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Solvent-Solute Interaction in an L_α Phase Formed With Water, Ethylene Glycol and Lecithin

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The variation in the order parameter with the ethylene glycol/water ratio was determined by deuterium HMR groups in ethylene glycol were determined in an *L* liquid crystalline phase of lecithin, water and ethylene glycol. In addition, the interlayer spacing was determined by small angle X-ray diffractometry.

The results were described using a simple two-component adsorption model and showed no substantial variation between water and ethylene glycol of their binding affinity to the polar groups of the lecithin ^{31}P NMR results supported earlier proton NMR spectra interpretation of an increased disorder of the amphiphiles with *greater amount* enhanced of ethylene glycol/water ratio.

Keywords: lyotropic liquid crystals, NMR, lyotropic liquid crystals, order parameter solvent, non-aqueous lyotropic liquid crystals, surfactants, association structures, surfactants, solubilization, biological membranes, order parameters

INTRODUCTION

The lamellar liquid crystalline phase formed by lecithin with water has been studied extensively as a model for biomembranes. The structural and the dynamic aspects of the amphiphile have been thoroughly

investigated through a wide variety of physical techniques. After the pioneering efforts of Chapman and coworkers,[1,2] NMR has become a standard technique to characterize the dynamics and in some favorable cases, the structural aspects of these mesomorphic phases. ^{2}H-NMR studies of the selectively deuterated amphiphiles were in part responsible for the mean field theoretical[3,4] development of the phase transition (gel phase—L_α phase) associated with the chain melting phenomenon. By comparison, our understanding of the solvent solute interaction in the mesomorphic phases is still at a rather primitive level. ^{2}H-NMR studies of the solvent have attracted relatively little attention,[5–7] partly because, the main solvent used in these model membrane systems is water, which has an order parameter 0, and partly because the discovery of the L phase based on lecithin with nonaqueous solvents occurred only recently.[8] Following this discovery of the nonaqueous L_α phase formed with lecithin and ethylene glycol, we have conducted a series of systematic studies of the solvent order parameters using ^{2}H-NMR as a primary probe.[9–12] Order parameters as well as relaxation rates are composition dependent in these systems. These results are generally rationalized in terms of a fast exchange of the solvent molecule between two states; a bound state on the polar headgroup and a free state in the interstitial region. Further support for this model was provided by theoretical calculations[13–15] of solvent binding with the polar headgroup. The results indicate that typically one or two solvent molecules are strongly bound ($E_{\text{binding}} \sim 20$ Kcal mole^{-1}). The association energy is derived in part from hydrogen-bonding and in part from solvent induced changes in geometry of the amphiphile. Thus, solvent specific sites on the polar headgroup are expected.

With methods at hand to characterize the L_α phase in terms of order parameters and relaxation rates, with ethylene glycol or water as a solvent, we found it attractive to study the competitive processes in systems containing both ethylene glycol and water in combination as a mixed solvent. To this end, we examine the ^{2}H-NMR evidence for the competitive *vs.* specific binding of the solvent in this paper. We have chosen ethylene glycol-d_4 as a probe for the ^{2}H-NMR study in order to circumvent chemical exchange contributions to the spin lattice relaxation and overlapping signals (in case of EG-OD or D_2O). Since it was known from the previous studies that the L_α phase formed by ethylene glycol with lecithin has a lower amphiphile thickness than the corresponding aqueous counterpart, we have monitored the interlayer spacing using wide angle X-ray studies of the interlayer thickness.

EXPERIMENTAL

Materials

Lecithin *L*-a-(egg) was obtained from Avanti Polar Lipids, Inc. (Birmingham, AL 35216) and was used without further purification. Ethylene glycol-d_4, $HOCD_2CD_2OH$, (99%D) was purchased from Stohler Isotope Chemicals (Azusa, CA 91702).

Sample preparation

Two series of samples were prepared; 70% lecithin: 30% solvent and 55% Lecithin: 45% solvent. Ethylene glycol-d_4 and water mixtures covering the entire composition range were used as solvent. Samples were mixed and checked for homogeneity according to the procedure described earlier.[10]

NMR data

^{2}H-NMR spectra were obtained from a homebuilt NMR spectrometer operating at 9.18 MHz. The spectrometer is interfaced with a Nicolet 1180E data processor and a phase sensitive quadrature detection scheme was used. Typical 90 degree pulse width were 8–10 μsec. and the dead time of the spectrometer is 50 μsec. All spectra were recorded at 21°C. Deuteron NMR spectra of ethylene glycol-d_4 in all samples exhibited a "powder pattern" characteristic of a spin $I = 1$ in an axially symmetric environment. Typical sweep widths were 5–10 kHz. T_1 values were determined using the standard inversion recovery method, the T11R pulse sequence in the NTCFT software T_1 values were further processed according to the T131R routine to verify the complete inversion of the magnetization in T_1 experiments. The maximum variation of the T_1 values was typically less than 10%. The measurements were repeated after two months on identical samples to verify the reproducibility of the results. ^{31}P-NMR spectra were recorded on a CXP-200 spectrometer operating at 81 MHz.

X-ray data

Interlayer spacings were determined by reflection measurements using Ni filtered Cu radiation ($\lambda = 1.542$) and a Tennelec position sensitive detector. The data was collected in the RCA (Canberra)

data acquisition system (1024 channels). The system was interfaced with an IBM PC (XT) computer which is programmed to perform necessary data transfer and subsequent calculations of the interlayer spacing (10). Typical acquisition times are a few minutes.

RESULTS

In Figure 1, we present a plot of interlayer spacing *vs.* mole fraction of water in the solvent. The amounts of lipid were fixed at 70% and at 55% (W/W) in two separate series. These results parallel those of

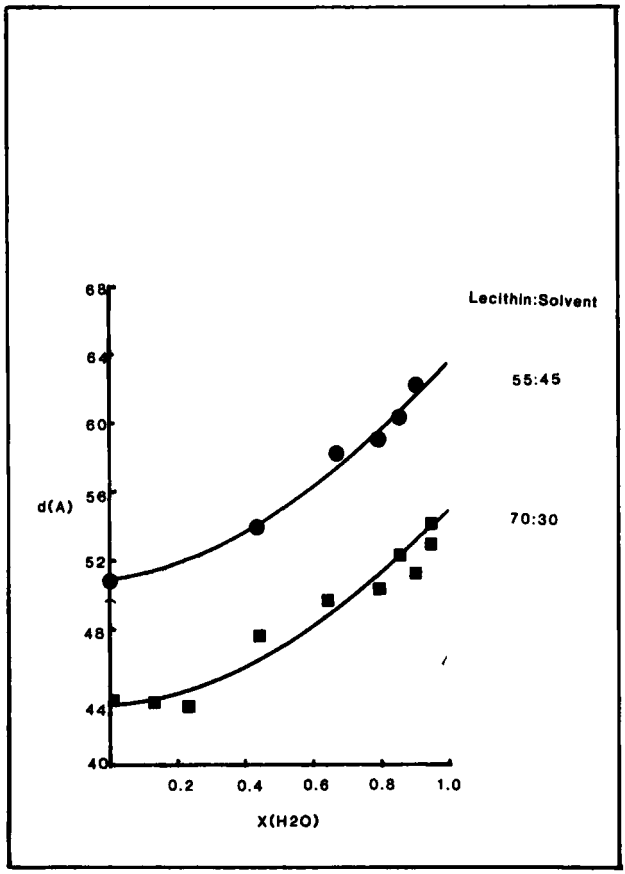

FIGURE 1 Interlayer spacing versus solvent composition [$H_2O/(H_2O + EG)$] in a lamellar liquid crystal from lecithin, water, ethylene glycol. Lecithin/solvent weight ratios: ● = 55/45, ■ = 70/30.

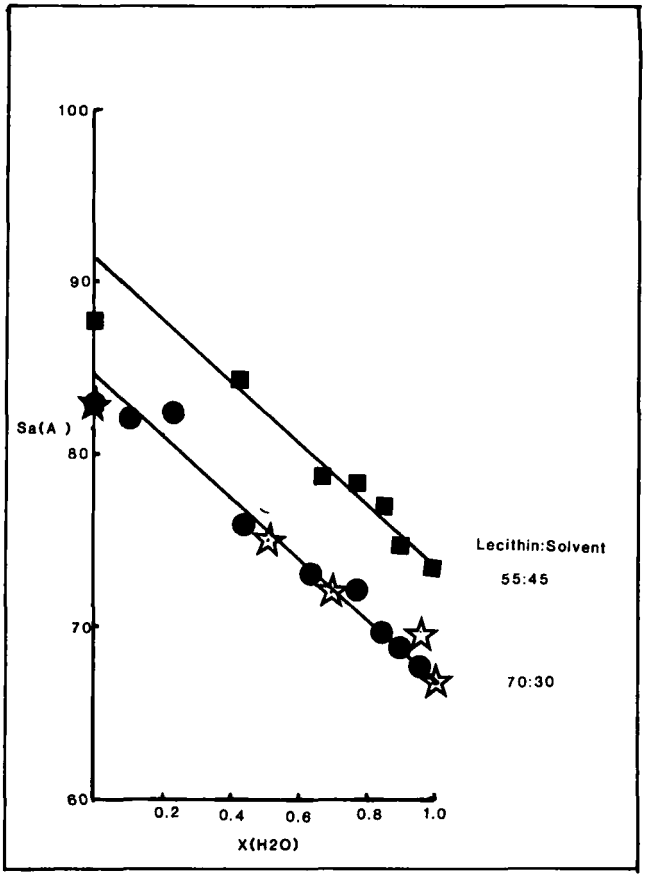

FIGURE 2 Lecithin cross sectional area for the two series in Figure 1. Lecithin/solvent weight ratios: ● = 55/45, ■ = 70/30, calculated according to Reference 17 ☆.

the previous study[8] which showed larger interlayer spacings for the aqueous system as compared to the nonaqueous system. However, the interesting feature of the plot is that the spacing increases nonlinearly as the mole fraction of water is increased. The data were used to calculate the surface cross sectional area of the polar headgroup as shown in Figure 2. In addition, for the 70:30 phase, the figure gives the area of the polar headgroup calculated from the "wobble model"[16] as discussed below.

The plot of the quadrupolar splitting of the methylene deuterons of ethylene glycol *vs.* solvent molar ratio is shown in Figure 3. The gradual decrease in the quadrupolar splitting with increasing water content is consistent with the notion that ethylene glycol molecules

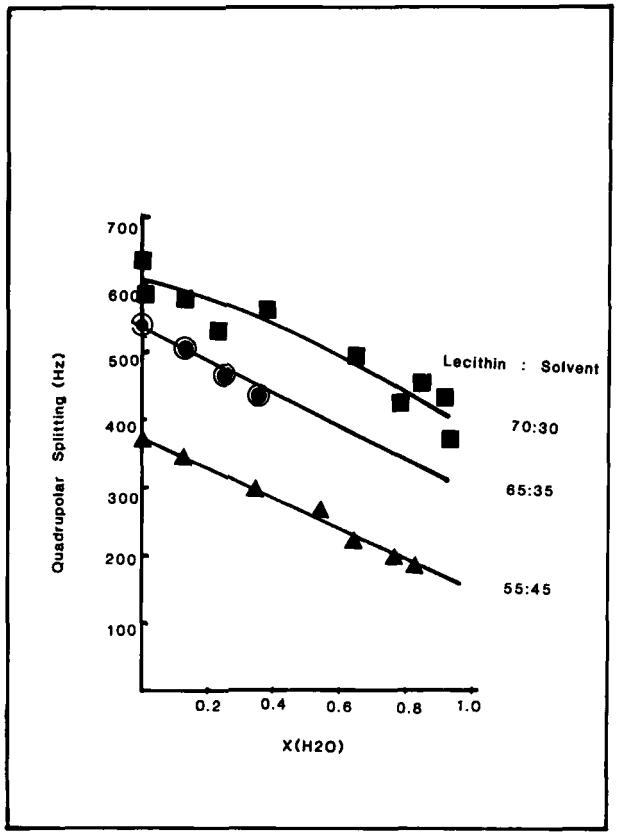

FIGURE 3 Quadrupolar splitting versus solvent composition [$H_2O/(H_2O + EG)$] in a lamellar liquid crystal from lecithin, water, ethylene glycol. Lecithin/solvent weight ratios: ⊙ = 65/35, ■ = 70/30 and ▲ = 55/45.

attached to the polar groups of lecithin are partly replaced by water. However, it is essential to note that the nature of the observed change in the quadrupolar splitting indicates that the binding constants for both solvents are of the same order of magnitude. In addition, a specific site of higher binding energy for ethylene glycol can be ruled out since that would have resulted in an increase in the average quadrupolar splitting with the more "loosely bound" ethylene glycol molecules being replaced by water molecules. Note also that the slopes $ds/dX_{(H_2O)}$, for the three lipid compositions are similar.

In Figure 4, the relaxation rate for the methylene deuterons is plotted as indicated above. Also included in the figure is the corresponding plot of the relaxation rate of the methylene deuterons of

ethylene glycol in an isotropic aqueous solution with no amphiphiles present. Although the order of magnitude of the quadrupolar splittings indicates that the solvent is somewhat isotropic, the relaxation data clearly show that there exists substantial differences between correlation times for the motion of the solvent molecules in the isotropic phase and those in the L_α phases. Thus, the widely held view about the fluidity of the solvent in the interstitial region should be treated with caution.

In Figure 5, we present a similar plot of the ^{31}P NMR chemical shift anisotropy. The results were interpreted in terms of Klein's wobble model (16), with the wobble angle defined as in Figure 6. Table I shows the results using $\Delta\sigma$ = 69 ppm. The calculation is

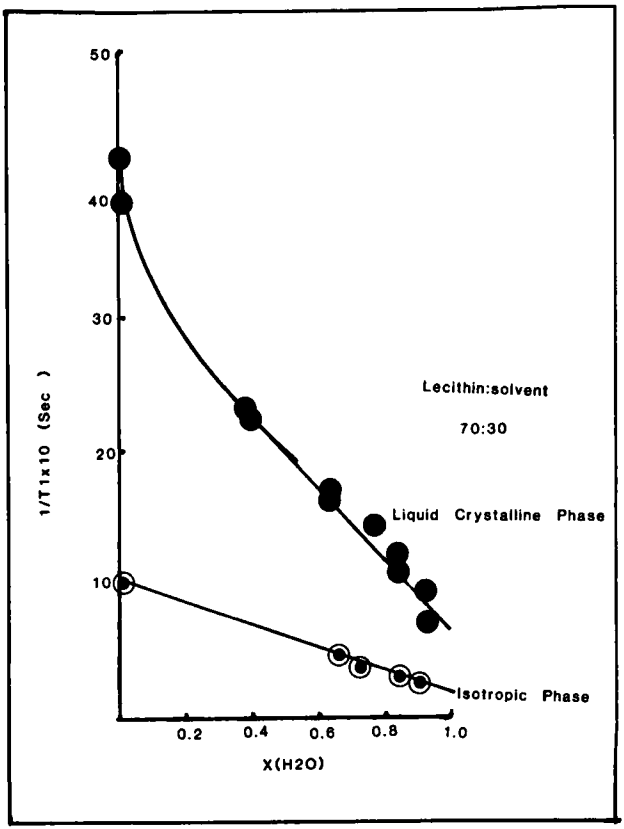

FIGURE 4 Relaxation rates for the methylene deuterons versus solvent composition [H_2O/(H_2O + EG)] in a lamellar liquid crystal from lecithin, water and ethylene glycol and in the isotropic solution of water and ethylene glycol ⊙.

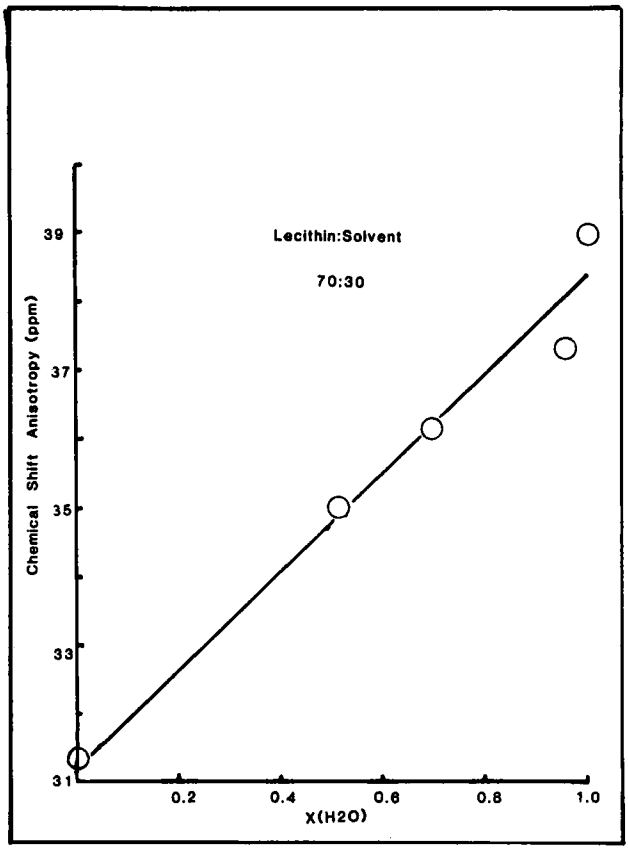

FIGURE 5 Chemical shift anisotropics versus solvent composition [H_2O/H_2O + EG)] in a lamellar liquid crystal from lecithin, water and ethylene glycol.

discussed in the next section. Again the results indicate that there is a gradual change in the dynamic state of the polar group as solvent composition is varied. Thus, at least on NMR and X-ray time scales, no abrupt changes were detected in the conformational state of the amphiphile.

DISCUSSION

Both X-ray and ^{31}P-NMR measurements, indicate a gradual transformation of the amphiphile thickness with monotonic variation of the water/ethylene glycol ratio. In the present article, theoretical

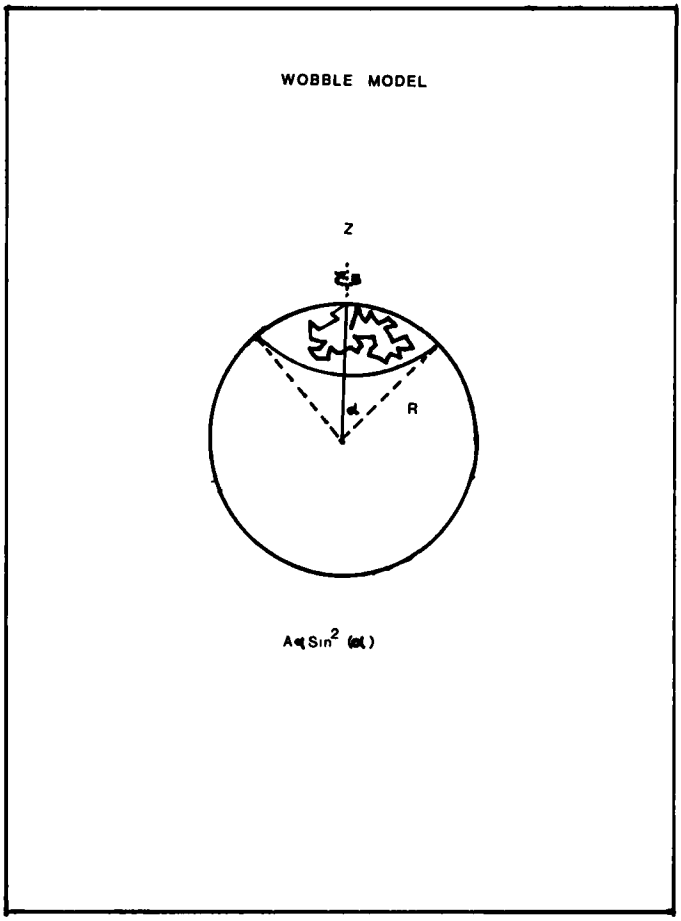

FIGURE 6 Geometry of the wobble model (see Reference 22). The rotation axis for the amphiphile is allowed to move randomly over that partial segment of the sphere defined by radius α. For this model, area ∝ $\sin^2\alpha$.

treatment of the problem (such as in Ref. 17) will not be made; instead, the changes will be related to the concept of solvent enhanced disorder of the amphiphile. The order parameters of individual methylene deuterons of the amphiphile have been successfully employed by Seelig et al.[18] to estimate the amphiphile thickness in the lamellar liquid crystalline phase. The effective thickness is given as follows:

$$\langle L \rangle = 1.25\left[n(1/2) - \sum S_{XD} \right]$$

TABLE I

Parameters characterizing observed ^{31}P chemical shift anisotropies for a 70/30 lecithin/solvent liquid crystalline phase in terms of the wobble model

Solvent composition (H_2O)	Chemical shift anisotropy obs(ppm)	Wobble angle (degrees)	Lecithin cross sectional area $(A)^2$
0.0	31.02	55.11	81.5
0.56	34.49	51.84	75.1
0.75	36.16	50.26	71.9
0.88	37.27	49.20	69.8
1.0	38.94	47.61	66.5

in which $\langle L \rangle$ is the mean square thickness of the amphiphilic layer and ΣS_{XD}, the sum of the methylene deuteron order parameters, is taken over the entire chain. In these systems, the order parameters S_{XD} are normally negative and increased motion causes the magnitude of S_{XD} to decrease. Thus it is clear that the above expression predicts a decrease in the amphiphile thickness as the chains become more disordered due to a reduction in the segmental order parameters across the chain length. In the present study, the individual order parameters are not available but the observed decrease in phosphorus NMR chemical shift anisotropy with increasing EG content at a fixed solvent weight ratio may be taken as indicative of an overall decrease in the amphiphile order parameters. The amphiphile chains are more disordered in the nonaqueous medium than in the aqueous medium as shown by our recent proton NMR studies.[19] However, the ^{31}P results are complicated by the fact that the chemical shift anisotropy is governed by two order parameters;[20,21] therefore, the present results should be viewed as support for the proton NMR results.[19]

This naturally leads to the question of the physical reason for the disorder. Further insight into the structural aspects is gained from surface area considerations of the amphiphile. Development along the lines of packing concepts introduced by Ninham et al.[22] would immediately suggest that a decrease in the amphiphile thickness should be accompanied by a concomitant increase in the apparent surface area per amphiphile molecule. An increase in the amphiphile surface area may be rationalized as a natural consequence of enhanced penetration of solvent molecules between the amphiphilic molecules as the relative composition of the ethylene glycol in the solvent medium is increased. The plot in Figure 2 suggests that the observed cross

sectional area is a simple weighted average described by the following equation

$$A_{obs} = A_{EG}X_{EG} + A_{H_2O}X_{H_2O} \quad (1)$$

where A_i denotes the apparent lipid cross sectional area and X_i the mole fraction of the appropriate component. This approach indicates that the specific cross sectional areas (after appropriate extrapolation at $X_{H_2O} = 0$ and 1 in the plot) are dependent on solvent to lipid ratio. This is not surprising, since at higher solvent to lipid ratio, the extent of swelling (penetration) is known to increase.

Further insight into the dynamics of the solvation is obtained from a simplified consideration of the composition dependence of the ^{31}P-NMR chemical shift anisotropy. The model we shall employ is the so-called "wobble model."[16] The primary motion of the amphiphile is assumed to be a rapid reorientation about the long axis, which results in an axially symmetric line shape for ^{31}P with chemical shift anisotropy $\Delta\sigma_R = 69$ ppm. The rotation axis of the PO_4 group is further assumed to undergo random excursion on the restricted surface of a sphere defined by the so-called wobble angle α, as shown in Figure 6. The observed chemical shift anisotropy in the presence of the wobble, $\Delta\sigma_w$, is further reduced by motional averaging according to

$$\Delta\sigma_W = \Delta\sigma_R(1 - 3W)/2 \quad (2)$$

where

$$W = (1 + \cos\alpha + \cos^2\alpha)/3 \quad (3)$$

For both motions, the frequency of the motion is assumed to be large compared to the chemical shift anisotropy.

The phosphorus chemical shift anisotropy may be used in a rather crude way to estimate the dependence of the lecithin surface area on solvent composition. Such a calculation presupposes a unique axis of rotation. Seelig et al.[17] have shown that the chemical shift anisotropy is scaled by an order parameter, which is taken to be that of the methylene deuterons in the glycerol moiety. The model is equivalent to multiple rotations.

We note that $\Delta\sigma$ values for aqueous lamellar phases are typically 40–45 ppm, whereas those for phases containing EG vary between 20 and 40 ppm depending upon Lecithin/EG ratio. The data in Table

I show monotonic variation between 31 and 39 ppm for mole fraction of water in the solvent between 0 and 1. These data suggest that the nonaqueous phase has a larger lecithin surface area than the corresponding aqueous phase.

The cross sectional area of the polar group from the X-Ray study may be compared with those from ^{31}P NMR data by use of the "wobble model." The value of α is obtained by use of eqns. 2 and 3 from the data shown in Table I. The cross sectional area is obtained from the simple geometric model shown in Figure 6. For this model the surface area is given roughly as

$$\text{Area} = \pi R^2 \sin^2\alpha \tag{4}$$

The composition (water/EG ratio) dependence of R is unknown, and for simplicity, we assume R to be constant. A value $R = 6.2$ Å scales the area properly for the aqueous lamellar phase. Areas for mixed solvent phases were calculated using the same value for R, and the results are shown in Table I.

The comparison of the NMR data with the X-ray data (Figure 2) shows that there is excellent agreement between the two methods. An alternative explanation of the observed results is that there is a gradual change in packing of the polar headgroups. It is possible that neutron diffraction studies might allow a conclusion to be drawn concerning these alternatives.

Solvent ^2H NMR studies

The concept of solvent binding to the polar groups is rather well established through theoretical calculations and composition studies of the solvent order parameters. Thus, the observed order parameter variation in Figure 3 may well be described by a two component adsorption model.[23] If we further impose a requirement that all the binding sites on the lipid are filled at all relative solvent compositions, the final expression takes a particularly simple form:

$$(EG)_{bnd} = b_1(EG)/(b_1(EG) + b_2(H_2O)$$

where b_1 and b_2 determine the relative binding affinities of ethylene glycol and water respectively. The NMR parameters measured in this study presumably reflect the fractions of solvent in the binding sites. Thus the qualitative features of the composition dependent variation

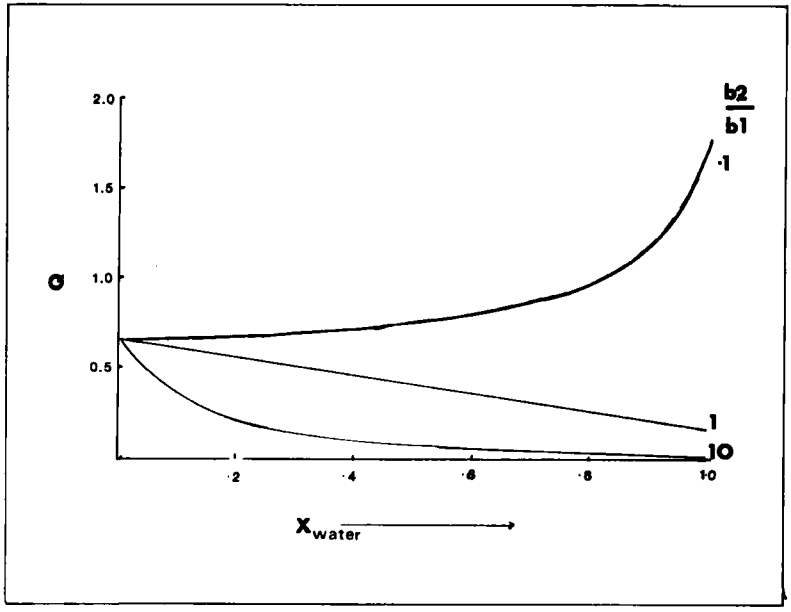

FIGURE 7 Simulation of order parameter obtained from a 2-side model. In this model the concentration of bound species is given as follows: C-bound (ethylene glycol (EG)) is B1 × (EG) ÷ B1 (EG + B2) H_2O. This model assumes that on the binding sides on the head groups and filled at all times. From this equation it is straight forward; to calculate fraction of ethylene glycol bound as the function of water contact.

of the NMR parameters can be determined from this simple model; they are presented in Figure 7.

Quite clearly, the data reflect no substantial variation of the binding affinity from water to ethylene glycol. We would like to emphasize that there are several conceptual shortcomings of such a simple model. The so-called "bound species" need not have a composition independent order parameter. The ^{31}P NMR data suggest that the polar groups are more ordered as the ratio of water to ethylene glycol is increased (at a fixed lipid to solvent ratio). If such a trend is imposed on the order parameter of the bound species, then the values of b_1 would certainly be overestimated. Indeed, it is not possible to reconcile both T_1 and the order parameter in terms of a consistent number of bound molecules.

In order to treat order parameter variation rigorously, one has to resort to a molecule dynamics simulation of the order parameter. Such an approach has been recently presented by Heinzinger *et al.*[24] These authors estimate order parameters and the diffusion coefficient

of water bound between two walls assuming a Lennard-Jones type interaction potential. The predicted values of the order parameter of molecules near the wall are typically .01 and they decrease strongly as the separation between the molecule and wall increases. Intuitively, this is expected since the bulk water is not ordered. Marcelja *et al.*[25] have predicted such behavior from a phenomenological treatment of the effect of lipid-lipid repulsive forces on water held within the lipid bilayer. Though the molecular dynamics simulation represents a first step, in practice such simulation for a lipid bilayer presents a formidable problem in accounting for electrostatic as well as hydrogen bonding types of interaction. It should be noted that the order parameter of EG in the limit of infinite dilution is non zero and depends roughly upon the inverse of the interlayer spacing.[11]

One of the interesting features of this study is the composition dependence of T_1. It is fairly well established that the solvent deuterons are on the high temperature arm of the T_1 minimum. Hence, we write[26] for T_1:

$$1/T_1 \propto \tau_c$$

It must be then concluded that the correlation time changes by a factor of six by simply changing the relative amount of water present both in the liquid crystalline phase and in the isotropic solution. Such a change would be expected from a simple viscosity consideration since the macroscopic viscosity changes by factor of 17 in going from pure ethylene glycol to water. There have been numerous studies[27,28] which have indicated an almost linear variation of the molecule correlation time with the macroscopic viscosity. Thus, a likely conclusion, consistent with observed T_1 values, is that the two dimensional thin liquids (solvent thickness, d_S = 9 Å) have extraordinarily high apparent viscosities. However, extreme care is required in defining what is meant by viscosity as measured by the NMR T_1 results. In the case of quadrupolar nuclei, it is the rotational motion that modulates the relevant interaction tensors. The inadequacy of the correlation between viscosity (and microviscosity) and the translational and rotational diffusion coefficients of small molecules is discussed by Huntress.[29] In view of the fact that the surface viscosities are orders of magnitude higher than predicted from bulk viscosities and the "viscosities" of penetrating solvent molecules are an unknown quantity, the results appear reasonable.

Another possible explanation of the observed correlation time variation is in terms of the so-called hydration force invoked in aqueous

lamellar lecithin based liquid crystals. The extraordinary repulsive forces[30-32] that are presumed to exist between the adjacent lamellae would certainly increase the correlation time of the molecules sandwiched between the bilayers. Large decreases in correlation times have been observed for hydrogen bonding liquids under high pressure.[28] The observed effects are similar in magnitude to those observed in the present study, and thus one may interpret our results in terms of pressure induced viscosity in the interstitial medium.

Finally, we emphasize that the effect of alignment of the solute (non-zero order parameter) on relaxation is minimal in the present case as the order parameter of the solvent molecules, indicate that the motion is essentially isotropic. Also, ODF or SLRS[33,34] contributions to the spin lattice relaxation are negligible since they enter as a function of S^2.

References

1. N. J. Salsbury and D. Chapman, *Biochim. Biophys. Acta,* **163**, 314 (1968).
2. D. Chapman, *Quart. Rev. Biophys.* and references sited therein, **8**, 185 (1975).
3. S. Marcelja, *Biochim. Biophys. Acta,* **367**, 165 (1974).
4. J. F. Nagle, *Annu. Rev. Phys. Chem.*, **31**, 157 (1980).
5. E. G. Finer, *J. Chem. Soc. Faraday Trans. II*, **69**, 1590 (1973).
6. J. Seelig and F. Borle, *Biochim. Biophys. Acta*, **735**, 131 (1983).
7. K. Arnold, L. Pratsch and K. Gawrisch, *Biochim. Biophys. Acta,* **728**, 121 (1983).
8. N. Moucharafieh and S. E. Friberg, *Mol. Cryst. Liq. Cryst.*, **49**, 231 (1979).
9. D. W. Larsen, S. E. Friberg and H. Christenson, *J. Am. Chem. Soc.*, **102**, 6565 (1980).
10. D. W. Larsen, S. B. Rananavare, M. El Nokaly and S. E. Friberg, *Finn. Chem. Letters*, 6-8, 96-104 (1982).
11. D. W. Larsen, S. B. Rananavare and S. E. Friberg, *J. Am. Chem. Soc.* (In press).
12. S. B. Rananavare, Ph.D. Thesis, 1983, Univ. of Missouri-St. Louis, Mo., 269 pp.
13. T. Flaim, S. E. Friberg and P. L. M. Plummer, *J. Biol. Phys.*, **9**, 201 (1981).
14. A. Pullman and B. Pullman, *Quart. Rev. Biophys.*, **7**, 505 (1975).
15. H. Frishleder, S. Gleichmann, R. Krahl, *Chem. Phys. Lipids*, **19**, 144 (1977).
16. S. J. Kohler and M. P. Klein, *Biochemistry*, **16**, 519 (1977).
17. M. Kreissler, B. Lemaire and P. Bothorel, *Biochim. Biophys. Acta*, **735**, 23 (1983).
18. H. Schindler and J. Seelig, *Biochemistry*, **14**, 11 and 2283 (1975).
19. D. W. Larsen, S. B. Rananavare, M. El Nokaly, F. S. Starry and S. E. Friberg (submitted for publication).
20. J. Seelig, *Biochim. Biophys. Acta*, **515**, 105 (1978).
21. A. Saupe, Angew, Chem. Int. ed., **7**, 2 and 108 (1968).
22. J. N. Israelachvili, D. J. Mitchell and B. W. Ninham, *J. Chem. Soc. Faraday Trans.* Ii, **72**, 1525 (1976).
23. A. W. Adamson, "*Physical Chemistry of Surfaces*," (4th ed.), Intersciences Publishers, New York, 1982, pp. 121.
24. R. Sonnenschein and K. Heinzinger, *Chem. Phys. Letts.*, **102**, 6 and 550 (1983).
25. S. Marcelja and N. Radic, *Chem. Phys. Letts.*, **42**, 1 and 129 (1976).
26. A. Abragam, "*Principles of Nuclear Magnetism*," Clarendon Press, Oxford (1961).

27. N. Bloembergen, "*Nuclear Magnetic Relaxation*," Frontiers of Modern Physics series, W. A. Benjamin, Inc., New York (1961).
28. M. Wolfe and J. Jonas, *J. Chem. Phys.*, **71**, 8 and 3252 (1979).
29. W. Huntress Jr., "*Advances in Magnetic Resonance*", **4**, 1 (1969).
30. V. A. Parsegian, N. Fuller and R. P. Rand, *Proc. Natl. Acad. Sci.* USA, **76**, 6 and 2750 (1979).
31. D. LeNeveu, R. P. Rand, and V. A. Parsegian, Nature (London), **259**, 601 (1976).
32. L. Guldrand, B. Jonsson and H. Wennerstrom, *J. Colloid Interface Sci.*, **89**, 2 and 532 (1982).
33. J. H. Freed, *J. Chem. Phys.*, **66**, 4183 (1977).
34. D. Jaffe, R. L. Vold, and R. R. Vold, *J. Chem. Phys.*, **78**, 8 and 4852, (1983).