdy enclosure inside which the cell can function, but it can also perform a large number of biological functions. For instance, it can function as a discriminating gate, enabling nutrients and other essential agents to enter and waste products to leave. The cytoplasmic cell membrane can "pump" substances from one side of the membrane where such substance's concentration is low to the other where it is much higher. Thus the cytoplasmic membrane selectively regulates the flux of nutrients and ions between the cell and its external environment. The cells of higher organisms have, in addition to a cytoplasmic membrane, a number of internal membranes that isolate the structures termed organelles, which play various specialized roles (1).

1.2. Structure of Biological Membranes

All of the biological membranes mentioned above are remarkably similar in their basic structural features. It is clear that any model formulated to describe membrane structures must be able to account for such an extraordinary range of functions.

Membranes are composed almost entirely of two classes of molecules: proteins and lipids (polysaccharides etc. are also associated with biological membranes). The proteins provide the membrane with its distinctive functional properties, whereas the lipids give the gross structural properties of the membrane (1). A dipalmitoyl-3-sn-phosphatidylcholine (DPPC) lipid molecule is shown in Fig. 1. The lipids found in membranes are
Figure 1. A schematic representation of Dipalmitoyl-3-sn-phosphatidylcholine lipid molecule.
amphipathic, meaning that one end of the molecule is hydrophobic, or insoluble in water, and the other end hydrophilic, or water-soluble. The hydrophilic region of the lipid molecule is polar, and the hydrophobic region is non-polar. In most membrane lipids the nonpolar region consists of hydrocarbon chains of fatty acids: hydrocarbon molecules with a carboxyl group (COOH) at one end. In a typical membrane lipid two fatty acid molecules are chemically bonded through their carboxyl ends to a backbone of glycerol. The glycerol backbone, in turn, is attached to a polar head group consisting of phosphate and other groups. Phosphate-containing lipids of this type are called phospholipids, which are found in all membranes.

Because the two parts of a membrane lipid have incompatible solubilities, the lipid molecules in the presence of water spontaneously organize themselves in the form of a variety of lyotropic mesophases characterized by the existence of long range order and short range disorder (1-4). The convincing evidence for the existence of these mesophases have been provided by X-ray studies (5), nuclear magnetic resonance (NMR) studies (6-7), and other physicochemical techniques (8-12).

Of particular interest is the lamellar liquid crystal (L\(_\alpha\)) phase where the lipid molecules form bilayers and alternate in a regular lattice with layers of water and counter ions as shown in Fig. 2. In this way the hydrophobic region of each molecule is shielded from water, whereas the hydrophilic polar head groups find themselves in a lower electrostatic energy by associating intimately with water. The hydrocarbon chains associate themselves in the form of bilayers as a result of the above interactions and the attractive Van der Waal's forces.

Most bilayers in lipid-water system in lamellar liquid crystalline phase naturally arrange themselves into an onion ring configuration, so
Figure 2. (a) The phospholipid bilayer forms the fundamental structural matrix of a membrane. The lipids are arranged chain to chain so that only the hydrophilic polar heads (the circles) are exposed to the aqueous solution on both sides of the membrane. Lipid molecules can diffuse laterally at a high frequency, but can rarely execute a flip-flop transition from one layer to the other. The wiggly lines represent the hydrocarbon chain.
(b) Spherical bilayer (vesicle).
that nowhere in the bilayers the hydrophobic tails are in contact with water. These bilayers form the basic structure of most biological membranes.

1.3. **Model Membranes**

Due to the complexity of real biological membranes, the NMR spectrum obtained is a superposition of a large number of powder patterns which are not well resolved. Thus, in order to understand the fundamental physical properties which determine the proper function of membranes, workers in the field look for simpler membrane systems that could be used as "models". Since lipids are the fundamental "building blocks" of the cell membrane, model membrane systems have been made with lipids obtained from natural sources such as egg yolk lecithin (13-14) as well as from synthetic techniques such as DPPC (15). These model membranes are similar (not identical) in their structural properties to biological membranes and they can be much better characterized physically and chemically than membranes from living cells, which have a complicated assortment of different lipids. The phase transitions are also much sharper with purified lipids of a single homogenous type, this makes quantitative measurement and theoretical interpretation much easier. Furthermore, NMR spectra given by model membranes are simpler to interpret than those given by natural ones. There have been numerous studies on model and biological membranes concerning the importance of the hydrophobic parts of the phospholipid molecules in determining the general properties of the interior of the phospholipid bilayer (5-7, 16).

1.4. **Membrane Fluidity and Transport Carriers**

Since the phenomenon of life occurs inside the cell, which is enclosed by a membrane, how do substances pass through the membrane to provide the cell with life-supporting nutrients? The hydrophobic nonpolar fatty acid
hydrocarbon chain region of a phospholipid bilayer is physically incompatible with small water-soluble substances such as metal ions, sugars and amino acids, and thus acts as a barrier through which they cannot flow freely. Bangham et al of the Agricultural Research Council in Cambridge, England, and Chappell et al of the University of Cambridge (17) measured the rate at which glucose passes through the phospholipid-bilayer walls of liposomes (vesicles) and found that it was far too low to account for the rate at which glucose penetrates biological membranes. They demonstrated that a highly selective carrier protein exists in biological membranes to facilitate the passage of metal ions and small polar molecules through the permeability barrier presented by the phospholipid bilayer.

Since transport carriers must be mobile in order to move substances from one side of the cell membrane to the other, it is necessary for the region containing the fatty acid chains to have a high degree of fluidity in which each bilayer behaves as a two-dimensional fluid with the lipid chains preferentially oriented along the normal to the bilayer surface. Within the bilayer the hydrocarbon chains of the lipid molecules are flexible (melted) and the molecules undergo rapid lateral diffusion and rotation about their long axis. Different parts of the hydrocarbon chain can also undergo small and rapid angular excursions such as bending, twisting and flopping perpendicular to the molecular axis (the long axis of the molecule). X-ray diffraction patterns of membrane systems above the transition temperature are diffuse and quite similar to those obtained from long-chain liquid hydrocarbons found in paraffin, which indicates that the fatty acids of membranes are in fact disordered at physiological temperature (5).

1.5. Involvement of Lipid Component of Membrane in Phase Transition

Experiments have conclusively demonstrated that the phase transition
in membrane systems (such as Acholeplasma laidlawii and model membranes) is due exclusively to the lipid component of the membrane (18-19). Therefore physical techniques that elucidate structure, and theories that deal with phases are appropriate tools to study this phenomenon. The basic nature of the phase transition in multibilayer dispersions is revealed by calorimetry, X-ray diffraction and nuclear magnetic resonance (16-18). Calorimetric data (18-19) showed that the transitions in lipid bilayers mainly involve hydrocarbon-chain disordering (phase transitions involving structural changes in lipid bilayers such as lamellar to hexagonal phase transition had been observed by X-ray technique (5)).

X-ray diffraction and other physical techniques studies show that, with low and varying water concentration, lipids have a rich variety of phase behaviour in addition to the phase transition in the presence of excess water, which is of primary biological interest (18).

1.6. Biological Significance of Phase Transition in Membrane System

The order–disorder change in the dynamical state of hydrocarbon chains in bilayer membranes gives rise to a phase transition that occurs in many cell membranes as well as model membranes. Much work (18) has been done on the cytoplasmic membrane of Acholeplasma laidlawii, which is a primitive organism with a large surface-to-volume ratio. After the cell was grown, at a particular temperature $T_g$, the membrane was extracted and calorimetric measurements were made. Data show a specific heat anomaly, about 20°C broad, centered near or slightly below $T_g$. When these cells were grown at different growth temperatures $T_g'$, the calorimetric anomaly is shifted towards the temperature $T_g'$. This suggests that the phase transition is not just some physical phenomenon that happens to occur, but that it has real biological relevance. There are many other examples
of physically induced biological changes related to the membrane phase transition. Organisms that exist in cold environments have membrane components giving rise to reduced phase-transition temperatures.

1.7. **Study of Membrane System by NMR Technique**

Nuclear magnetic resonance is a very useful technique for studying the structure and the dynamical state of the membrane systems (3, 20). By structure, we mean the average orientation of the hydrocarbon chains, the polar groups, and the amplitudes of fluctuation of the lipid segments. The study of the dynamical state of a membrane system is concerned with the rate of segmental motions and the rate of diffusion of the lipids within each monolayer. In terms of experimental NMR parameters, the problems of membrane structure and dynamical properties of the bilayer are solved if the complete set of segmental second rank order parameter tensors (see Section 2.3a, Chapter 2) and the relaxation times are measured, and if a consistent molecular interpretation of the experimental data can be provided. The order parameter $S_{CD}$ of a deuterium bond vector and, for a special case, $S_{HH}$ of a H-H bond vector can be measured from the quadrupole splitting, $\Delta v_{Q}$, in the deuterium nuclear magnetic resonance (DMR) spectrum and from the nuclear dipolar splitting in the proton magnetic resonance (PMR) spectrum respectively, since they are directly proportional to the splittings (Appendix A). The order parameter can then be interpreted in terms of statistical models for the bilayer structure. The onset of motions or the phase transition from one form of lattice structure to the other with a higher degree of lattice symmetry (such as a cubic phase) will, due to motional averaging, reduce the order parameters $S_{CD}$ and $S_{HH}$, and consequently the splittings in the spectra. The other NMR parameters such as the line width of a singlet absorption spectrum are also reduced due to
motional averaging of dipolar or quadrupolar interactions. Therefore, phase transitions or onset of motions in these systems are detected by the abrupt change in the NMR parameters as the temperature is varied.

Much work has been done on the conformations and motions of the lipid molecules in membrane system (21-30). Davis et al (23) studied the hydrocarbon chain disorder in the potassium palmitate-water system. In the liquid crystalline phase the C-D order parameters of the first few methylene chain segments were found to increase with increasing temperature to a maximum of 100°C and then decrease at higher temperatures. In contrast, the C-D order parameters for the rest of the methylene chain segments decreased with increasing temperature. In the same system Higg and Mackay (30) have determined the complete order parameter tensors for the α-methylene group by measuring the α-CH₂ dipolar splittings in an otherwise perdeuterated chain and the α-CD₂ splittings (23) in the specifically deuterated chains. The temperature dependence of the α-CH₂ splittings was similar to that of the α-CD₂ splittings.

Burnell et al (31) studied the ordering of water in the potassium palmitate/D₂O system. They found that the order parameter of the deuterium in D₂O had the same temperature dependence as the first few methylene pairs as established by Davis et al described previously. This correlation was ascribed to the lipid-water interaction via hydrogen bonding between the water and the polar heads near the lipid-water interface.

1.8. Water in Membrane System

Water (H₂O) constitutes a major component of cells of all living organism, and plays an important role in life process at the cellular level. The ordering of water at the lipid-water (D₂O) interface can be observed because of the interactions between the nuclear quadrupole moments of
deuterium in D$_2$O and the electric field gradients at the nuclear sites. The anisotropy experienced by water molecules in the lipid-water system is far smaller than that in the hydrocarbon chains, but is still large enough to give a well resolved quadrupole splitting for water deuterons in D$_2$O.

In the DMR spectra of D$_2$O in lamellar phases of egg yolk lecithin, egg phosphatidylethanolamine, ox brain sodium phosphatidylserine (32) and dipalmitoyl phosphatidylcholine (33), a sharp central line was observed. Finer et al ascribed this sharp central line to the presence of isotropic water. One should be cautious of this explanation. As pointed out by Wennerström et al (34) and Lindblom et al (35) that for some samples, the sharp central line was independent of the added water concentration, and may be due to double quantum transitions, which is unobservable at low RF power, while it is much more intense than the powder pattern at high RF field strengths. Thus it is very easy to determine the nature of the peak by investigating the dependence of the signal intensity on the RF field strength.

Application of Finer's method of analysis (36), Finer and Darke were able to distinguish 2, 3 and 4 different kinds of water for egg phosphatidylethanolamine, egg lecithin and sodium phosphatidylserine respectively. These water types were identified as tightly bound inner hydration shell, weakly bound water, trapped water, with exchange between them being rapid on the NMR time scale, and free water. The characteristic splitting of the main hydration shell of egg yolk lecithin is 0.37 kHz, and that of the inner hydration shell or the most tightly bound shell of the same system is 6.9 kHz, which are considerably smaller than the splitting of 170 kHz typically found for polycrystalline ice and hydrates (56).
1.9. Motivation for the Research Project

Deuterium magnetic resonance of D$_2$O in EYL/D$_2$O system has been done by Finer et al (32) and others. All of them measured the quadrupole splittings of DMR spectra of water (D$_2$O) as a function of temperature. This method of evaluating DMR spectra is subjected to a systematic error and is difficult to apply when the spectra are broadened.

In this research, the moments (Appendix A) will be calculated from the DMR spectra of D$_2$O in the EYL/D$_2$O and E. Coli/D$_2$O systems, and the splittings from the moments thus determined. The latter will be compared to the splittings directly obtained from measuring the separations between the doublet peaks of the spectra.

According to the data (Alex Mackay, unpublished) obtained from PMR studies of the protiated outer membrane of E. Coli/D$_2$O and EYL/D$_2$O in excess water, the temperature dependence of the second moments and the $M_4/M_2^2$ calculated from the PMR spectra of the two systems showed an anomalous discontinuity at 4°C that is not observed in Davis' data (37) obtained from DMR study of the deuterated outer membranes of E. Coli. Since PMR is sensitive to intermolecular motions while DMR is not, the data seem to suggest that the anomalous discontinuity was due to onset of lateral diffusion of the phospholipid molecules. Because pure D$_2$O freezes at 4°C, the lateral diffusion below 4°C might have been stopped by freezing of the water. The possible correlation between the disappearance of the lateral diffusion and freezing of the water will be confirmed or dismissed by the results of my experiments.
2.1. Basic Principles of Nuclear Magnetic Resonance

Nuclei having a non-zero nuclear spin possess a magnetic dipole moment \( \vec{\mu} \), which is related to the nuclear spin \( \vec{I} \) by 

\[
\vec{\mu} = \gamma \vec{\mathbf{I}}
\]

where \( \gamma \) is the gyromagnetic ratio, and \( \vec{\mathbf{I}} \) is a vector operator. In a static magnetic field \( \vec{H}_0 \), a magnetic nucleus may exist in one of the \( 2I + 1 \) spin states with spin quantum number \( m = -I, -I + 1, \ldots, I \) and energy levels 

\[
E_z(m) = -\gamma \hbar H_0 m = -\hbar \omega_0 m,
\]

which are the eigenvalues of the Zeeman Hamiltonian 

\[
H_z = -\vec{\mu} \cdot \vec{H}_0 = -\gamma H_0 I_z
\]

where \( I_z \) is the Z-component of the nuclear spin operator \( \vec{I} \). Thus, when an appropriate radio frequency electromagnetic radiation (RF field) is applied to the nucleus perpendicular to the applied magnetic field \( \vec{H}_0 \), a transition between two spin states is induced by a coupling of the magnetic dipole moment to the RF field, and the phenomenon of nuclear magnetic resonance occurs.

2.2. Quadrupolar Interactions and The First Order Perturbation

In addition to a magnetic dipole moment, a nucleus of spin \( I > \frac{1}{2} \) possesses an electric quadrupole moment which has its origin in a nonspherically symmetric nuclear charge distribution. Consequently, the nucleus has an electrostatic interaction with its environment when it is in an electrostatic field gradient (EFG) which does not possess too high a degree of symmetry. This interaction depends on the orientation of the nuclear spin.
Hence, the total Hamiltonian for a nucleus with spin $I > \frac{1}{2}$ in an applied magnetic field $\vec{H}_0$ is given by

$$H = H_Z + H_Q$$  \hspace{1cm} \{2.3\}

where $H_Z$ is the Zeeman Hamiltonian, and $H_Q$ is the quadrupolar Hamiltonian due to the interaction between the quadrupole moment and the electric field gradient existing at the nuclear site. Here we have ignored chemical shift and dipole-dipole interaction terms etc.

If the coupling between the nuclear quadrupole moment and EFG is much smaller than the coupling of the nuclear magnetic dipole moment to the applied static magnetic field, it can be shown (38-41) that the first order perturbation to the Zeeman energy levels due to $H_Q$ are given in frequency units by

$$E_m(1) = \frac{2}{4\hbar I(2I-1)} \left( \frac{3\cos^2 \theta - 1}{2} + \frac{\eta}{2}\sin^2 \theta \cos 2\phi \right) \{3m^2 - I(I+1)\}$$  \hspace{1cm} \{2.4\}

where the angles $\theta, \phi$ specify the magnetic field direction with respect to the principal coordinate system of the EFG as shown in Fig. 3a, $m$ is the magnetic quantum number in the representation where $I_Z$ is diagonal, and $\frac{e^2qQ}{\hbar}$ is the quadrupole coupling constant involving the product of the ZZ component of the EFG $q = V_{zz}/e$ in the principal coordinate system, and the quadrupole moment $eQ$ associated with the spin $I$ nucleus. The quantity:

$$\eta = \frac{|V_{yy}|}{|V_{xx}|}/|V_{zz}|$$  \hspace{1cm} \{2.5\}

is called the asymmetry parameter, where $V_{xx} = \frac{\partial^2 V}{\partial x^2}$ etc., and $V$ is the net electrostatic potential at the nuclear site. Conventionally, the EFG principal axes are ordered so that

$$|V_{zz}| \gg |V_{yy}| \geq |V_{xx}|$$  \hspace{1cm} \{2.6\}
Figure 3. (a) Orientation of the static magnetic field with respect to the principal coordinate system of the electric field gradient. (b) Schematic representation of the geometry and the coordinate system in a lipid bilayer. $\theta$ is the angle between the magnetic field $\vec{H}_o$ and the bilayer normal $\hat{n}$, $\theta$ is the angle between the C-D bond direction and $\vec{H}_o$, and $\theta_n$ is the angle between the C-D bond direction and $\hat{n}$. 
and, consequently, $0 \leq \eta \leq 1$. In its principal axis system, the EFG tensor at the nuclear site is determined by the parameters $q$ and $\eta$. The asymmetry parameter $\eta$ is a measure of the deviation of the EFG from axial symmetry. The parameter $Q$, which is a measure of the deviation of the nuclear charge distribution from spherical symmetry, is the property of the nucleus alone, and is the same for all compounds in which a given nucleus is found. For a prolate spheroidal distribution (football like) $Q$ is positive, for an oblate spheroid (flattened at the poles and bulging at the equator) $Q$ is negative. $Q$ vanishes for a spherically symmetric charge distribution. Thus the energy levels of the total Hamiltonian in frequency units as a result of the first order perturbation solution of eqn. (2.3) are given by

$$E_m = E_m^{(0)} + E_m^{(1)}$$

where $E_m^{(0)} = E_{2m}/2\pi = (-\gamma H /2\pi)m = -\nu_o m$, and $\nu_o$ is the Larmor frequency.

Let us define a parameter $\nu_Q$ as

$$\nu_Q = \frac{3}{4} \frac{e^2 qQ}{h(2I-1)} = \frac{1}{2\pi} \frac{3}{4} \frac{e^2 qQ}{h(2I-1)}$$

Then eqn. (2.7) is written as

$$E_m = -\nu_o m + \frac{\nu_Q}{3} \left( \frac{3 \cos^2 \theta - 1}{2} + \frac{\eta}{2} \sin^2 \theta \cos 2\phi \right) \{3m^2 - I(I+1)\}$$

2.3. Deuterium Magnetic Resonance (DMR)

a) Deuteron on the hydrocarbon chain.

Assume that the amphiphilic membrane system is in the rigid lattice phase so that the chain motion is suppressed. Let us consider one of the deuterons in the $n^{th}$ position of the hydrocarbon chain as depicted in Fig. 3b. Since the deuterium nucleus has a spin $I = 1$, its energy levels as given by the general perturbation result expressed in eqn. (2.9) are
and the corresponding resonance frequencies are

\[ E_{-1} = \nu_o + \frac{1}{3} \nu_q Q_n \left( \frac{3 \cos^2 \theta - 1}{2} + \frac{\eta}{2} \sin^2 \theta \cos 2\phi \right) \]

\[ E_0 = -\frac{2}{3} \nu_q Q_n \left( \frac{3 \cos^2 \theta - 1}{2} + \frac{\eta}{2} \sin^2 \theta \cos 2\phi \right) \]

\[ E_{+1} = -\nu_o + \frac{1}{3} \nu_q Q_n \left( \frac{3 \cos^2 \theta - 1}{2} + \frac{\eta}{2} \sin^2 \theta \cos 2\phi \right) \]

where \( \nu_q = (2\pi)^{-1} \frac{3}{4} e^2 q_n Q / h \), and \( q_n \) is the ZZ component of the EFG at the deuteron site in the \( n \)th position of the chain. Thus the NMR spectrum arising from the particular deuteron will consist of two sharp peaks centered about the central frequency \( \nu_o \) and separated by

\[ \Delta \nu_n = \nu_q Q_n (3 \cos^2 \theta - 1) + \eta \nu_q Q_n \sin^2 \theta \cos 2\phi \]

Notice that, in the absence of cylindrical symmetry (\( \eta \neq 0 \)) the resonance frequencies and the splitting depend on the angles \( \theta \) and \( \phi \). The effect of non-zero \( \eta \) on the powder pattern spectrum has been explored in great detail by Barnes (41) and Cohen et al (40).

Suppose that the membrane system in a lamellar liquid crystalline phase is oriented so that all the optical axes (in a lipid bilayer they are the normals to the bilayer surfaces) of the microdomains in the macroscopic sample are parallel to each other, making an angle \( \theta \) with the applied magnetic field \( \mathbf{H}_0 \). If the lipid molecules in the oriented sample undergo a rapid anisotropic motion with a correlation time \( \tau_c \) much shorter than the
inverse of the static quadrupolar splittings, then the reorientation of the C-D bond will modulate \( \theta \) and hence \( \theta_n \) as depicted in Fig. 3b, and the angular dependent factors in eqn. \( \{2.15a\} \) is taken as the time average. Thus:

\[
\Delta \nu_n = \nu_Q n <3 \cos^2 \theta - 1> + \eta \nu_Q n <\sin^2 \theta \cos 2\phi>
\]

(2.15b)

The EFG at a deuteron site on a hydrocarbon chain in liquid crystalline phase has, to a good approximation, a cylindrical symmetry*, thus the asymmetry parameter \( \eta \) is practically zero. Therefore, neglecting \( \eta \), eqn. \( \{10b\} \) is reduced to a much simpler form:

\[
\Delta \nu_n = \nu_Q n <3 \cos^2 \theta - 1>
\]

(2.15c)

The anisotropic motion of the \( \text{CD}_2 \) in the \( n \)th position of the hydrocarbon chain can be separated into two independent components: (a) reorientation of the \( \text{CD}_2 \) group about a symmetry axis and (b) fluctuations of the direction of this axis (the average direction of this axis has been shown to be normal to the lamella in related systems (42)). With the motions separated into these two independent components, and using the well known addition theorem for spherical harmonics (Abragam book, p. 454), \( <3 \cos^2 \theta - 1> \) can be written

\[
<3 \cos^2 \theta - 1> = \frac{3 \cos^2 \theta - 1}{2} (3 \cos^2 \theta - 1)
\]

(2.16)

and the first order quadrupole splitting in the DMR spectrum arising from the deuteron in the \( n \)th position of the hydrocarbon chain is given by

\[
\Delta \nu_n = \nu_Q n \frac{3 \cos^2 \theta - 1}{2} (3 \cos^2 \theta - 1)
\]

(2.17)

---

*The \( Z \) principal axis of the EFG at the site of a deuterium nucleus on the hydrocarbon chain is almost always within a few degrees of the C-D covalent bond direction and that the asymmetry parameter is \( \eta \leq 0.05 \) (41)
where the angles $\theta_n$ and $\Omega$ are defined in Fig. 3. The formulae \{2.15a\} and \{2.17\} are the fundamental equations for the evaluation of DMR spectra.

We define a C-D bond order parameter $S_{CD_n}$ as

$$ S_{CD_n} = \frac{3\cos^2 \theta_n - 1}{2} $$

{2.18}

where the subscript $n$ on $D$ denotes the deuteron in the $n^{th}$ position along the hydrocarbon chain. Then eqn. \{2.17\} is simplified to

$$ \Delta \nu_n = \nu_Q S_{CD_n} (3\cos^2 \theta - 1) $$

{2.19}

To a good approximation, all the hydrocarbon chain - CD$_2$ deuterons are assumed to be chemically equivalent and hence will have the same quadrupole coupling constant. Furthermore, since the quadrupole coupling $\nu_Q$ of the deuteron on the hydrocarbon chain is predominantly of intramolecular origin (C-D bond), it depends mainly on the state of the covalent C-D bond. Thus $\nu_Q$ depends on temperature through bond vibration. However, in the temperature range of interest in this study, the temperature variation of $\nu_Q$ is expected to be negligibly small, and thus can be assumed constant in that range. Consequently, the order parameter for the methylene deuterons can be obtained directly from the measured quadrupole splittings using eqn. \{2.19\} if $\nu_Q$ is known.

An oriented sample is difficult to prepare, so instead one usually uses a powder sample made of many small crystals oriented randomly with respect to the laboratory frame whose Z-axis is oriented along the applied static magnetic field direction. In our case, the bilayer normals are randomly oriented with equal probability for all directions, and the superposition of the lines arising from the different orientations gives rise
to a broad absorption curve characteristic of a powder pattern of the form

\[ f_n(\omega) = \frac{1}{2} [g_n(\omega) + g_n(-\omega)] \]  \{2.20\}

where

\[ g_n(\omega) = \left( \frac{1}{6\Delta\omega_n} \right)^{\frac{3}{2}} \left\{ (\frac{\Delta\omega_n}{2} - \omega)^{-\frac{1}{2}} + (\frac{\Delta\omega_n}{2} + \omega)^{-\frac{1}{2}} \right\} \quad 0 \leq \omega \leq \frac{\Delta\omega_n}{2} \]

\[ = \frac{1}{6\Delta\omega_n} (\frac{\Delta\omega_n}{2} + \omega)^{-\frac{1}{2}} \quad \frac{\Delta\omega_n}{2} < \omega \leq \Delta\omega_n \]

\[ = 0 \quad \text{otherwise} \]  \{2.21\}

\[ \Delta\omega_n = \frac{3}{4} \frac{e^2 q}{\hbar} S_{CD_n} = 2\pi v S_{CD_n}, \text{ and } S_{CD_n} \text{ is given by eqn. } \{2.18\}. \]

The spectrum has two intense peaks separated by an amount given by the splitting in the DMR spectrum of the corresponding sample oriented at \( \theta = 90^\circ \), i.e.

the peaks separation in the powder pattern is

\[ \Delta\omega_n = 2\pi |v S_{CD_n}| \]  \{2.22\}

as given by eqn.\{2.19\} with \( \theta = 90^\circ \). This first order powder pattern spectrum is shown in Fig. 4a.

When dipolar line broadening is taken into account, the powder pattern assumes the form (43)

\[ f_n(\omega) = \frac{AN_n}{T_{2n}} \int_0^{\pi} \left( \frac{1}{T_{2n}} + (\frac{\omega + 2\pi v}{T_{2n}})^2 S_{CD_n} P_2(\cos\theta) \right)^{-1} \]

\[ + \left( \frac{1}{T_{2n}} + (\frac{\omega - 2\pi v}{T_{2n}})^2 S_{CD_n} P_2(\cos\theta) \right)^{-1} \sin\theta d\theta \]  \{2.23\}

where \( N_n \) is the number of deuterons in the \( n^{\text{th}} \) position of the hydrocarbon chains, \( T_{2n} \) is the spin-spin relaxation time of the deuterons in that position, and \( A \) is a normalization constant. The function \( P_2(\cos\theta) \) is the
Figure 4. (a) Theoretical 1st order powder pattern for a deuterium nucleus in a symmetric electric field gradient ($\eta = 0$). The dashed lines show the individual components of the $m = -1 \leftrightarrow m = 0$ and $m = 0 \leftrightarrow m = +1$ transitions, while the solid line indicates the sum of the two components. Note that the separation of the 180° "shoulders" is twice the doublet separation $\Delta v$.
(b) The same spectrum as (a) but quadrupolar and dipolar line broadening has been taken into account. The dotted curve is the unbroadened spectrum in (a).
Legendre polynomial defined as \( P_2(\cos \theta) = \frac{(3 \cos^2 \theta - 1)}{2} \). Notice that the intrinsic splitting in this broadened powder pattern is \( \Delta \omega_n = 2\pi |\nu_{Q_n} S_{CD_n}| \).

This broadened first order powder pattern is depicted in Fig. 4b. For a perdeuterated lipid system consisting of \( M \) non-equivalent deuteron positions along the chains, the resultant spectrum consists of a superposition of \( M \) broad absorption curves given by (43)

\[
f(\omega) = \sum_{n=1}^{M} f_n(\omega)
\]

each with two sharp edges \( \langle \theta_0^\circ \rangle \) separated by an angular frequency \( \Delta \omega_n = 2\pi |\nu_{Q_n} S_{CD_n}| \).

In an isotropic liquid, if the motion is fast compared to the static splitting frequency, the order parameter is averaged to zero and no splitting is observed. However, since the motion in a lamellar liquid crystal system is in general anisotropic, \( S_{CD_n} \neq 0 \) and a splitting of the first order spectrum will usually be observed.

b) DMR on \( D_2O \).

The principles of deuteron magnetic resonance of \( D_2O \) are the same as those outlined in the last section, except that in deuteron sites, although the major contribution to the electric field gradient is from the intramolecular \( O-D \) bond, there can be contributions which are of intermolecular origin such as the charge distribution in the vicinity of the polar head groups as well as other origins. Furthermore, a decrease of \( \nu_Q \) with increasing hydrogen-bond strength has been observed (44). Therefore the quadrupole coupling constant \( \nu_Q \) could be different from site to site.

Further complication also arises due to chemical exchange between deuterons in water molecules in different sites. If the chemical exchange
rate is much more rapid than the quadrupole splitting, the observed splitting is a weighted average over the different sites and is given by (36, 45-46)

$$\Delta \nu = \left| \sum_i P_i \nu_Q^i S_i \right|$$  \hspace{1cm} \{2.24\}

where $P_i$ is the probability (defined by the fraction of nuclei in site $i$) that a nucleus is in site $i$ with a characteristic quadrupole coupling constant $\nu_Q^i$ and order parameter $S_i$. The largest contribution to $\nu_Q^i$ is assumed to come from the intramolecular O-D bond (44), which means that $\nu_Q^i$ remains approximately the same for the different sites. If $\nu_Q^i$ does not vary significantly with temperature either, then, as shown by eqn. \{2.24\}, the measured splittings are approximately proportional to an average order parameter

$$<S> = \sum_i P_i S_i$$  \hspace{1cm} \{2.25\}

with a constant of proportionality equal to $\nu_Q \approx \nu_Q^i$. Thus $\Delta \nu$ can then be written as

$$\Delta \nu = \left| \sum_i P_i \nu_Q^i S_i \right| = \left| \nu_Q \right| \left| \sum_i P_i S_i \right|$$  \hspace{1cm} \{2.26\}

which can provide useful information for a qualitative interpretation of the quadrupole splittings of the first order DMR spectrum of water system.

2.4. Proton Magnetic Resonance

For the purpose of comparing the results of $D_2O$ in the protiated EYL/$D_2O$ sample (in 22% water by weight) obtained through DMR technique to those obtained from the proton magnetic resonance of EYL in the same sample, a brief review of PMR theory is presented here.

The total Hamiltonian of $N$ protons system is given by (39)
where the first term is the Zeeman energy and the second term is the sum of the pairwise interaction energy between two magnetic dipoles $\mu_j$ and $\mu_k$. When the protons in the N-body spin system form small groups within which the proton separations are distinctly smaller than those between two neighbouring groups, to a first order approximation one may consider such a group as an isolated system and calculate its energy levels in the presence of an applied field $H_o$, treating the rest of the protons in the system as being a perturbation on these energy levels, because the dipole-dipole interaction decreases rapidly with distance.

a) A two-spin system

For a pair of strongly coupled protons on a $\alpha$-CH$_2$ group in an otherwise deuterated hydrocarbon chain in a sample oriented at an angle $\theta$, and assuming that the lattice is rigid and dipolar broadening by the neighbouring protons is neglected, solution of eqn. (2.27) gives a doublet of infinitely sharp peaks centered about the Larmor frequency $\omega_0 = \gamma H_o$ with separation between them given by

$$\Delta \omega = \frac{3}{2} \frac{\gamma^2 h}{r^3} (1 - 3\cos^2 \theta) \quad \{2.28\}$$

each of which are broadened by the dipolar interaction with the neighbouring dipoles. The dipolar energy levels of the two-spin system oriented at $\theta$ and the corresponding first order spectrum are given in Fig. 5.

For a powder sample, a similar argument as that in Section 2.3 on DMR (47-48) will give a well-known Pake doublet, which is identical with that
Zeeman Energy

\[ E = \gamma \hbar H_o \]

Dipolar Coupling

\[ + \frac{\gamma^2 \hbar^2}{4r^3}(1 - 3\cos^2 \theta) \]

\[ - \frac{\gamma^2 \hbar^2}{2r}(1 - 3\cos^2 \theta) \]

\[ + \frac{\gamma^2 \hbar^2}{4r^3}(1 - 3\cos^2 \theta) \]

\[ E = -\gamma \hbar H_o \]

Figure 5. (a) Zeeman energy (left) and dipolar coupling (right) of the triplet states of a two-spin system oriented at an angle \( \theta \).

(b) The first order spectrum of the two-spin system oriented at \( \theta \) whose energy levels are given by (a). The dashed lines are the unbroadened resonance lines and the solid curves are dipolar broadened doublets.
shown in Fig. 4 with the peak separation replaced by $\Delta \omega = \frac{3}{2} \frac{\gamma^2}{r^3} |S_{HH}|$, where $S_{HH}$ is the component of the second order, symmetric, traceless order parameter tensor along the H-H direction.

b) PMR on a protiated lipid system

For a perdeuterated lipid system, the $\alpha$-deuterium NMR signal is easily distinguished from the other deuteron resonances, due to the large $\alpha$-deuteron quadrupolar splitting. In contrast, in a non-deuterated lipid system in liquid crystalline phase, the intra-methylene and inter-methylene proton dipolar interactions are comparable, and are much larger than the range of proton chemical shifts. Consequently, the spin systems have complex lineshapes (30). For instance, for phospholipid bilayer vesicles the observed PMR lineshape is a superposition of Lorentzian curves (49-50), and for the lamellar bilayer in liquid crystalline mesophase the PMR lineshape is characterized by extremely broad wings with a singularity at the central (Larmor) frequency (51). Theories have been developed by Bloom et al (52) and Wennerström (53) to explain the lineshape observed in liquid crystal lamellar bilayer. The logarithmic lineshape given by the theory is shown in Fig. 6, curve d.
Figure 6. PMR lineshapes for values of $m_2(0)/\omega_A^2(0)$ to be (a) $10^{-4}$, (b) $10^{-2}$, (c) $10^{-1}$ and (d) 1 (Bloom, et al (52)). Spectrum (d) is the type of absorption lineshape first observed by Lawson and Flautt (51).
Chapter 3

Experimental

3.1. The Materials

a) The D$_2$O (99.7% enrichment) was purchased from Merck Sharpe and Dohme (Montreal).

b) The protiated EYL (egg yolk lecithin), type 3E was purchased from Sigma Corporation and used without further purification.

3.2. Samples Preparation

a) To get rid of the water in the EYL, we dissolved the material in benzene and recrystallized it by blowing nitrogen through the solution at room temperature at a rate fast enough to minimize exposure to light and prevent it from oxidation. The sample was then kept under vacuum at room temperature overnight to eliminate the last traces of benzene. The protiated EYL sample was made by weighing the corresponding molar amount of D$_2$O into the sample tube which contained the dried EYL. The sample was mixed by stirring with a spatula and sealed. It was then wrapped in tin foil and kept in a refrigerator at -20°C.

b) Description of the preparation of E. Coli sample has been given by Davis et al (37).

3.3. NMR Apparatus

a) The deuterium spectra were taken at 34.44 MHz in a high resolution superconducting solenoid supplied by Nalorac, Inc., Concord, California with a Bruker SXP4-100 NMR spectrometer. The transient digitization and averaging was accomplished with a Nicolet 1090AR digital oscilloscope interfaced to an Intel 8080A microprocessor-based data acquisition system. The Fourier transforms and moment calculations were done with a BNC-12 minicomputer. The
BNC-12 computer is equipped with a Diablo Disk Drive (series 31 single density) and was used for storage and analysis of the data. The Spectrometer is capable of putting out a train of up to 4 RF pulses of controlled amplitude and whose phases and lengths can be varied independently.

A programable timer (Nicolet 293 I/O controller) interfaced to the computer was used to automate the NMR measurements. Thus the triggering of the individual RF pulses, the spacing between them, and the repetition rate were computer controlled.

The probe consisted of a RF coil into which the sample was inserted. The coil (together with the sample) was enclosed in a cylindrical copper oven. Cooling was achieved by blowing coolant (liquid nitrogen) through the oven. The temperature gradient across the sample volume was estimated to be considerably less than 1°C.

3.4. NMR Measurements

The conventional method of obtaining NMR spectra consists of applying a 90° RF pulse and then Fourier transforming the free induction decay (FID). During the application of the RF pulse, the receiver of the NMR spectrometer gets saturated and a certain length of time (called the recovery time or dead time) has to elapse before it returns to its normal operating conditions. Therefore the early part of the FID cannot be observed due to the recovery time of the receiver. The usual method, delaying data acquisition until the receiver has recovered, results in loss of the information contained in the early part of the FID (which is very important for wide lines) and invariably leads to distortion of the spectrum (54). It also introduces first order phase shifts and a poorly defined base line. To circumvent this problem the NMR spectra were obtained using the solid echo by the method of Davis et al (54). This method consists of applying a 90° pulse (whose phase
is $0^\circ$ with respect to the reference frequency) followed by another $90^\circ$ pulse whose phase is shifted by $90^\circ$ with respect to the first pulse at a time $\tau$ (typically 100-200 $\mu$s) later. An echo is formed at $2\tau$ due to the refocusing of the nuclear magnetization. By Fourier transforming the echo starting at $t = 2\tau$ the full spectrum is obtained.

To enhance signal to noise, an alternating phase pulse sequence:

$$(90^\circ)_{\theta=0} - \tau - (90^\circ)_{\theta=90^\circ} - \tau - (90^\circ)_{\theta=180^\circ} - \tau - (90^\circ)_{\theta=90^\circ}$$

was used, where the second quadrupolar echo pulse sequence was phase shifted by $180^\circ$ with respect to the reference frequency. Five hundred scans for DMR on $\text{D}_2\text{O}$ and 1000 scans for PMR on EYL are sufficient to obtain good signal averaging. The signals were detected in quadrature.
Chapter 4

The Results

4.1. DMR Results

For convenience, the egg yolk lecithin/D\textsubscript{2}O in 22\% (by weight) of water (D\textsubscript{2}O), egg yolk lecithin/D\textsubscript{2}O and E. Coli/D\textsubscript{2}O in excess water are respectively abbreviated to EYL/22\%WD\textsubscript{2}O, EYL/EXCD\textsubscript{2}O and EC/EXCD\textsubscript{2}O. All the results mentioned here will be discussed more fully in Chapter 5 where the figures are placed.

a) Quadrupole splittings

When line broadening is absent, the quadrupole splittings can be obtained from the peak positions in the DMR spectra. However, in the presence of dipolar broadening, the positions of the maximum intensity are no longer coincident with the positions of the 90° edges of the quadrupole powder pattern (Appendix A). In this situation, the "splittings" are empirically defined as the average over two measurements; namely, the separation between the peaks and the separation between two points on the outer edges of the powder pattern at 75\% of the peak amplitude of the experimental DMR spectra. By the method described above, quadrupole "splittings" were measured directly from the DMR spectra of the EYL/22\%WD\textsubscript{2}O sample, the representatives of which are shown in Fig. 9. In the region between -6°C and -10°C, the lineshapes are singlets, and only the line widths at half-maximum intensity were measured. The results of the measurements are presented in Fig. 22.

b) Evaluation of the DMR spectra in terms of their moments

The values of the moments $M_n$ (Appendix A) can readily be calculated from the DMR spectra such as those shown in Fig. 7-9. Although in the
experiment the first eight moments were routinely calculated by integrating over the spectrum, only the first few moments of the spectra \( M_0 \) to \( M_4 \) are reliable in the liquid crystalline phase, and at lower temperature only \( M_0 - M_4 \) can be accurately determined, because the accuracy of the higher moments depends critically on the fidelity of the broad part of the spectrum. The results of the calculations of the integrated signal intensities \( M_0 \), the first, the second and the fourth moments for the three samples (namely, the EYL/22\%WD\(_2\)O, the EYL/EXCD\(_2\)O and the EC/EXCD\(_2\)O) are given in Fig. 10-13 and Fig. 16-18.

In order to compare the results of quadrupole splittings obtained by direct measurements from the DMR spectra of the EYL/22\%WD\(_2\)O sample (Fig. 22), the first moment \( M_1 \) (which gives the average splitting) as given by eqn. A.6 in Appendix A, namely,

\[
M_1 = \frac{4\pi}{3\sqrt{3}} \langle \Delta v \rangle
\]

was used to calculate the quadrupole splittings in the DMR spectra of the EYL/22\%WD\(_2\)O in the region from 5°C to 25°C. Below 5°C line broadening may become too severe to render validity to the equation given above. The results of the calculations are shown in Fig. 22.

4.2. PMR Results

The second and the fourth moments were calculated from the PMR spectrum of the EYL/22\%WD\(_2\)O system, and the results are shown in Fig. 23.

4.3. Sources of Error

There is no severe problem of distortion in the DMR spectrum of D\(_2\)O due to 90° pulse length, because the spectral width of D\(_2\)O is small (as
compared to that of PMR and DMR spectrum of the lipids). For our PMR experiment, we obtained a pulse length of 0.9 microsecond for the 90° pulse, which is short enough to rotate all magnetizations in all parts of the NMR spectrum through the same angle.

One of the significant systematic error in the DMR experiment for D$_2$O was magnetic inhomogeneity, which cannot be totally eliminated. Another source of error arose from image formation due to inaccurate quadrature phasing. For instance, the asymmetries in the base of the spectra of the EC/EXCD$_2$O at -1°C to 10°C as shown in Fig. 7 were due to the formation of images.

†This work was done in collaboration with Dr. A. L. Mackay.
Chapter 5

Discussion And Conclusion

5.1. DMR Spectra of D$_2$O

a) Spectra of D$_2$O in the EYL/EXCD$_2$O and EC/EXCD$_2$O systems

As shown in Fig. 7-8, the DMR spectra of D$_2$O in the EYL/EXCD$_2$O (whose water concentration lies between 50% and 60%) are qualitatively similar to those in the EC/EXCD$_2$O (whose water concentration > 90%). Unlike the DMR spectra of D$_2$O in the EYL/22%WD$_2$O, which consist of powder pattern doublets with quadrupole "splittings" in the order of 1 kHz in the temperature range between -3°C and 25°C as shown in Fig. 9, the quadrupole "splittings" in these spectra are absent. For T $\geq$ -2°C, the spectra of the EYL/EXCD$_2$O consist of an extremely narrow singlet characteristic of isotropic water. Like the EYL/EXCD$_2$O, the spectra of the EC/EXCD$_2$O for T $\geq$ -1°C also consist of a very narrow singlet.

Quantitatively, the spectra of D$_2$O in the EYL/EXCD$_2$O and that in the EC/EXCD$_2$O are different. The observed line widths of the spectra of the EYL/EXCD$_2$O range from 81 Hz at -2°C to 110 Hz at 20°C, while those of the EC/EXCD$_2$O stay constant at 30 Hz for T $\geq$ -1°C. Thus, the line widths of the DMR spectra of the EC/EXCD$_2$O are smaller than those of the EYL/EXCD$_2$O by a factor of 3 to 4.

The reduction in quadrupole splittings from 1 kHz for the spectra of the EYL/22%WD$_2$O (Fig. 9) to the zero splitting in the spectra of the EYL/EXCD$_2$O (Fig. 8) can be understood as follows:

Case I. Assume that the system is in water concentration C $\leq$ the maximum hydration (40%). All concentrations are expressed by grams of water per gram of lipid and water mixture. For a crude approximation, the two-site model proposed by Wennerström et al (34) is adopted for the system. The two
sites correspond to the trapped water (water which is incorporated between bilayers, but is weakly associated to the polar head groups (34b)) and bound water (water which is strongly bound to the surfaces formed by the polar head groups). The splitting in the DMR spectrum of trapped water cannot be resolved, while that for the bound water has a value in the order of several kHz. Since the maximum water incorporated between bilayers in the liquid crystalline lamellar phase is 40% by weight (16), all the water in the EYL/22%WD_{2}O system in liquid crystalline lamellar phase is incorporated into the bilayers, and there is practically no bulk water (free isotropic water that is not incorporated between bilayers and exchanges slowly with water that is incorporated between bilayers (32)). In the lamellar phase where C < 40%, the bilayers swell in two ways (namely, increase in thickness of the bilayers and moving-apart of the polar head groups) as water is added, so that all the water, trapped or bound, is increased. However, in our crude model, we assume that the change in the bound water is negligible so that only the amount of trapped water is increased when the total water content of the system is increased (54). On the basis of this model, and, since the exchange between the two sites is fast as indicated by the existence of only a single powder pattern (rather than two or more superimposed spectra) shown in Fig. 9a, the quadrupole splitting \( \Delta \nu \) in the observed DMR spectrum of water deuteron in the EYL/22%WD_{2}O is given by eqn. \{2.24\}:

\[
\Delta \nu = \Sigma_{i=t,b} P_{i} \nu_{Q}^{i} S_{i} = \left[ \frac{W - W_{b}}{W} \Delta \nu_{t} + \frac{W_{b}}{W} \Delta \nu_{b} \right] \tag{5.1}
\]

where \( \Delta \nu_{t} \ll \Delta \nu_{b} \); W and \( W_{b} \) are the mole fractions of the total \( D_{2}O \) content of the system and bound water, respectively; \( \Delta \nu_{b} = \nu_{Q}^{b} S_{b} \) is the characteristic splitting in the DMR spectrum of \( D_{2}O \) when it is strongly bound to the interface
formed by polar head groups, while $\Delta \nu_t = \nu_Q t_s$ is the characteristic splitting in the DMR spectrum of $D_2O$ when it is in the trapped water site. The above equation for $\Delta \nu$ demonstrates that, for low water concentration, the observed splitting is predominantly that of $\Delta \nu_b$, since $\Delta \nu_t \ll \Delta \nu_b$. As more water is added to the system, the fractional contribution from $\Delta \nu_b$ ($\Delta \nu_b$ may depend on water concentration, but here we assume it to be constant) becomes smaller. Consequently, an overall reduction in the splitting of the observed spectrum occurs with increasing water concentration until the splitting cannot be resolved. In a more realistic model, more than one type of bound site should be assumed.

**Case II.** For $C > 40\%$, two phases of $D_2O$ are formed: the maximum amount of water that is incorporated into the bilayers, and bulk or isotropic excess free water in a separate phase exchanging slowly with the maximum water of hydration. In this situation, the observed spectrum is a superposition of the two spectra arising from the water within the bilayers and the bulk water. The second moment of the observed spectrum is given by (Appendix B)

$$M_2 = \frac{M_2^H}{1 + A_{EX}/A_H} + \frac{M_2^{EX}}{1 + A_H/A_{EX}}$$

(5.2)

Here $M_2^H$ and $M_2^{EX}$ are respectively the second moments of the spectra of the water of maximum hydration and the excess free water. $A_H$ and $A_{EX}$ are the integrated intensities of the spectra of the water of maximum hydration $f_H$ and of the excess free water $f_{EX}$:

$$A_H = \int_{-\infty}^{\infty} f_H(\Omega) \, d\Omega$$

(5.3)

$$A_{EX} = \int_{-\infty}^{\infty} f_{EX}(\Omega) \, d\Omega$$

(5.4)
For $C \to \infty$, $A_{EX} \to \infty$ and, consequently, eqn. \{5.2\} is reduced to

$$M_2 = M_2^{EX}$$

i.e. the second moments and the line widths are mainly that of the isotropic excess free water as observed in the DMR spectrum of the EC/EXCD$_2$O system (Fig. 7, 13 and 15). Notice that the second moments $M_2$ and the line widths for $T \geq -1^\circ$C shown in Fig. 13 and 15 are mainly those of the magnet inhomogeneity ($-30 \text{ Hz} \pm 20\%$).

For $T \leq -2^\circ$C, the DMR spectrum of D$_2$O in the EC/EXCD$_2$O consists of a broad and structureless line whose line width varies from 240 to 700 Hz. The spectrum for the EYL/EXCD$_2$O in the region $T \leq -3^\circ$C is also broad and its line width varies from 380 to 400 Hz. Notice that the enormous drop in the DMR signal intensity shown in Fig. 10 is due to disappearance of the DMR signal of all the excess free water as a result of the freezing out of this water (this will be discussed later on). The spectrum of the frozen excess free D$_2$O (the ice) is too broad to be detectable. Thus, the DMR spectra observed in this temperature region arise solely from the water incorporated between the bilayers. The observed broad lines can be understood in terms of eqn. \{5.2\}. When the excess free water has been frozen out, $A_{EX} = 0$, and, because the ice signal cannot be detected, the equation is effectively reduced to

$$M_2 = M_2^H$$

$M_2^H$ is expected to be larger than $M_2^{EX}$, and consequently a broader line width

---

\*The ice spectrum has a quadrupole splitting of 160 kHz. To observe the entire DMR spectrum of polycrystalline ice, a spectral width of at least 300 kHz is required. The spectral width used for my water DMR experiments was only 10 kHz for the EC/EXCD$_2$O and the EYL/EXCD$_2$O systems, and 20 kHz for the EYL/22%WD$_2$O.
Figure 7. Deuterium nuclear magnetic resonance spectra of D$_2$O in the E. Coli/D$_2$O system in excess water (≥ 90% by weight) obtained at (a) 20°C, (b) 4°C, (c) -1°C, (d) -2°C and 34.42 MHz, 300 scans using the quadrupolar echo and quadrature detection method. Repetition rate = 1 second, spectral width = 10 kHz. All the spectra are 5 kHz plot.
Figure 8. Deuterium nuclear magnetic resonance spectra of D₂O in the egg yolk lecithin/D₂O system in excess water concentration (>>40%) obtained at (a) 18°C, (b) 4°C, (c) -2°C, (d) -3°C and 34.42 MHz, 500 scans using the quadrupolar echo and quadrature detection method. Spectral width = 10 kHz. All the spectra are 5 kHz plot.
below the freezing point. The absence of structure in the observed spectrum in this region may be caused by inhomogeneous broadening; the presence of ice in this temperature region may have created different regions into which the unfreezable water is distributed. Water in different regions exchanges slowly. Each of these regions may have the same $T_2$, but the order parameter of the O-D bond direction in the $D_2O$ molecules varies from region to region, so that water in different regions gives rise to DMR spectrum with different quadrupole splitting. A superposition of all of these spectra arising from water in all the regions yields a spectrum whose structure cannot be resolved.

b) Spectra of $D_2O$ in the EYL/22\%WD$_2O$

For temperatures between 25°C and -3°C, the DMR spectrum of $D_2O$ in the EYL/22\%WD$_2O$ clearly exhibits the well known doublet powder pattern with quadrupole "splitting" varying from 1.3 kHz for the system in lamellar liquid crystalline phase ($L_\alpha$) to 0.72 kHz at -3°C. The quadrupole splitting (in the order of 1 kHz) in the doublet powder pattern obtained for this system is much less than that typically found (55) for polycrystalline ice and hydrates (in the order of 150 kHz). The large reduction in the splitting is an indication of the greater time averaging of the electric field gradients due to a rapid but anisotropic reorientation of the water molecules associated with the lecithin polar groups while diffusing along the interface. The validity of

\[ \text{Structure in the spectra in the region } T \leq -2^\circ C \text{ for the EC/EXCD}_2O \text{ and } T \leq -3^\circ C \text{ for the EYL/EXCD}_2O \text{ is expected, since, as the trapped water decreases, and, as will be shown later, the squeezed out water in these systems is frozen as soon as it is squeezed out, eqn. (5.1) in this chapter predicts that the DMR signal of the bound water, which has characteristic splitting of several kHz, should predominate.} \]
this interpretation is further supported by the work of Finer et al (32) and Charvolin et al (56).

The presence of sharp edges in the doublet powder patterns shows that the asymmetry parameter $\eta = 0$. The growth of the signal at the centre of the spectrum is discernible at $-3^\circ C$, which becomes more pronounced in the spectrum at $-4^\circ C$. The spectrum at $-5^\circ C$ is definitely a superposition of two spectra: one is the powder pattern doublet due to the tightly bound water and the other is characteristic of free isotropic water. In the spectrum at $-10^\circ C$, the identity of the powder pattern doublet is lost. This spectrum consists of a narrow singlet with an intrinsic line width of 90 Hz. The sharp central component of the spectrum at $-5^\circ C$ arises from the isotropic "free" water which has been squeezed out from the bilayers. As shown in Fig. 9g-i, the zero degree shoulders and 90 degree edges are absent in the powder patterns for $T \leq -15^\circ C$. The splittings in these spectra range from 160 to 360 Hz, which are very small values. However, due to lack of experimental certainty about the origin of the broadening mechanism, we shall not attempt to interpret the observed spectra in this region. Note that the drop in signal to noise in the spectrum for the EYL/22%WD$_2$O at $-25^\circ C$ is not due to drop in the DMR signal intensity as can be seen from Fig. 11, but due to the broadening of the line.*

*The area of the spectrum $M_0$ (integrated signal intensity), which is proportional to the spin population, should be constant except for a small change in the Boltzmann factor, because the spin population is unchanged during the experiment.
Figure 9. DMR spectra of D₂O in the EYL/22%D₂O obtained at (a) 25°C, (b) 4°C, (c) -3°C, (d) -4°C, (e) -5°C, (f) -10°C, (g) -15°C, (h) -20°C, (i) -25°C and 34.46 MHz, 1000 scans accumulation for signal averaging using the quadrupolar echo and quadrature detection method. Repetition rate = 1/2 second, spectral width = 20 kHz. All the spectra are 5 kHz plot. Spectrum in Figure 10-j is the same as that in Figure 10-i except it is a 10 kHz plot.
5.2. **The Moments of the DMR Spectra**

a) The integrated signal intensities ($M_0$) of the DMR spectra of $D_2O$ in the EYL/EXCD$_2$O and EC/EXCD$_2$O

The integrated signal intensity ($M_0$) vs $T$ for $D_2O$ in the two systems are shown in Fig. 10. In the temperature range defined by $T > -2^\circ C$, the signal intensity of $D_2O$ in both systems increases as temperature decreases. This is partly due to the Boltzmann factor (signal intensity is proportional to the population difference in spin states, which is in turn proportional to the Boltzmann factor), and partly due to the fact that signal intensity is sensitive to change in $Q$ of the RF coil containing the sample, because as $T$ decreases, $Q$ increases, so does the signal intensity. It is also sensitive to the change in the dielectric constant of the water and the lipids.

The enormous drop in intensity of the DMR signal of $D_2O$ in the EYL/EXCD$_2$O is due to freezing out of all the excess free water in the system. Note that, within experimental error, the $M_0$ vs $T$ is linear for $T > -2^\circ C$, thus it is permissible to extrapolate the $M_0$ vs $T$ line down to $T = -3^\circ C$. The ratio of the value of $M_0$ actually measured at $T = -3^\circ C$ to the extrapolated value of $M_0$ at the same temperature is 0.15. This means that the water that remained unfrozen at $-3^\circ C$ comprises 15% of the original total water content of the sample. Furthermore, for $T \leq -3^\circ C$, there is systematic and gradual reduction in signal intensity as temperature decreases, as shown by the smooth curve on the left of the $M_0$ vs $T$ discontinuity in Fig. 10. That is an indication of further freezing out of water. As will be substantiated in Section 5.2d, it is the freezing out of the water squeezed out from the bilayers that leads to the gradual decrease in the signal intensity in the region below the freezing point as temperature decreases.

The temperature dependence of the DMR signal intensity of $D_2O$ in the
Figure 10. Temperature dependence of the integrated signal intensity, \( M_o \), of deuteron magnetic resonance of \( \text{D}_2\text{O} \) in the EYL/EXCD\(_2\text{O} \) (circles) and the E. Coli/EXCD\(_2\text{O} \) (triangles).
EC/EXCD$_2$O is similar to that in the EYL/EXCD$_2$O. The excess free water freezes at $-1^\circ C$. This freezing point and that of D$_2$O in the EYL/EXCD$_2$O (which freezes at $-2^\circ C$) differ only by $1^\circ C$. The fraction of water that remained unfrozen at $-2^\circ C$ is 16% of the total water.

b) The integrated intensity of the DMR spectrum of D$_2$O in the EYL/22%WD$_2$O system

The general temperature dependence of the DMR signal intensity of D$_2$O in the EYL/22%WD$_2$O for $T \geq -10^\circ C$ (Fig. 11) is similar to those mentioned earlier. There is an enormous drop in intensity as temperature changes from $-10^\circ C$ to $-15^\circ C$, indicating that the water squeezed out from the bilayers freezes between $-10^\circ C$ and $-15^\circ C$. The fraction of water remaining unfrozen at $-15^\circ C$ is 0.50.

Note that the freezing point of the squeezed out isotropic water in this system is $8^\circ C$ lower than that of D$_2$O in the other two systems.

c) The first and the second moments of the DMR spectra of D$_2$O in the EYL/EXCD$_2$O and EC/EXCD$_2$O systems

The temperature dependence of $M_2$ of the DMR spectrum of D$_2$O in the EYL/EXCD$_2$O is shown in Fig. 13. To rationalize the observed $M_2$ for the system EYL/EXCD$_2$O, the model proposed in Appendix B is assumed for the distribution of water in this system. Since water concentration in the EYL/EXCD$_2$O lies between 50% and 60% (recall that water concentration $c$ is expressed as $c = W/(W+L)$, where L and W are respectively the weight of the lipids and of the water), a maximum amount $W_{FH}$ of the water which gives $W_{FH}/(W_{FH}+L) = 0.4$ is incorporated into the lipid bilayers in liquid crystalline lamellar phase, forming the water of maximum hydration. The rest of the water forms the isotropic excess free water existing in a separate phase and exchanging slowly with the water incorporated between the bilayers. In such a model, the second moment, $M_2($EYL/EXCD$_2$O), of the observed NMR spectrum of the water in the EYL/EXCD$_2$O is
Figure 11. Temperature dependence of the integrated signal intensity, $M_0$, of deuteron magnetic resonance of D$_2$O in the EYL/22%WD$_2$O.
given by eqn. {B.1} in Appendix B:

\[ M_2^{(EYL/EXCD_2O)} = fM_2^{EX} + (1 - f)M_2^H \]  \[ {5.5} \]

where

\[ f = \frac{1}{1 + A_H^*/A_EX} \]  \[ {5.6} \]

is the fraction of the water outside the bilayers. Here \( f \) is assumed to be independent of temperature. \( M_2^{EX}, M_2^H \) are respectively the second moments of the isotropic excess free water and the water of maximum hydration as defined in Appendix B. Assume that

\[ M_2^H = pM_2^{(EYL/22\%WD_2O)} \]  \[ {5.7} \]

where \( M_2^{(EYL/22\%WD_2O)} \) is the second moment of the DMR spectrum of \( D_2O \) in the EYL/22\%WD_2O, and that \( p \) is independent of temperature. Substituting eqn. {5.7} into eqn. {5.5} gives

\[ M_2^{(EYL/EXCD_2O)} = fM_2^{EX} + (1 - f)pM_2^{(EYL/22\%WD_2O)} \]  \[ {5.8} \]

Since the observed second moment \( M_2^{EX} \) of the free water is virtually that of the magnet inhomogeneity, \( M_2^{EX} \) is insensitive to change in temperature as can be seen in the \( M_2 \) vs T plot for the EC/EXCD_2O shown in Fig. 13 where the spectrum is dominated by the excess free water for \( T \geq -1^\circC \).

If the model under consideration is valid, then, according to eqn. {5.8}, we expect to obtain a straight line for a plot of the \( M_2^{(EYL/EXCD_2O)} \) versus \( M_2^{(EYL/22\%WD_2O)} \) with slope \( (1 - f)p \) and intercept \( fM_2^{EX} \). The plot of the \( M_2^{(EYL/EXCD_2O)} \) for \( T \geq -2^\circC \) (represented by points marked by o in Fig. 13) against the \( M_2^{(EYL/22\%WD_2O)} \) in the same temperature range (presented in Fig. 18) is shown in Fig. 14 where the \( M_2^{(EYL/EXCD_2O)} \) is indeed linearly related to the \( M_2^{(EYL/22\%WD_2O)} \). The slope and the intercept of the straight line obtained by
the principle of least squares fit to the experimental data are determined to be:

\[(1 - f)p = 0.073 \pm 0.0036 \quad \{5.9\}\]

\[fM_{EX}^2 = 0.63 \times 10^6 \text{ sec}^{-2} \pm 0.038 \times 10^6 \text{ sec}^{-2} \quad \{5.10\}\]

Let \(W_b\) be the amount of water found in the bound site, and \(W_{iso}^{mx}\) be the maximum amount of water added to the EYL/22%WD₂O to make it fully hydrated, i.e. \(W_{FH} = W_b + W_{iso}^{mx}\) is the amount of water so that \(C = W_{FH}/(W_{FH} + L) = 40\%\).

The upper and lower bounds for the proportional constant \(p\) can be calculated on the basis of the two-site model proposed in Section 5.1a and the following assumptions:

(a) The surface area per polar head group stays constant when more water is added to the system EYL/22%WD₂O, so that \(W_b\) remained constant and that all the water added to this system contributes only to the isotropic site.

(b) There is only one value of order parameter in the bound site, and thus only one value of splitting \(\Delta \nu_b\) for this site. The splitting in the isotropic site is zero.

Consequently, the average overall splittings observed in the systems EYL/22%WD₂O and EYL/40%WD₂O are respectively given by eqn. \{5.1\}:

\[<\Delta \nu> = \Delta \nu = \frac{W_b}{W_b + W_{iso}^{22}} \Delta \nu_b \equiv \frac{W_b}{W_{22}} \Delta \nu_b \quad \{5.11\}\]

\[<\Delta \nu> = \Delta \nu = \frac{W_b}{W_b + W_{iso}^{mx}} \Delta \nu_b \equiv \frac{W_b}{W_{FH}} \Delta \nu_b \quad \{5.12\}\]

Here \(W_{22} \equiv W_b + W_{iso}^{22}\) is the total water in the EYL/22%WD₂O and \(W_{iso}^{mx}\) is the isotropic water between the bilayers. \(W_{FH} \equiv W_b + W_{iso}^{mx}\) is the total amount of
water in the EYL/40%WD\(_2\)O.

The second moment \(M_2\) as given by eqn. A.3 or A.6 in Appendix A can be written as

\[
M_2 = K \langle \Delta v \rangle^2 = K \{\Delta v\}^2 \tag{5.13}
\]

where \(K\) is a constant.

Eqn. \{5.13\} gives \(M_2(\text{EYL/22%WD}_2\)O) and \(M_2^H \equiv M_2(\text{EYL/40%WD}_2\)O) the following expressions:

\[
M_2(\text{EYL/22%WD}_2\)O) = K \left(\frac{W_b}{W_{22}} \Delta v_b\right)^2 \tag{5.14}
\]

\[
M_2^H = K \left(\frac{W_b}{W_{FH}} \Delta v_b\right)^2 \tag{5.15}
\]

From eqn. \{5.7\}, \(p\) is given by

\[
p = \frac{M_2^H}{M_2(\text{EYL/22%WD}_2\)O}} = \left(\frac{W_{22}}{W_{FH}}\right)^2 \tag{5.16}
\]

Note that as assumption (a) is relaxed, \(M_2^H\) tends to increase with increasing \(c\), so that \(p\) given by eqn. \{5.16\} represents the lower bound, \(p_{1b}\), for \(p\). From the expressions for the water concentrations of the systems EYL/22%WD\(_2\)O and EYL/40%WD\(_2\)O:

\[
\frac{W_{22}}{W_{22} + L} = 0.22 \quad \text{and} \quad \frac{W_{FH}}{W_{FH} + L} = 0.4 \tag{5.17}
\]

\(W_{22}/W_{FH}\) was found to be 0.42 and \(p_{1b} = 0.18\). The assumption that the water added to the EYL/22%WD\(_2\)O does not contribute to the second moment, but it makes the average environment more isotropic puts an upper bound on \(p\) so that

\[
0.18 \leq p \leq 1 \tag{5.18}
\]

Eqn. \{5.9\} and inequality \{5.18\} give

\[
0.59 \pm 0.02 \leq f \leq 0.93 \pm 0.0036 \tag{5.19}
\]

and from \{5.10\} and \{5.9\}, the upper and the lower bounds on \(M_2^{\text{EX}}\) are given by:

\[
(0.68 \pm 0.014) \times 10^6 \text{sec}^{-2} \leq M_2^{\text{EX}} \leq (1.07 \pm 0.074) \times 10^6 \text{sec}^{-2} \tag{5.20}
\]
From eqn. \{5.5\} or \{5.8\}, it is clear that the increase in $M_2$ for the EYL/EXCD$_2$O with increasing temperature (this is also observed in EYL/22\%WD$_2$O as shown in Fig. 18), a behaviour unexpected when compared with most studies of motional narrowing, is due to lipid-water interaction, which imposes a constraint on the ordering of the time average $\mathbf{efg}$ at the lipid-water interface. The ordering effect is assumed to be such that at low temperatures, the principal axis $\mathbf{efg}$, which is along the O-D bond direction, assumes an orientation with respect to the bilayer normal $\mathbf{n}$, giving an average O-D bond order parameter which is smaller than that for the high-temperature configuration. As temperature changes from $-2^\circ C$ to $-3^\circ C$, there is a sharp increase in the value of $M_2$ by a factor of three, corresponding to the broadening of the DMR absorption line by the mechanism elucidated in Section 5.1a, namely, the sudden disappearance of the domination by the isotropic excess free water.

The variation of the $M_2$ of the DMR spectrum of D$_2$O in the EC/EXCD$_2$O as a function of temperature is similar to that for the EYL/EXCD$_2$O, except that for $T \geq -1^\circ C$, the $M_2$ is independent of temperature because the observed DMR signal is dominantly that of the isotropic excess free water as discussed in Section 5.1a. As shown in Fig. 13, the value of $M_2$ in the region $T \geq -1^\circ C$ for the EC/EXCD$_2$O, which is dominantly that of the magnet inhomogeneity, is found to be $0.45 \times 10^6$ sec$^{-2}$ $\pm$ $0.15 \times 10^6$ sec$^{-2}$, which is smaller than the lower bound of the $M_2^{EX}$ for the EYL/EXCD$_2$O given by the inequality \{5.20\}. This is not unexpected, because $M_2^{EX}$ depends on the sample and its geometry and the state of the magnet inhomogeneity in the space occupied by the sample. Again, in this system, the discontinuity in the $M_2$ occurs at the temperature at which the excess free water is frozen out. The five-fold increase in the $M_2$ as temperature changes from $-1^\circ C$ to $-2^\circ C$ reflects the
Figure 12. Temperature dependence of the first moment $M_1$ of the DMR spectrum of $D_2O$ in the EYL/EXCD$_2O$ (circles) and the E. Coli/EXCD$_2O$ (triangles).
Figure 13. Temperature dependence of the second moment $M_2$ of the DMR spectrum of D$_2$O in the EYL/EXCD$_2$O (circles) and the EC/EXCD$_2$O (triangles).
Figure 14. Second moments, $M_2(\text{EYL/EXCD}_2\text{O})$, of the DMR spectra of $D_2O$ in the system EYL/EXCD$_2$O plotted against the second moments, $M_2(\text{EYL/22\%WD}_2\text{O})$, of $D_2O$ in the EYL/22\%WD$_2$O. The solid line is the least squares fit to the experimental data (circles) where a two parameter fit of the form

$$M_2(\text{EYL/EXCD}_2\text{O}) = fM_2^{\text{EX}} + (1-f)pM_2(\text{EYL/22\%WD}_2\text{O})$$

was used.
Figure 15. Temperature dependence of the line width $\Delta\nu$ of the DMR spectrum of D$_2$O in the E. Coli/EXCD$_2$O (circles) and EYL/EXCD$_2$O (triangles).
Figure 16. Temperature dependence of the fourth moment $M_4$ of the DMR spectrum of $\text{D}_2\text{O}$ in the EYL/EXCD$_2\text{O}$ (circles) and E. Coli/EXCD$_2\text{O}$ (triangles).
disappearance of the domination by the excess free water due to freezing out of the water. Similar remarks can be made about the temperature dependence of the first moment $M_1$ (Fig. 12) and the line width $\Delta \nu_2$ (Fig. 15) of the DMR spectrum of the EYL/EXCD$_2$O and EC/EXCD$_2$O.

d) The first and second moments of the DMR spectra of D$_2$O in the EYL/22%WD$_2$O

Comparison of the $M_2$ vs T for the EYL/22%WD$_2$O with that for the EYL/EXCD$_2$O presented in Fig. 13 and 18 shows that the values of the $M_2$ for the first system are 6 (at -2°C) to 9 (at 20°C) times larger than those for the second. This difference is expected, because when more water is added to a system with maximum hydration, the amount of isotropic water increases, and thus, as pointed out in Section 5.1a and Appendix B, the average environment for the D$_2$O molecules becomes more isotropic due to the increased domination by the excess free water, leading to the smaller $M_2$ as dictated by eqn. {B.1} in Appendix B.

The observation of the phenomenon of lipid-water interaction in this system (EYL/22%WD$_2$O) is much more pronounced than that in the EYL/EXCD$_2$O and EC/EXCD$_2$O. In a system where the water concentration is well below the maximum hydration, eqn{5.1} derived from the two-site model in this chapter indicates that the bound water, which is involved in the lipid-water interaction, dominates the observed DMR signal, giving rise to spectra with splittings in the order of several kHz. Consequently, the $M_2$ of the spectrum is very sensitive to the O-D bond orientational order parameter whose magnitude depends on the lipid-water interaction.

In the region $-10^\circ$C $\leq T \leq -3^\circ$C, the most striking contrast between the behaviour of the $M_2$ for this system (EYL/22%WD$_2$O) and the EYL/EXCD$_2$O is that, as temperature decreases, the former decreases whereas the latter increases.
Furthermore, as temperature decreases, the $M_2$ (and correspondingly the line width as shown in Fig. 22) for the EYL/22%WD$_2$O in the region decreases much more rapidly than that for the same system in the region $T \geq -3^0C$. The temperature dependence of the $M_2$ for the EYL/22%WD$_2$O in this region is not unexpected, because the trapped water in this region is gradually squeezed out from the bilayers. The squeezed out isotropic water remains unfrozen down to $-10^0C$ as shown in Section 5.2b, and the DMR signal of that isotropic water becomes more and more dominant as temperature decreases, leading to a decline in the $M_2$ much more rapid than that expected from lipid-water interaction in the region $T \geq -3^0C$. On the other hand, as shown in Fig. 10, all the isotropic excess free water in the EYL/EXCD$_2$O has been frozen out in this region, and, in the range $-3^0C \leq T \leq -2^0C$, the lipid is fully hydrated. Thus, for $-10^0C \leq T \leq -3^0C$, the trapped water is expected to be squeezed out from the bilayers gradually as temperature is lowered. However, the behaviour of the $M_2$ for the EYL/EXCD$_2$O is obviously incompatible with the existence of such isotropic water. This means that the isotropic water is frozen as soon as it is squeezed out from the bilayers. This hypothesis is supported by the evidence provided by the decrease in the DMR signal intensity for the EYL/EXCD$_2$O in this region as shown in Fig. 10. Thus, we expect that the $M_2$ of the spectrum for the EYL/EXCD$_2$O at $-10^0C$ should be considerably larger than that of the spectrum for the EYL/22%WD$_2$O at the same temperature. This is indeed the case as shown in Fig. 13 and Fig. 18, where the ratio of $M_2$ for the EYL/EXCD$_2$O to that for the EYL/22%WD$_2$O is 53:5 or 11. The increase in $M_2$ of the spectrum for the EYL/EXCD$_2$O in the range from $-3^0C$ to $-10^0C$ as temperature decreases may be ascribed to the following mechanisms: (a) The squeezing out of the trapped water results in a population reduction in the isotropic sites between the bilayers, while the population in the anisotropic sites may
remain fixed. Consequently, according to eqn (5.1) in this chapter, the $\Delta \nu_b$ in that equation gains more and more weight, while the contribution from the $\Delta \nu_t$ fades away, resulting in the increase in $M_2$ as temperature decreases.

(b) As temperature is lowered, the average 0-D bond order parameter at each site increases, giving rise to a broader line width. (c) When temperature is decreased, the motions of the lipid polar head group, to which the water molecules are bound, are reduced (36), resulting in a lesser motional averaging of the electric field gradients and the dipolar interaction, so that the spin-spin relaxation time $T_2$ is shortened. Consequently, a lifetime line broadening occurs. It is very difficult to distinguish between the process described in (a) and that in (b).

The $M_2$ vs T plot for the EC/EXCD$_2$O (Fig. 13) in the region between -3°C and -10°C is similar to that for the EYL/EXCD$_2$O, and is also dictated by the same mechanism as supported by the experimental facts shown in Fig. 10.

The freezing of the squeezed out water in the EYL/EXCD$_2$O and EC/EXCD$_2$O in the region mentioned above is in great contrast to the squeezed out water in the EYL/22%WD$_2$O, which did not freeze until below -10°C. This difference is not surprising, because, in the neighborhood of -2°C, large surfaces of ice are formed due to the freezing of the excess free water in the EYL/EXCD$_2$O and EC/EXCD$_2$O. These surfaces enhance freezing of water pushed out from the bilayers in the temperature region from -2°C to -10°C. In the EYL/22%WD$_2$O sample, no such ice surfaces exist at -2°C, so that there is no surface of crystallization in the region.

As temperature decreases, the $M_2$ for the EYL/22%WD$_2$O reaches a minimum at -10°C. From the spectra of this system in the region from -3°C to -10°C as shown in Fig. 9c-f, it is evident that the presence of isotropic water squeezed out from the bilayers is one of the most likely mechanisms
Figure 17. Temperature dependence of the first (triangles) and the fourth (circles) moments, $M_1$, $M_4$, of the DMR spectrum of $D_2O$ in the EYL/22%WD$_2O$. 
Figure 18. Temperature dependence of the second moment $M_2$ of the DMR spectrum of $D_2O$ in the EYL/22%WD$_2$O.
responsible for the minumum. A complete phase diagram of egg yolk lecithin-water system has been provided by Small and Chapman (57) and Reiss-Husson (58). However, since the phase line $T_c$ is ill-defined, it is dangerous to draw any conclusion regarding the phase behaviour of our sample (the EYL/22%WD$_2$O) solely on the basis of their phase diagram. Unfortunately, our PMR experiment did not cover the region below -10°C, so that no information concerning the phase behaviour of our EYL/22%WD$_2$O sample is available. Due to lack of experimental certainty, it is very difficult to know whether a phase transition is also involved at -10°C.

e) The temperature dependence of the $\Delta_2$ and the $M_4/M_2^2$ for the EYL/22%WD$_2$O, EYL/EXCD$_2$O and EC/EXCD$_2$O.

The temperature dependence of the parameters $\Delta_2$ and $M_4/M_2^2$ for the three systems are plotted in Fig. 19-21.

The shapes of the $\Delta_2$ vs T and $M_4/M_2^2$ vs T for the EYL/EXCD$_2$O and EC/EXCD$_2$O are very similar. A sharp drop in $\Delta_2$ and $M_4/M_2^2$ occurs as temperature changes from -2°C to -3°C and -1°C to -2°C for the EYL/EXCD$_2$O and EC/EXCD$_2$O, respectively. The values of these parameters for temperatures below the freezing point are smaller than those at temperatures above it.

The shapes of the $\Delta_2$ vs T and $M_4/M_2^2$ vs T for the EYL/22%WD$_2$O are very different from those for the other two systems. A sharp anomalous increase in the values of $\Delta_2$ and $M_4/M_2^2$ occurs around -10°C. Note that the anomalous increase in either $\Delta_2$ or $M_4/M_2^2$ (Appendix A) does not correspond to the coexistence of ice and water phases, since ice does not contribute to the moments of the spectrum. However, since the state of water depends on the environment (the lipids) in which the water exists, it is very likely that the anomalous behaviour of the two parameters for the EYL/22%WD$_2$O at -10°C corresponds to the coexistence of different phases of water.
Figure 19. Temperature dependence of the ratio $M_4/M_2^2$ (circles) and the relative mean square deviation $\Delta_2$ (triangles) of the DMR spectrum of $D_2O$ in the E. Coli/EXCD$_2O$. 
Figure 20. Temperature dependence of the ratio $M_4/M_2^2$ (circles) and the relative mean square deviation $\Delta_2$ (triangles) of the DMR spectrum of D$_2$O in the EYL/EXCD$_2$O.
Figure 21. Temperature dependence of the relative mean square deviation $\Delta_2$ (circles) and the ratio $M_4/M_2^2$ (triangles) of the DMR spectrum of $D_2O$ in the EYL/22%WD$_2O$. 
5.3. Comparison With Other Work

The temperature dependence of the first moment $M_1$ (which gives the average quadrupole splitting, and hence average order parameter), the second moment $M_2$ and the quadrupole splitting measured directly from the DMR spectrum of $D_2O$ in the EYL/22%WD$_2$O as shown in Fig. 18 and 22 have the same general shape as the quadrupole splitting vs temperature of the DMR spectrum for the EYL/9D$_2$O (19% by weight of water concentration) presented by Finer et al (32) as shown in Fig. 22, but they do not agree in detail. For convenience, let us denote EYL/9D$_2$O as EYL/19%WD$_2$O.

At $-20^\circ$C, the spectrum of the EYL/19%WD$_2$O (Fig. 22) has a quadrupole splitting of 1.8 kHz, while that of the EYL/22%WD$_2$O (Fig. 22) has a much smaller splitting of only 0.37 kHz, a difference by a factor of five. The splitting at $-10^\circ$C in the spectrum of the EYL/19%WD$_2$O is 0.81 kHz, and that of the EYL/22%WD$_2$O is only 0.1 kHz, a factor of eight smaller. But the splitting in the spectra at $25^\circ$C for these two systems are nearly identical; namely, 1.2 kHz for the splitting in the spectrum of the EYL/19%WD$_2$O, and that of the EYL/22%WD$_2$O is 1.02 kHz. A minimum in the $\Delta\nu$ vs T of the EYL/19%WD$_2$O and EYL/22%WD$_2$O occurs at $-2^\circ$C and at $-10^\circ$C, respectively, with a minimal value of 0.52 kHz for the former and 0.12 kHz for the latter. The phase diagrams provided by Small (57) and F. Reiss-Husson (58) indicate that the phase behaviour of the EYL/water system is sensitive to small change in water concentration, so the difference in the temperature at which the minimum in the $\Delta\nu$ vs T of the two systems occurs is not unexpected. Finer ascribed the minimum to the onset of chain melting. However, no such transition in the EYL/22%WD$_2$O is observed in the range from $-10^\circ$C to $48^\circ$C as demonstrated by our PMR data given in Fig. 23.
Figure 22. (a) Temperature dependence of the quadrupole splitting $\Delta \nu$ which is measured directly from the DMR spectrum of D$_2$O in the EYL/22%WD$_2$O (circles). The points marked by triangles are the quadrupole splittings $\Delta \nu_{M1}$ calculated from the first moment $M_1$ of the DMR spectrum of D$_2$O in the said system. Note that the calculated splittings are systematically larger than those obtained by direct measurement, indicating the existence of line broadening. If line broadening is absent, the two curves should be coincident. (b) The insert shows the quadrupole splitting vs $T$ in the DMR spectra of egg yolk phosphatidylethanolamine/21%WD$_2$O (triangles) and EYL/19%WD$_2$O = EYL/9D$_2$O (circles) presented by Finer et al (32).
5.4. PMR Results for the EYL/22%WD₂O

Temperature dependence of the $M_2$ and $M_4/M_2^2$ of PMR spectrum of EYL in the EYL/22%WD₂O system are given in Fig. 23.

The $M_2$ decreases monotonically and smoothly as temperature rises from -10°C to 48°C. The value of $M_2$ at 48°C is smaller by a factor of five as compared to that at -10°C. The reduction in $M_2$ at higher temperature region arises from motional narrowing due to increase in time averaging of the dipole-dipole interaction.

Within experimental error, the $M_4/M_2^2$ is independent of temperature. If we assume the existence of translational diffusion of the phospholipid molecules along the bilayer surfaces and rotation about the normal to the bilayers, and, furthermore, if the powder pattern lineshape is assumed to be given by a superposition of the lineshape

$$f(\omega, \theta) = \left\{2\pi M_2 \left(P_2(\cos\theta)\right)^2\right\}^{1/2} \exp\left\{-\frac{(\omega-\omega_0)^2}{2M_2 \left\{P_2(\cos\theta)\right\}^2}\right\}$$

by summing over the orientation angle $\theta$ (52), we obtain 6.45 for the value of the $M_4/M_2^2$, which is consistent with the data for Fig. 23 within experimental error. Thus, this particular value of $M_4/M_2^2$ may suggest that there are still a lot of motions at a temperature as low as -10°C. Furthermore, since $M_4/M_2^2$ is very sensitive to coexistence of phases (Appendix A), the absence of anomalous increase in that parameter indicates that there is no phase transition in the entire region under investigation.

However, as shown in Fig. 24, the preliminary PMR results for EYL in the EYL/EXCD₂O (Alex Mackay, unpublished) exhibit some unusual behaviour near 4°C.

**

This work was done in collaboration with Dr. A. L. Mackay.
which could be associated with a change in the ordering of the water molecules. This phenomenon also occurs in the EC/EXCD₂O at 4°C as shown in the same figure (the $M_4/M_2^2$ for this system is not shown). Since the DMR data on the E. Coli outer membrane (37) does not show any anomaly at 4°C (Fig. 25), and no anomaly is observed in the EYL/22%WD₂O as shown above, the phenomenon observed by Alex Mackay in the two systems in excess water† has been ascribed to the onset of translational diffusion of the phospholipid molecules. The possible correlation between this phenomenon and the freezing out of water in the two systems in excess water has been ruled out on the evidence provided by my DMR results presented in this thesis.

†When the write up of this thesis is completed, Alex Mackay repeated the PMR experiment for the EYL/EXCD₂O sample, and found that the results are not reproducible.
Figure 23. Temperature dependence of the ratio $M_4/M_2^2$ (circles) and the second moment (triangles) of the PMR spectrum of the EYL/22%WD$_2$O.
Figure 24. Temperature dependence of the second moment $M_2$ of PMR spectrum of (a) the outer membrane of E. Coli in the EC/EXCD$_2$O (circles), (b) the EYL in the EYL/EXCD$_2$O (triangles), (c) temperature dependence of $M_4/M_2^2$ of the PMR spectrum of the EYL in the EYL/EXCD$_2$O (squares) (Alex Mackay's unpublished data).
Figure 25. (a) Second moments vs temperature of the DMR spectrum of outer membranes of E. Coli grown on a medium containing perdeuterated palmitic acid (triangles).

(b) The parameter $\Delta_2$ vs temperature of the DMR spectra of the outer membranes of E. Coli grown on a medium containing oleic acid as well as perdeuterated palmitic acid (37).
Moments Of Nuclear Magnetic Resonance Spectra

When line broadening in NMR absorption spectra becomes substantial, the sharp spectral features disappear. Thus, the true or intrinsic quadrupole splittings (see Section 2.3a, Chapter 2), and consequently the orientational order parameters cannot be determined precisely from the usual spectroscopic technique; namely by measuring the separation between the peaks (peak splitting) in a spectrum directly. That is, the intrinsic splittings can no longer be identified with the peak splittings. However, the intrinsic splittings can be determined by matching each experimental spectrum with a computer simulated spectrum (Bloom et al, 1978a, unpublished), where a known broadening is introduced into a powder pattern of known splittings.

Alternatively, the moments of a NMR absorption spectrum provide a systematic way of determining the order parameter distribution.

The $n^{th}$ moment of a spectrum is defined as

$$M_n = \int_{-\infty}^{\infty} (\omega - \omega_0)^n f(\omega - \omega_0) \, d\omega / \int_{-\infty}^{\infty} f(\omega - \omega_0) \, d\omega \tag{A.1}$$

where $f(\omega - \omega_0)$ is the lineshape of the NMR absorption spectrum centered about the Larmor frequency $\omega_0$ (in angular frequency units).

Moments of Deuterium Nuclear Magnetic Resonance Spectrum

For a first order quadrupole powder pattern, the DMR spectrum $f(\omega - \omega_0)$ is symmetric about the angular Larmor frequency, $\omega_0$, i.e. $f(|\omega - \omega_0|) = f(-|\omega - \omega_0|)$ (Abragam, 1961), consequently the odd moments of this lineshape vanish. It is convenient to use the moments of the half-spectrum defined as
where \( \Omega = \omega - \omega_0 \). In this case both odd and even moments exist. Notice that for even moments, eqn\{A.1\} is reduced to eqn\{A.2\} because of the symmetry in \( f(\Omega) \).

The DMR lineshape \( f(\Omega) \) of a simple system having only one type of deuterium site (and hence one value of order parameter) is a single quadrupole powder pattern given by eqns. \{14\} and \{15\} in Section 2.3a, Chapter 2, and the moments of this spectrum as defined by eqn\{A.2\} has been shown (Bloom et al, 1978a) to be

\[
M_n = A_n \left( \frac{3}{4} \frac{e_{4Q}}{n} \right)^n S_{CD}^n
= A_n (2\pi)^n (\Delta \nu)^n \quad \{A.3\}
\]

when line broadening can be neglected, i.e. when there is no line broadening or the intrinsic line width is much smaller than the quadrupole splitting. The coefficient \( A_n \) can be calculated from the expression for the spin 1 powder pattern lineshape (Bloom et al, 1978a). The first two coefficients are given as \( A_1 = 2/3 \sqrt{3} \) and \( A_2 = 1/5 \). In eqn\{A.3\}, \( S_{CD} \) is the C-D bond order parameter previously defined (Chapter 2), and \( \Delta \nu \) is the quadrupole splitting in the powder spectrum. Thus, for a single spin \( I = 1 \) quadrupole powder pattern, when broadening is negligible, the moments of the spectrum are functions only of the quadrupole splitting \( \Delta \omega = 2\pi \Delta \nu \), and the measurement of any one of the moments is equivalent to a measurement of the quadrupole splitting.

In deuterium-labelled phospholipids in biological membranes, large numbers of inequivalent deuterium sites may exist, because most biological
membranes have a variety of phospholipids corresponding to different polar head groups and combinations of pairs of acyl chains having various lengths and degrees of saturation. These differences may give rise to variations in the value of C-D bond order parameter $S_{CD}$ among the different lipids even if all lipids were labelled at the same position on a hydrocarbon chain. Larger variations in $S_{CD}$ occur between gel and liquid crystal regions of the sample. At physiological temperature, these different thermodynamic phases are expected to coexist in biological membranes, which are relatively inhomogeneous since they consist of mixtures of lipids and proteins. Furthermore, the lipid-protein interface itself in a biological membrane system may give rise to observable variations in $S_{CD}$. Thus, the information required to characterize the orientational order of such a complex system is not simply an order parameter $S$, but rather an order parameter distribution function $P(S)$.

The $n^{th}$ moment of the order parameter distribution $P(S)$ is defined as

$$S_n = \int_0^\infty S^n P(S) \, dS \tag{A.4}$$

where, for a quasi-continuous distribution of $S$, $P(S)dS$ is the probability of finding an orientational order parameter between $S$ and $S + dS$ for the deuterium sites of the complex system.

For a distribution of order parameters characterized by $P(S)$, the resultant DMR spectrum is a superposition of powder patterns. It has been shown (Bloom et al, 1978, unpublished) that
\[
M_n = A_n \left( \frac{3}{4} \frac{e^2 q Q}{n} \right)^n S_n \\
= A_n (2\pi r)^n <S_{CD}^n > \\
= A_n (2\pi)^n <(\Delta \nu)^n > \quad \{A.5\}
\]

where
\[
\nu_Q = \frac{3}{4} \frac{e^2 q Q}{\hbar} \\
\Delta \nu = \nu_Q S_{CD}
\]

For the first two moments, eqn.\{A.5\} gives
\[
M_1 = \frac{4\pi}{3\sqrt{3}} <\Delta \nu> = \frac{4\pi}{3\sqrt{3}} \nu_Q <S_{CD} > \\
M_2 = \frac{4\pi}{5} <(\Delta \nu)^2 > = \frac{4\pi}{5} \nu_Q^2 <S_{CD}^2 > \quad \{A.6\}
\]

The relative mean square deviation from the mean \( \Delta_2 \) of the distribution of orientational order parameters is defined as
\[
\Delta_2 = \frac{S_2 - S_1^2}{S_1^2} = \frac{<S_{CD}^2> - <S_{CD}^2>^2}{<S_{CD}^2>} \\
= \frac{M_2}{1.35M_1^2} - 1 \quad \{A.7\}
\]

A simple system having only a single order parameter (and consequently, its DMR spectrum consists of only a single powder pattern) has the property that \( S_n = S_{1}^n = S_{CD}^n \) giving \( \Delta_2 = 0 \) (neglecting line broadening). In this simple case, eqn. \{A.5\} is reduced to eqn. \{A.3\}.

The parameter \( \Delta_2 \) is a very useful one in that it characterizes the width of the distribution of quadrupole splittings. This parameter is very sensitive to inhomogeneity in the kind of sample under consideration such as
the coexistence of phases (59).

**Proton Magnetic Resonance**

Unlike deuterium-labelled phospholipids in model and biological membrane systems where deuteron-deuteron dipolar interactions are negligible, $\Delta_2$ for specifically protonated or protiated phospholipids in membrane system is difficult to interpret, because in this system proton-proton dipolar interactions are large and cannot be ignored. However, the ratio of the fourth to the square of the second moments of a PMR spectrum is still useful and has physical meaning. For example, consider a protiated system in a state in which there is neither translational diffusion nor rotation of the phospholipid molecules, then the PMR lineshape is assumed to be a simple Gaussian given by

$$f(\omega) = (2\pi M_2)^{-1/2} \exp\left\{-\left(\omega - \omega_0\right)^2 / 2M_2\right\}$$

which gives $M_4/M_2^2 = 3$. In a lamellar liquid crystalline phase, both of these motions are present. If the lineshape of the oriented sample is given by (52)

$$f(\omega, \theta) = (2\pi M_2(0))^{-1/2} \exp\left\{-\left(\omega - \omega_0\right)^2 / 2M_2(0)(P_2(\cos\theta))^2\right\}$$

where $\theta$ is the angle between the magnetic field $H_0$ and the axis of symmetry for the motion (the normal to the bilayer), $P_2(\cos\theta)$ is the second order Legendre polynomial $(3\cos^2\theta - 1)/2$, and $M_2(0)$ is the second moment of the spectrum when the sample is oriented at $\theta = 0$. The second and the fourth moments of the powder pattern arising from eqn. (A.9) are respectively $\frac{1}{3} M_2(0)$ and $\frac{3}{35} M_4(0)$, consequently, $M_4/M_2^2 = 6.45$. The parameter $M_4/M_2^2$ is very sensitive to coexistence of phases such as gel and liquid crystalline phases. An anomalous behaviour in the temperature dependence of $M_4/M_2^2$ over the region of co-existence of phases has been observed in the DPL/D$_2$O system (Alex Mackay, unpublished).
Appendix B

Contributions To The Second Moment

Let us consider a lipid/water system in a water concentration, c, which is much higher than 40% by weight (the maximum hydration). In this system, a maximum amount (by weight), $W_{FH}$, of the water which gives $W_{FH}/(W_{FH} + \text{Lipids}) = 40\%$ is incorporated into the bilayers (33), while the rest of the water forms what is called bulk or isotropic excess free water existing in separate phase and exchanging slowly with the water incorporated between the bilayers (32-33, 36, 61). Thus, the observed NMR spectrum of the water, $F(\Omega)$, is a superposition of two spectra: a spectrum $f_H(\Omega)$ arises from the water associated with the lipid, and the other $f_{EX}(\Omega)$ is the NMR absorption spectrum of the excess free water; that is:

$$F(\Omega) = f_H(\Omega) + f_{EX}(\Omega)$$ \hspace{1cm} \{B.1\}

From the definition of moments given in Appendix A,

$$M_2 = \frac{\int_{-\infty}^{\infty} \Omega^2 F(\Omega) \, d\Omega}{\int_{-\infty}^{\infty} F(\Omega) \, d\Omega}$$

$$= \frac{\int_{-\infty}^{\infty} \Omega^2 f_H(\Omega) \, d\Omega + \int_{-\infty}^{\infty} \Omega^2 f_{EX}(\Omega) \, d\Omega}{\int_{-\infty}^{\infty} f_H(\Omega) \, d\Omega + \int_{-\infty}^{\infty} f_{EX}(\Omega) \, d\Omega}$$

$$= \frac{M_2^H}{1 + A_{EX} / A_H} + \frac{M_2^{EX}}{1 + A_H / A_{EX}}$$ \hspace{1cm} \{B.2\}

$$= fM_2^{EX} + (1 - f)M_2^H$$ \hspace{1cm} \{B.3\}

where

$$M_2^H = \frac{\int_{-\infty}^{\infty} \Omega^2 f_H(\Omega) \, d\Omega}{\int_{-\infty}^{\infty} f_H(\Omega) \, d\Omega}$$ \hspace{1cm} \{B.4\}
\[ M_2^{EX} = \int_\Omega^2 f_{EX}(\Omega) \, d\Omega / \int f_{EX}(\Omega) \, d\Omega \]  
\{B.5\}

\[ A_H = \int f_H(\Omega) \, d\Omega \]  
\{B.6\}

\[ A_{EX} = \int f_{EX}(\Omega) \, d\Omega \]  
\{B.7\}

\[ f = 1 / \{1 + A_H / A_{EX}\} \]  
\{B.8\}

For \( c > 40\% \) by weight, addition or reduction in the amount of water in the system will not alter the amount of water incorporated between the bilayers, consequently, \( A_H \) and \( M_2^H \) are constant. Thus, for \( c \to \infty \), \( A_{EX} \to \infty \), and, consequently,

\[ M_2 \to M_2^{EX} \]

On the other hand, when \( c \to 40\% \) by weight, \( A_{EX} \to 0 \). Hence:

\[ M_2 \to M_2^H \]
References


(2) Helmut Hanser, in: Lipids, ed. by Felix Froukis, Plenum Press.

(3) Joachim Seelig, Quarterly Reviews of Biophysics 10, 3 (1977), 353-418.


(17) C. Fred Fox, in: The Structure of Cell Membrane, Scientific American, 30-38.

(18) D. Chapman, Quart. Rev. Biophys. 8, 185 (1975).


(35) Göran Lindblom, Nils-Ola Persson and Gösta Arvidson, Lyotropic Liquid Crystals and the Structure of Biomembranes, Chapter 9, ed. by S. Friberg.
(37) Jim H. Davis, Christine P. Nichol, Gerold Weeks and Myer Bloom, Biochemistry, 18, 10 (1979), 2103.


(46) H. Wennerström, Molecular Physics, 24 (1972), 69-80.


(56) Jean Charvolin and Paul Rigny on the "Deuteron Relaxation Study Of D₂O Motions In A Lyotropic Liquid Crystal", Chemical Physics Letters.

