Rhodes, D. G., Blechner, S. L., Schoen, P. E., & Yager, P. (1987) *Biophys. J. 51*, 527a.
Rhodes, D. G., Blechner, S. L., Yager, P., & Schoen, P. E. (1988) *Chem. Phys. Lipids 49*, 39-47.
Rosenblatt, C., Yager, P., & Schoen, P. E. (1987) *Biophys. J. 52*, 295-301.
Rudolph, A. S., & Burke, T. G. (1987) *Biochim. Biophys. Acta 902*, 349-359.
Rudolph, A. S., Burke, T. G., & Sheridan, J. P. (1987) *Biophys. J. 51*, 185a.
Sayre, D. (1952) *Acta Crystallogr. 5*, 843.
Schnur, J. M., Price, R., Schoen, P., Yager, P., Calvert, J. M., Georger, J., & Singh, A. (1987) *Thin Solid Films 152*, 181-206.
Schoen, P. E., & Yager, P. (1985) *J. Polym. Sci., Polym. Phys. Ed. 23*, 2203-2216.
Schoen, P. E., Yager, P., Sheridan, J. P., Price, R., Schnur, J. M., Singh, A., Rhodes, D. G., & Blechner, S. L. (1987) *Mol. Cryst. Liq. Cryst. 153*, 357-366.
Servuss, R. M. (1988) *Chem. Phys. Lipids 46*, 37-41.
Shannon, C. E. (1949) *Proc. Inst. Radio Eng. N.Y. 37*, 10-21.
Sheridan, J. P. (1988) *Nav. Res. Lab. Memo. Rep.* 5975.
Singh, A., Thompson, R. B., & Schnur, J. M. (1986) *J. Am. Chem. Soc. 108*, 2785.
Snyder, R. G., Hsu, S. L., & Krimm, S. (1978) *Spectrochim. Acta 34a*, 395.
Tardieu, A., Luzzati, V., & Reman, F. C. (1973) *J. Mol. Biol. 75*, 711-733.
Torbet, J., & Wilkins, M. H. F. (1976) *J. Theor. Biol. 62*, 447-58.
Weast, R. C., & Astle, M. J., Eds. (1977) *CRC Handbook of Chemistry and Physics*, CRC Press, Boca Raton, FL.
Yager, P., & Schoen, P. (1984) *Mol. Cryst. Liq. Cryst. 106*, 371-381.
Yager, P., Schoen, P. E., Davies, C., Price, R., & Singh, A. (1985) *Biophys. J. 48*, 899-906.
Yager, P., Price, R. P., Schnur, J. M., Schoen, P. E., Singh, A., & Rhodes, D. G. (1988) *Chem. Phys. Lipids 46*, 171-179.

On the Use of Deuterium Nuclear Magnetic Resonance as a Probe of Chain Packing in Lipid Bilayers†

N. Boden,* S. A. Jones, and F. Sixl
School of Chemistry, The University, Leeds LS2 9JT, U.K.
Received July 10, 1990; Revised Manuscript Received November 2, 1990

ABSTRACT: The packing of hydrocarbon chains in the bilayers of lamellar (L_α) phases of soap/water and phospholipid/water mixtures has been studied by deuterium NMR spectroscopy and X-ray diffraction. A universal correlation is shown to exist between the average C-D bond order parameter $\bar{S}_{CD}$ of hydrocarbon chains and the average area per chain a_{ch}, irrespective of the chemical structure of the surfactant (hydrophilic group, number of chains per molecule, and chain length), composition, and temperature. The practical utility of the correlation is illustrated by its application to the characterization of the distribution of various hydrophobic and amphiphilic solutes in bilayers. The distribution of hydrocarbons within a bilayer is shown to depend upon their molecular structure in a manner which highlights the nature of the molecular interactions involved. For example, benzene is shown to be fairly uniformly distributed across the bilayer with an increasing tendency to distribute into the center at high concentrations. In contrast, the more complex hydrocarbon tetradecane preferentially distributes into the center of the bilayer at low concentrations, while at higher concentrations it intercalates between the surfactant chains. Alcohols such as benzyl alcohol, octanol, and decanol all interact similarly with the bilayer in so far as they are pinned to the polar/apolar interface, presumably by involvement of the hydroxyl group in a hydrogen bond. But the response of the surfactant chains to the void volume created in the center of the bilayer is dependent upon the distance of penetration of the alcohol into the bilayer. For benzyl alcohol, the shortest molecule, this void volume is taken up by the disordering of the chains, while for decanol, the longest molecule, it is absorbed by interdigitation of the chains of apposing monolayers. For octanol, the chain interdigitation mechanism is dominant at low concentrations, but there is a transition to chain disordering at high concentrations. Finally, it is shown that the correlation provides a useful test for statistical mechanical models of chain ordering in lipid bilayers.

Membranes are quasi two-dimensional, multicomponent fluids. The underlying structural element is a bimolecular layer of amphiphilic lipids. This bilayer has consequently been the subject of extensive experimental and theoretical studies. It consists of a core of hydrocarbon chains which is separated from the water by a layer of densely packed hydrophilic headgroups. The packing density of the hydrocarbon chains within the bilayer is commonly described in terms of either the bilayer thickness d_l or the average area per molecule a_m at the bilayer/water interface. Most of our knowledge concerning molecular packing in lipid bilayers has been obtained from X-ray measurements of the bilayer repeat distance d_o in lamellar L_α phases according to a procedure first described by Luzzati (1969): d_l is calculated from $d_l = \phi_l d_o$ and a_m from $a_m = 2V_l/Nd_l$, where ϕ_l, V_l, and N are respectively the volume fraction of lipid, the partial molar volume of lipid, and the

† We thank the University of Leeds for a "Pool Post" for F.S. and the Science and Engineering Research Council for a research studentship to S.A.J. and for financial support to purchase equipment.

0006-2960/91/0430-2146$02.50/0

Avogadro number. These measurements must, however, be conducted on well-defined liquid-crystalline phases, where the water content is low (typically, less than 40% by weight of water). At these low water contents there are uncertainties about the values of the partial molar volumes, although density measurements of soap/water (Ekwall et al., 1963; Mandell et al., 1968; Laggner & Stabinger, 1976) and phospholipid/water (White et al., 1987; Tardieu et al., 1973) lamellar phases seem to support the generally made assumption that V_l and V_w are independent of composition and equal to the values for, respectively, lipid in excess water and bulk water. It is also essential to know precisely the composition of the sample: uncertainties of composition can be a significant source of errors. For instance, it is most likely that the discrepancies of the structural data reported for DPPC/water mixtures by different laboratories (Inoko & Mitsui, 1978; Janiak et al., 1976; Lis et al., 1982) are the result of uncertainties in compositions of the samples. To circumvent these problems, McIntosh and Simon (1986) have recently suggested an approach based upon the measurement of electron density profiles. However, the interpretation of electron density profiles in terms of chain packing is made difficult by the inherently low resolution and because assumptions have to be made about the structure of the bilayer/water interface. Apart from these technical difficulties, there are practical constraints on the applicability of the X-ray method. The method is not applicable to biologically more interesting systems such as, for example, single-walled cellular membranes or dispersions of lipid vesicles. In a different context, studies of the interaction of solute molecules with lipid bilayers are complicated by the need to aportion the total surface area of the membrane into the contributions arising from the various individual components. For instance, it is known that local and general anaesthetics are preferentially solubilized in the polar headgroup region of phospholipid bilayers (Boden et al., 1988; Boulanger et al., 1981; Kuroda & Fujiwara, 1987) and, as a result, the chain packing at the interface will be quite different from that within the bulk of the bilayer. Clearly, a more direct probe of the hydrocarbon chain packing in a bilayer is needed which, unlike X-ray diffraction, would be applicable to systems of unknown composition. The objective of this work is to show that measurements of deuterium NMR quadrupole splittings for CD_2 groups in hydrocarbon chains can be used to this end.

The basis of our approach is as follows. The packing of the hydrophilic groups at the bilayer/water interface imposes a constraint on the motion of the chains, which, in turn, gives rise to the characteristic variation of the partially averaged value $\Delta\tilde{\nu}_i$ of the deuterium quadrupole splitting of the CD_2 group as a function of its position i along the chain (Seelig & Seelig, 1980). Thus, the "ordering" of the chains is an intrinsic property of the system and must reflect the density of packing of the chains. That this is so has been demonstrated experimentally by Mely et al. (1975): the quadrupole splittings measured in the lamellar phase of the potassium dodecanoate/water system were found to decrease as the average area per chain a_{ch} was increased. Now, it would be useful to have an explicit expression relating the $\Delta\tilde{\nu}_i$ to a_{ch}. Unfortunately, either current statistical mechanical models of chain ordering are unsuitable for such practical purposes, or as we shall demonstrate later, they agree only semiquantitatively with experimental measurements. We, therefore, need to circumvent this situation with an empirical approach as proposed in this paper. New X-ray diffraction and ^2H NMR measurements for the lamellar phases of the dimyristoylphosphatidylcholine (DMPC)/water and the potassium oleate/water systems are combined with published data for a variety of other phospholipid/water and soap/water lamellar phases to yield an empirical correlation between the average CD bond order parameter $\bar{S}_{CD}$ of hydrocarbon chains and a_{ch}. This approach is analogous to the empirical usage of chemical shifts in high-resolution NMR spectroscopy. It will turn out that the correlation between $\bar{S}_{CD}$ and a_{ch} is universal and independent of the chemical structure of the surfactant (polar headgroup, chain length, number of chains per lipid molecule), composition, and temperature. This correlation is of practical utility, apart from its use for testing theoretical models of chain ordering. This is demonstrated by using it to expose the way in which various kinds of hydrocarbons and alcohols interact with and are distributed in bilayers of DMPC and potassium oleate, chosen to reveal the effects of the greater complexity of the hydrophilic group in the former case. It could also be used to study chain packing in lipid bilayers of unknown composition, such as, for example, in real biological membranes or in dispersions of phospholipids in excess water.

Materials and Methods

Synthesis. DMPC, perdeuteriated in the *sn*-2 chain, was synthesized and purified according to standard procedures (Gupta et al., 1977). Ordinary DMPC was purchased from Fluka. Potassium oleate was prepared by neutralizing a solution of pure oleic acid in ethanol with potassium ethoxide. All the surfactants were dried under high vacuum over phosphorus pentoxide for at least 2 days. Other materials (benzene, cyclohexane, tetradecane, benzyl alcohol, octanol, octanol-d_{17}, and decanol) were distilled under vacuum.

Sample Preparation. Samples for NMR measurements were directly prepared by weighting the components into NMR tubes of 5-mm o.d. DMPC was dried under vacuum over phosphorus pentoxide for 2 days before the appropriate amount of water was added with a microsyringe. The tubes were sealed under nitrogen gas, and their contents thoroughly mixed by repeatedly spinning the sample back and forth in a bench centrifuge while the sample was maintained in its lamellar L_α phase (40–50 °C for DMPC and 20–25 °C for potassium oleate). Samples for X-ray measurements were indirectly prepared as described above and then transferred into Lindemann tubes of 0.5-mm i.d.

Measurements. ^2H NMR spectra were accumulated at 61.5 MHz on a Bruker WH-400 spectrometer with single pulses of 30-μs duration. Temperature control was via the standard air-flow system, being accurate to an estimated ±1 °C.

Small-angle X-ray diffraction measurements were carried out with a home-built pinhole camera, monochromatic Cu Kα radiation of vavelength 0.154 nm, and point collimation. Sample temperature was controlled to within 0.01 K. Molecular areas a_m were calculated from the bilayer repeat d_o as described by Luzzati (1969). Partial molar volumes of DMPC were taken to be identical with the values calculated from the partial specific volumes of the lipid in excess water (Nagle & Wilkinson, 1978).

Thin-layer chromatography indicated that no degradation of the samples had occurred during the experiments.

Results

Figure 1a shows typical examples of ^2H NMR spectra of lamellar phases of DMPC-d_{27}/water mixtures for two different hydrations. The spectra are seen to be distorted since a high-resolution spectrometer, having insufficient pulse power, has been used. It is well established, however, that this distortion does not affect the values of partially averaged quadrupole splittings $\Delta\tilde{\nu}$ as obtained from the separation between

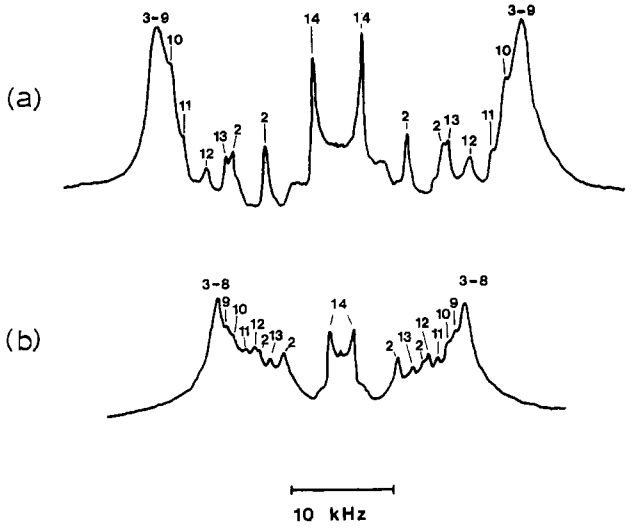

FIGURE 1: ^{2}H NMR spectra of DMPC-d_{27}/water lamellar phases at 40 °C. Mole ratio of water to lipid $R_{w/l}$: (a) 5.0 and (b) 25.0. The average interfacial areas per chain a_{ch}, as measured by X-ray diffraction, are (a) 0.252 nm^2 and (b) 0.313 nm^2.

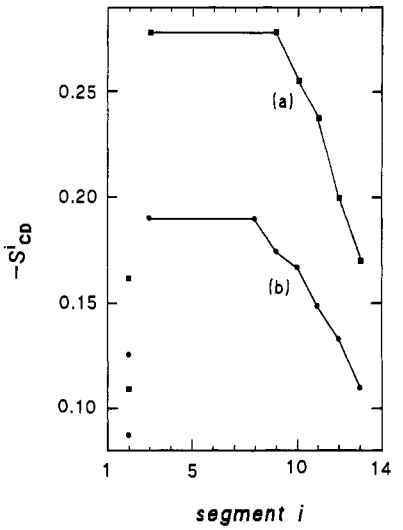

FIGURE 2: Variation of the segmental C–D bond order parameters S^i_{CD} along the *sn*-2 chain of DMPC-d_{27}. The order profiles a and b correspond respectively to spectra a and b in Figure 1, from which the order parameters were calculated by use of $\Delta\tilde{\nu} = 3/4\chi_D S^i_{CD}$ and assuming a value of 170 kHz for χ_D.

the peak singularities in the Pake powder spectrum (Gally et al., 1981). An unambiguous assignment of the individual peaks to the corresponding methylene groups would only be possible if specifically deuteriated fatty acid chains were used. In particular, there is some uncertainty about the values of $\Delta\tilde{\nu}$ for the methylene segment next to the carboxylate group in the *sn*-2 chain. The deuterons of this segment are known to give two separate signals which overlap with resonances from methylene groups further down the chain (Seelig & Seelig, 1974a). The plots of the segmental order parameters S^i_{CD} versus segment n in Figure 2 were, therefore, obtained by comparison with published results for specifically deuteriated phospholipids (Oldfield et al., 1978; Seelig & Seelig, 1974b) and by assuming that S^i_{CD} decreases monotonically toward the end of the chain. The resulting order profiles (Figure 2) clearly show a decrease in the values of all the S^i_{CD} when the mole ratio of water to lipid $R_{w/l}$ increases from 5 to 25. This observation is consistent with the X-ray diffraction experiment, which, over the same range of $R_{w/l}$, indicates an increase in the average area per chain a_{ch} from 0.252 to 0.313 nm^2. But

Table I: Summary of Values for the Average CD Bond Order Parameter $\bar{S}_{CD}$ and the Average Area per Chain a_{ch} at the Bilayer/Water Interface[a]

lipid	$R_{w/l}$	temp (°C)	a_{ch} (nm^2)	ref	$-\bar{S}_{CD}$	ref
DMPC	5	40	0.252	*b*	0.256	*b*
	9	35	0.273	*b*	0.214	*c*
	12	30	0.278	*b*	0.215	*b*
	12	40	0.286	*b*	0.194	*b*
	25	30	0.307	*b*	0.197	*b*
	25	40	0.313	*b*	0.170	*b*
	>25	30	0.310	*b*	*sn*-1 0.192	*d*
					sn-2 0.199	
DPPC	>27	45			*sn*-1 0.198	*e*
					sn-2 0.203	
potassium laurate	3.1	31	0.307	*f*	0.190	*f*
	3.1	74	0.328	*f*	0.173	*f*
	4.9	27	0.345	*f*	0.169	*f*
	4.9	36	0.351	*f*	0.163	*f*
	4.9	51	0.360	*f*	0.154	*f*
	4.9	86	0.390	*f*	0.151	*f*
	4.9	110	0.410	*f*	0.139	*f*
potassium palmitate	6.3	86	0.390	*g*	0.128	*h*
	6.3	104	0.408	*g*	0.125	*i*
potassium stearate (9%) in potassium oleate	5.2	20	0.354	*j*	0.154	*j*
	5.7	20	0.361	*j*	0.147	*j*
	6.4	20	0.369	*j*	0.142	*j*
	6.9	20	0.378	*j*	0.140	*j*
	7.7	20	0.385	*j*	0.139	*j*
rubidium stearate	8.1	86	0.409	*g*	0.137	*k*

[a] In the case of DMPC the values given for $\bar{S}_{CD}$ refer to the *sn*-2 chain, unless stated otherwise. $R_{w/l}$ is the mole ratio of water to lipid. The published values of a_{ch} for DPPC bilayers were found to be to variable for inclusion into this table. [b] This work. [c] Pope and Dubro (1986). [d] Füldner (1980). [e] Seelig and Seelig (1974b). [f] Mely et al. (1975). [g] Gallot and Skoulios (1965). [h] Davis and Jeffrey (1977). [i] Davis et al. (1976). [j] Jones (1982). [k] Jeffrey (1989).

it is at variance with recently published low-resolution electron density profiles of phopholipid bilayers, which failed to detect any changes in the bilayer thickness d_l upon changes of hydration (McIntosh & Simon, 1986). The results for other compositions and temperatures are summarized in Table I together with data taken from the literature for all the soap/water and phospholipid/water lamellar phases for which complementary ^{2}H NMR and X-ray data are available. The orientational order of the chains is expressed in terms of the average C–D bond order parameter $\bar{S}_{CD}$, where the average is taken over all of the $n - 2$ methylene segments, except in the case of phospholipids where values of S^i_{CD} for the C_2 segment of the *sn*-2 chain are not included.

In the case of phospholipids with two hydrocarbon chains per molecule there exists the possiblity that the two chains are motionally inequivalent. However, the results for DMPC and DPPC in Table I show that $\bar{S}_{CD}$ is in fact very similar for both the *sn*-1 and *sn*-2 chains. Thus, the value of $\bar{S}_{CD}$ for the *sn*-2 chain can also be taken as a good representation for the averaged order parameter of the *sn*-1 chain.

In order to correlate $\bar{S}_{CD}$ and a_{ch}, we have chosen to plot $-\bar{S}_{CD}$ versus $1/a_{ch}$ (Figure 3). Intuitively, a $1/a_{ch}$ dependence of $-\bar{S}_{CD}$ seemed to be most plausible, although there is no physical argument which would predict the observed linear relationship between $-\bar{S}_{CD}$ and $1/a_{cd}$. The straight line in Figure 3 represents a least mean squares fit to the experimental points, and its functional form is

$$-\bar{S}_{CD} = a + b/a_{ch} \quad (1)$$

where $a = -0.049 \pm 0.012$, $b = 0.073 \pm 0.004$ nm^2, and the correlation coefficient is $r = 0.972$. A linear correlation of comparable quality was obtained from a plot of log $-\bar{S}_{CD}$ versus $-\log a_{ch}$ (Boden et al., 1988).

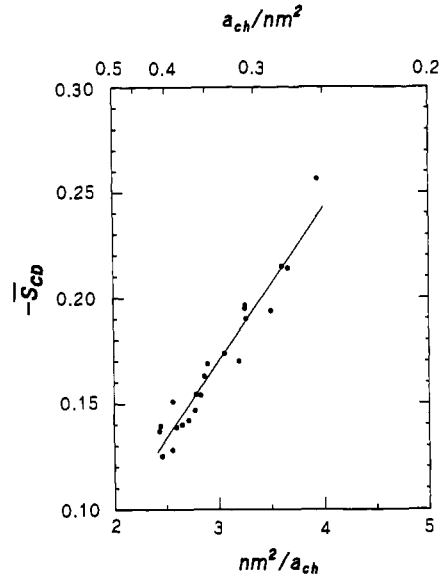

FIGURE 3: Plot of the average CD bond order parameters $\bar{S}_{CD}$ against the inverse of the average chain areas a_{ch} for the soap/water and phospholipid/water lamellar phases listed in Table I. The straight line represents a least mean squares fit of the experimental points, and its explicit form is given by eq 1.

The correlation between $-\bar{S}_{CD}$ and a_{ch} in Figure 3 is quite remarkable considering the variety of chemical structures and the wide intervals of composition and temperatures involved. We note, in particular, that the value of $-\bar{S}_{CD}$ is only a function of a_{ch} and is independent of the total number of segments (C_n) in the chain and of the number of chains in the molecule. This result is consistent with the observation that the average area per molecule at the bilayer/water interface a_m is independent of chain lengths for both soap/water (Gallot & Skoulios, 1965) and phospholipid/water (Lewis & Engelman, 1983) lamellar phases. The values of a_{ch} in Figure 3 extend over the entire range of stable lamellar L_α phases. The smallest value of 0.25 nm^2 corresponds to the area at which the van der Waal's attraction between the chains induces a transition to a crystalline lattice. The corresponding average order parameter of −0.25 must, therefore, be near to the maximum attainable value for an L_α phase. The largest value of 0.41 nm^2 is most probably determined by the chain packing free energy. Calculations based on mean field theories (Szleifer et al., 1986; Gruen, 1985b) show minima in the chain free energy versus a_{ch} curves when $a_{ch} \approx 0.40$ nm^2. The free energy cost associated with a further expansion of the headgroup area results in a transition to a hexagonal phase (rod-shaped aggregates) in the case of soaps. In the case of phospholipids a transition to a dispersion of vesicles usually occurs before this limiting value of a_{ch} is reached.

The results presented in Figure 3 also provide some, albeit indirect, evidence for the validity of the Luzzati analysis of X-ray diffraction data. On the basis of low-resolution electron density profiles of phospholipid bilayers, McIntosh and Simon (1986) have argued that inaccuracies in the compositions of lamellar phases and/or the usage of incorrect values for the partial molar volumes V_l and V_w leads to unreliable structural data. However the problem of erroneous compositions is readily eliminated by the good correlation between $\bar{S}_{CD}$ and a_{ch} shown in Figure 3. Moreover, there is, in general, good agreement between the values of the molecular areas a_m reported by different laboratories. For instance, our results for the DMPC/water system (Table I) agree to within ±0.01 nm^2 with measurements by Janiak et al. (1979) and Lis et al. (1982) over the entire range of composition. The DPPC/water system represents a notable exception where published structural data disagree significantly (Inoko & Mitsui, 1978; Janiak et al., 1976; Lis et al., 1982). This discrepancy must indeed arise from erroneous compositions or from the presence of impurities. A more serious problem concerns the possibility that the partial molar volumes V_l and V_w could vary significantly as a function of composition. All of the density measurements so far of soap/water (Ekwall et al., 1963; Mandell et al., 1968; Laggner & Stabinger, 1976) and phospholipid/water (White et al., 1987; Tardieu et al., 1973) lamellar phases have shown that the values of V_l and V_w are in fact independent of composition. The data presented in Figure 3 suggest that these results are of general validity. For example, for DMPC bilayers at 30 °C and a hydration of $R_{w/l}$ of 25, a_{ch} = 0.307 nm^2. At this hydration, the molar volume of bulk water must be a good approximation to the parial molar volume of the water in the lamellar phase. Moreover, we also know the specific volume of the lipid in excess water (Nagle & Wilkinson, 1978). The value obtained for a_{ch} ought, therefore, to be quite reasonable. Now the value of −0.197 for $\bar{S}_{CD}$ is, within experimental error, the same as that for potassium dodecanoate at 31 °C ($\bar{S}_{CD}$ = −0.190). The correlation in Figure 3 predicts that the average areas per chain mush be similar. Indeed, the values obtained for a_{ch} from the X-ray data are identical at 0.307 nm^2. Yet, for potassium dodecanoate the value of $R_{w/l}$ is only 3.0. At this hydration substantial differences between the value of the molar and partial molar volumes would be expected if such differences were significant. Clearly, they are not. Thus, the results presented in Figure 3 seem to confirm the validity of the Luzzati procedure and the assumption of constant partial molar volumes over an extensive concentration range.

Discussion

General Comments. The most significant feature of the above results is that the state of a planar lipid bilayer is completely specified by a relationship between only two variables, the average CD bond order parameter $\bar{S}_{CD}$ and the average area of the chains a_{ch} (eq 1). This is not an entirely unexpected result since the density of packing of the lipid molecules imposes the necessary motional constraint on the chains of which S_{CD} is a measure. It is worth emphasizing, however, that this relationship is universal and independent of molecular structure, i.e., the structure of the polar headgroups, the number of chains per molecule, or the length of the chains. To further illustrate this point, we compare the order profiles in bilayers of DMPC and potassium laurate when a_{ch} = 0.307 nm^2 (Figure 4). The variation along the chain of the segmental order parameters S^i_{CD} is quite different. The order parameter "plateau", where S^i_{CD} is approximately constant, is much shorter for potassium laurate and the decay of S^i_{CD} toward the end of the laurate chain is steeper. Nevertheless, the values of $\bar{S}_{CD}$ are, within 5%, indentical (DMPC, $\bar{S}_{CD}$ = −0.197; potassium laurate, $\bar{S}_{CD}$ = −0.190). Thus, the chains accommodate the packing constraints, as reflected in the value of a_{ch}, subjected to a characteristic value of $\bar{S}_{CD}$. The detailed and energetically most favorable way of satisfying these constraints will, however, depend on the structure of the lipid molecule. It will also depend on the geometry of the lipid aggregates. The order profiles observed in cylindrical aggregates (hexagonal phases) and in spherical micelles, though being qualitatively similar to those in bilayers, have quite different shapes (Gruen, 1985b; Szleifer et al., 1986; Ben-Shaul & Gelbart, 1985). Nevertheless, by using volume-weighted mean values of a_{ch} (Gruen, 1985b), we find that eq 1 is still applicable to cylindrical and, presumably, spherical micellar

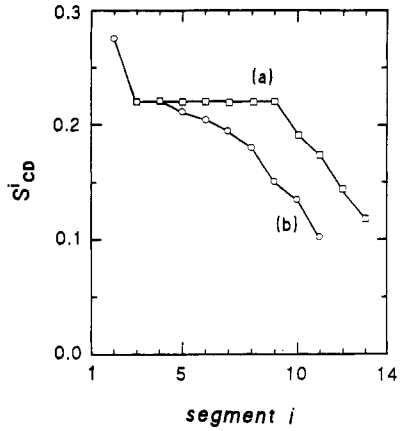

FIGURE 4: Variation of the segmental CD bond order parameters S^i_{CD} along the aliphatic chains of (a) DMPC-d_{27} and (b) specifically deuteriated potassium laurate. The value for the cross-sectional area a_{ch} of the aliphatic chains is 0.307 nm^2 for both (a) and (b). The average CD bond order parameters $\bar{S}_{CD}$ are (a) −0.197 and (b) −0.190. Experimental conditions: (a) $R_{w/l}$ = 25, temperature 30 °C; (b) $R_{w/l}$ = 3.1, temperature 31 °C. Data for potassium laurate were taken from Mely et al. (1975).

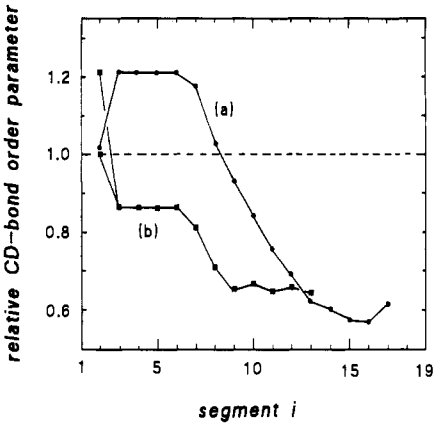

FIGURE 5: Effect of benzyl alcohol on the order profiles in bilayers of (a) potassium oleate and (b) DMPC. A "relative" order parameter is the ratio of a segmental order parameter in the presence of solute to the corresponding quantity in the pure lipid/water lamellar phase. A value larger than 1.0 indicates an increase; a value smaller than 1.0, a decrease, in the CD bond order parameter. Experimental conditions: (a) $R_{w/l}$ = 7.6, $R_{s/l}$ = 1.23, temperature 21 °C; (b) $R_{w/l}$ = 12.0, $R_{s/l}$ = 0.8, temperature 30 °C.

aggregates (Boden and Sixl, unpublished results).

Application of Equation 1 to Multicomponent Bilayers. (1) Reporter Molecules as Probes of Chain Ordering in Lipid Bilayers. Equation 1 is expected to have wide practical utility in studies of lipid bilayers. In particular, it clarifies the protocol for interpretation of quadrupole splittings in bilayers composed of a mixture of chemically different lipids. Consider, for example, the ideal case of a random mixture of two lipid species having quite different polar headgroups. If the bilayers are perfectly planar, i.e., devoid of morphological defects, and if the hydrocarbon chains are of identical length, then the cross-sectional areas a_{ch} and, thus, the average order parameters $\bar{S}_{CD}$ must have the same values for both molecules. The application of spin-labeled and deuteriated fatty acids as probes of molecular chain ordering in lipid bilayers is only possible because of this principle. For instance, ^{2}H NMR measurements of DPPC/palmitic acid mixtures, where either of the two components was perdeuteriated, have shown that the order profile of the fatty acid is virtually identical with that of the phospholipid (Pauls et al., 1983). This means that the carboxylate group of the palmitic acid must be close to the C_1 or C_2 position of the glycerol backbone of DPPC. The molecule is presumably held in this position by the formation of a H-bond. A similar kind of interaction has been suggested to occur in lecithin/cholestrol mixtures (Franks, 1976). It is quite possible that dissociation of a proton from the carboxylate group could result in vertical displacement of the molecule and in a concomitant stretching or compression of the chain. The order parameters of the phospholipid and the fatty acid chains would then be modified and, thus, render fatty acids unsuitable as reporter molecules.

Lipid bilayers of biological origin and/or significance very often contain mono- and polyunsaturated fatty acids. Measurements of molecular order in these systems would, ideally, require the usage of unsaturated probe molecules. These are difficult to synthesize, however, and fully saturated molecules are normally employed. Our results for the potassium oleate/potassium stearate-d_{35} mixture suggest that the presence of a single double bond in one of the components is of no obvious significance. The values of a_{ch} and $\bar{S}_{CD}$ for this system are consistent with all the other data (see Figure 3); i.e., the ordering of the stearate chain is a correct indicator for the molecular packing of the alkyl chains.

(2) Distribution of Benzyl Alcohol in DMPC and Potassium Oleate Bilayers. Benzyl alcohol has been used frequently as a model compound for local anaesthetics. It is known to be solubilized in the polar/apolar interface of bilayers (Colley & Metcalfe, 1972; Pope et al., 1986) and to cause a lateral expansion of bilayer membranes (Turner & Oldfield, 1979). Figure 5 compares the effect of benzyl alcohol on the order profiles of DMPC-d_{27} and of 9% potassium stearate-d_{35}/potassium oleate bilayers. It can be seen that, as a result of steric interactions with the alcohol, the chain order increases for the first seven methylene segments of the potassium stearate chain. Molecular models show that the length (ca. 0.8 nm) of this ordered part of the chain corresponds very well with the size of a benzyl alcohol molecule. This is indicative of a specific interaction between the carboxylate groups and the alcoholic OH groups. The creation of free volume in the center of the bilayer gives rise to the observed decrease in the orientational order of the remaining segments of the chain. The sharp division between "ordered" and "disordered" chain segments suggests that the mobility of the benzyl alcohol molecules normal to the bilayer surface must be quite constrained with little penetration into the center of the bilayer. This observation is of interest when compared with the results for the DMPC/benzyl alcohol system. As shown in Figure 5, creation of free volume again causes a reduction of the order parameters of the corresponding chain segments. However, only for a single methylene group, $C(2)D_2$, do the quadrupolar splittings increase in the presence of benzyl alcohol. We have previously argued (Boden et al., 1988) that the most likely reason for this is the formation of a hydrogen bond between the OH group of benzyl alcohol and the phosphate group of the DMPC molecule: in this configuration the benzyl alcohol molecule does not extend beyond the C_2 segment of the lipid. The way in which benzyl alcohol is solubilized in DMPC and potassium oleate bilayers is, thus, very similar: the solute molecule being quite strongly held in the interface by specific interactions. But, since the interface of the DMPC bilayers is of far greater thickness (0.10–0.12 nm), the chains are unimpeded along almost their entire lengths. In consequence, the available data can be used to reveal how the benzyl alcohol molecule packs into the polar interface of the bilayer.

Figure 6 shows the variation of the molecule area (twice a_{ch}) of DMPC-d_{27} as a function of the mole ratio of benzyl

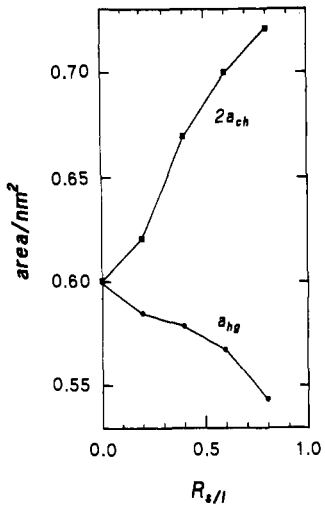

FIGURE 6: Twice the chain area a_{ch} and the headgroup areas a_{hg} of DMPC-d_{27} at various mole ratios of benzyl alcohol to phospholipid: $R_{w/l}$ = 25, temperature 30 °C. For calculation of a_{hg}, see text.

alcohol to DMPC $R_{s/l}$; it is seen to increase substantially from 0.60 to 0.72 nm^2 in the concentration range from $R_{s/l} = 0$ to $R_{s/l} = 0.8$. In contrast, the average area available to the polar headgroup a_{hg} decreases from 0.60 to 0.54 nm^2. a_{hg} has been calculated by subtracting the fractional area of the solubilized benzyl alcohol from the cross-sectional area of two chains:

$$a_{hg} = 2a_{ch} - R_{s/l}\ (0.22\ \text{nm}^2)$$

where 0.22 nm^2 is the cross-sectional area of one benzyl alcohol molecule (Boden et al., 1988). The decrease of a_{hg} probably represents the combined effects of two contributions. First, insertion of benzyl alcohol may cause a change in the orientation of the lipid headgroups. The phosphorus/nitrogen dipole of phospholipid molecules is known to be tilted by 15°–27° with respect to the bilayer surface (Hauser et al., 1981). This allows the PO_4^- and the $N(CH_3)_3^+$ groups of nearest neighbors to come into close proximity, and it optimizes the attractive electrostatic interactions between them. Binding of benzyl alcohol to the phosphate groups would be expected to attenuate these interactions with a consequent increase in the tilt angle and, thus, a decrease in a_{hg}. Second, the presence of water molecules is vital for the molecular structure and arrangement of lipid headgroups. Displacement of water molecules from their "binding sites" by benzyl alcohol would also decrease the value of a_{hg}. Evidence that this latter mechanism does indeed occur has recently been obtained by measurements of partial moelcular surface areas in the DMPC/benzyl alcohol/water system (Boden et al., 1988). These results clearly suggested that benzyl alcohol interferes with the hydration of the lipid headgroups.

It is well-known that the structural and motional properties of phospholipid headgroups in liquid-crystalline bilayers are difficult to characterize by conventional methods. It has not yet been possible, for instance, to interpret quadrupolar splittings of headgroup- deuteriated phospholipids in terms of structural parameters (Sixl & Watts, 1982, 1983). The above example illustrates how, in favorable cases, the density of packing of lipid headgroups can be derived from measurements of chain order parameters by ^{2}H NMR and by application of eq 1.

In contrast to the DMPC/benzyl alcohol/water system, the chain packing in potassium oleate/benzyl alcohol bilayers is seen to be a function of distance into the bilayer (Figure 5). The significance of the value of a_{ch}, obtained by application of eq 1, is seen from the following argument. A value for the

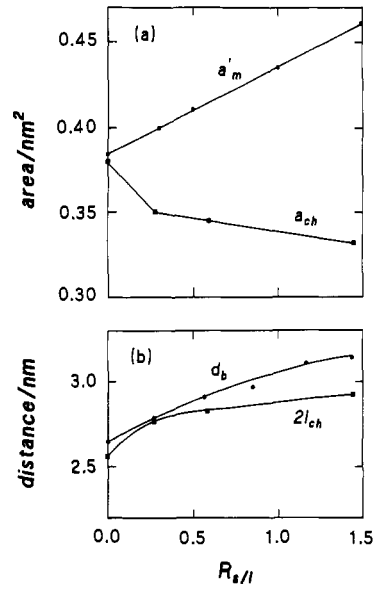

FIGURE 7: Variation of (a) the apparent molecular surface area a'_m and the chain area a_{ch} and (b) the hydrophobic thickness of the bilayer d_b and the average chain length l_{ch} as a function of solute concentration in the potassium oleate/benzene/water system: $R_{w/l}$ = 7.6, temperature 21 °C.

average chain length l_{ch} may be obtained by dividing the chain volume [0.486 nm^3 for $CH_3(CH_2)_{16}$][1] by a_{ch}. The values of $2l_{ch}$, thus obtained, are, over the entire range of concentrations, within 0.1 nm of the hydrophobic thickness of the bilayers d_b, as calculated from X-ray measurements. This explicitly demonstrates that the benzyl alcohol molecule does not accumulate at the center of the bilayer and is solely located at the interface. Moreover, it demonstrates the applicability of eq 1 to complex bilayer mixtures.

(*3*). *Solubilization of Hydrocarbons in Potassium Oleate/Water Bilayers.* Studies of the solubilization of hydrocarbons in bilayers have implications for a wide range of technological processes such as, for example, enhanced oil recovery and detergency. Hydrocarbons are also of considerable interest in membrane biology because of their potency as local anaesthetics and their usage in studies of black lipid films. All those hydrocarbons which are soluble in bilayer membranes will, of course, be solubilized in the apolar region of the bilayer. However, the way in which the solute molecules are distributed within this region and the resulting effect on the chain ordering is expected to depend on their molecular structure. To ascertain to what extent this is so and also to illustrate the utility of the correlation in Figure 3 in such studies, we have investigated the solubilization of benzene, cyclohexane, and tetradecane into the bilayers of the lamellar phase of the potassium oleate/water system.

Figures 7–9 summarize the results for, respectively, the potassium oleate/benzene/water, potassium oleate/cyclohexane/water, and potassium oleate/tetradecane/water systems. All the data were obtained for a constant water/potassium oleate mole ratio of 7.6. The plots show the variation of (a) the apparent molecular areas a'_m and the chain areas a_{ch} and (b) the hydrophobic thickness of the bilayer d_b and the average chain length l_{ch} as a function of solute concentration. a_{ch} was calculated from the quadrupolar splittings of perdeuteriated potassium stearate, incorporated (9% by weight) as a reporter molecule, and a'_m is the average interfacial area per soap molecule as calculated from X-ray dif-

[1] Chain volumes are calculated, throughout this paper, by using the formula given by Gruen (1985a).

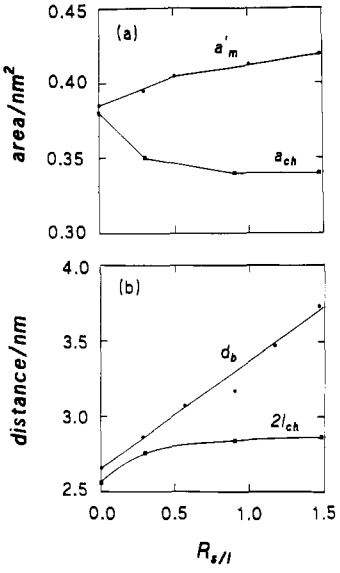

FIGURE 8: Variation of (a) the apparent molecular surface area a'_m and the chain area a_{ch} and (b) the hydrophobic thickness of the bilayer d_b and the average chain length l_{ch} as a function of solute concentration in the potassium oleate/cyclohexane/water system: $R_{w/l}$ = 7.6, temperature 21 °C.

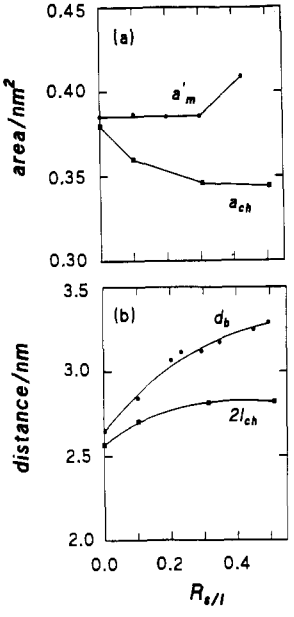

FIGURE 9: Variation of (a) the apparent molecular surface area a'_m and the chain area a_{ch} and (b) the hydrophobic thickness of the bilayer d_b and the average chain length l_{ch} as a function of solute concentration in the potassium oleate/tetradecane/water system: $R_{w/l}$ = 7.6, temperature 21 °C.

fraction measurements by assuming the partial molar volumes of the hydrocarbons are equal to their molar volumes.

The relative segmental order profiles are similar for all three solutes and have the form shown for benzene in Figure 10. The magnitudes of these relative order parameters were found to initially increase with $R_{s/l}$, but to change only very slowly for values beyond 0.3. This behavior is reflected in the magnitudes of a_{ch} and l_{ch}. The similarity in the behavior of all three solutes is quite striking: the value of a_{ch} is 0.345 nm² at $R_{s/l}$ = 0.5 in all three cases. In contrast, the variation of a'_m and d_b with $R_{s/l}$ is dependent on the solute, an indication that the distribution of the solute within the bilayer is dependent upon its molecular structure.

For benzene a'_m increases linearly with $R_{s/l}$ (Figure 7a), indicating that benzene molecules tend to pack uniformly

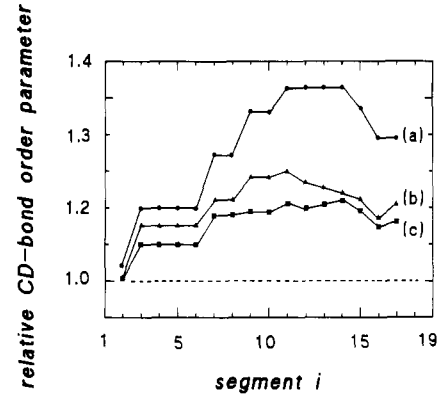

FIGURE 10: Effect of (a) decanol, (b) octanol, and (c) benzene on the chain order profile in potassium oleate bilayers. For definition of relative order parameters, see legend to Figure 5. Mole ratios of solute to soap: 0.38 (decanol), 0.29 (octanol), and 0.58 (benzene). Mole ratio of water to soap = 7.6 for all three systems; temperature 21 °C.

between the surfactant chains causing an expansion of the interface. For $R_{s/l} < 0.3$, $d_b \approx 2l_{ch}$, but at higher concentrations d_b becomes greater than $2l_{ch}$, showing that there is now a tendency for benzene molecules to distribute into the center of the bilayer.

Cyclohexane is seen (Figure 8) to behave similarly to benzene up to values of $R_{s/l}$ of 0.5. But, thereafter, the behavior is markedly different. The rate of increase of a'_m slows down, indicating a far stronger tendency for cyclohexane to distribute into the center of the bilayer. This is strikingly illustrated by the behavior of d_b which increases linearly with increasing $R_{s/l}$. The difference between these two solubilizates can be quantified by comparing the values of d_b and $2l_{ch}$ at $R_{s/l}$ = 1.5: $2l_{ch}$ has the same value (2.8 nm), but d_b is larger for cyclohexane (3.75 nm) than for benzene (3.15 nm).

The behavior of tetradecane is surprising in that a'_m remains constant up to $R_{s/l}$ = 0.3 and thereafter it increases (Figure 9). The concomitant decrease in a_{ch} over the same range of concentrations shows that some of the tetradecane is intercalated between the surfactant chains. but d_b is seen to initially increase much more rapidly than $2l_{ch}$, showing that tetradecane tends to preferentially distribute into the center of the bilayer at low concentrations. The increase of a'_m and the slow down in the rate at which d_b is increasing at higher concentrations indicate that an increasing amount is now intercalated between the surfactant chains. X-ray measurements (McIntosh et al., 1980, 1981) have shown that long-chain alkanes are also intercalated between the chains in phospholipid bilayers.

The differences in the behavior of the three solubilizates can be understood in terms of the interplay of the entropy of mixing which would favor a uniform distribution across the bilayer and the interaction between the solute and water which enhances the lateral compression in the bilayer. The latter brings about a reduction of the internal (conformational) entropy of the surfactant chains, and this is the origin of the increase in the value of l_{ch}. The importance of the interaction between solute and water is reflected by the fact that benzene, which has the higher affinity to water, has a greater propensity than either cyclohexane or tetradecane to pack between the surfactant chains. The internal entropy of the solubilizate is another important factor. For instance, the internal entropy of a tetradecane molecule intercalated between surfactant chains will be suppressed by the lateral compression of the bilayer, which will restrict the molecule to those conformations which are essentially parallel to the surfactant chains. This is why tetradecane tends to distribute into the center of the

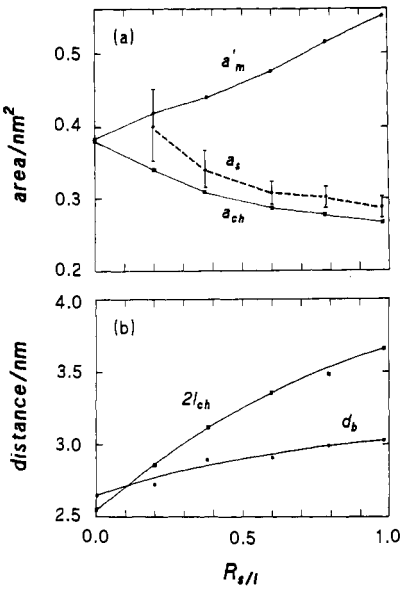

FIGURE 11: Variation of (a) the apparent molecular surface area a'_m and the chain area a_{ch} and (b) the hydrophobic thickness of the bilayer d_b and the average chain length l_{ch} as a function of solute concentration in the system potassium oleate/decanol/water. a_s is the cross-sectional area of the solute and has been calculated from a'_m and a_{ch} as described in the text. Bars indicate the error in the values of a_s arising from an uncertainty of ±0.01 nm^2 in the value of a'_m, $R_{w/l}$ = 7.6, temperature 21 °C.

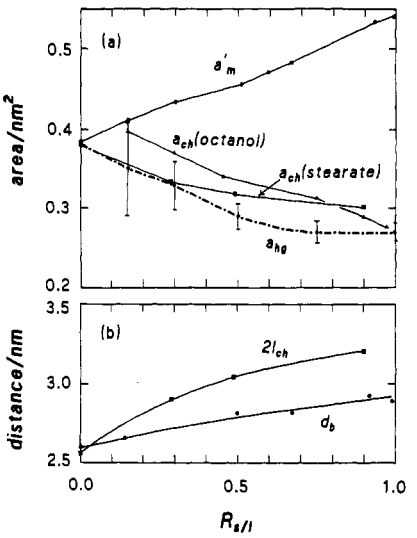

FIGURE 12: Variation of (a) the apparent molecular surface area a'_m and the chain area a_{ch}(octanol) and a_{ch}(stearate) and (b) the hydrophobic thickness of the bilayer d_b and the average chain length l_{ch} as a function of solute concentration in the potassium oleate/octanol/water system. a_{ch}(octanol) and a_{ch}(stearate) are the chain areas calculated from the quadrupolar splittings of, respectively, perdeuteriated octanol and perdeuteriated potassium stearate. The values of a'_m and a_{ch}(octanol) were used to calculate the headgroup area a_{hg} of the soap as described in the text. For explanation of error bars, see legend to Figure 11. $R_{w/l}$ = 7.6, temperature 21 °C.

bilayer at low concentrations. While at higher concentrations, tetradecane is forced to go between the surfactant chains with a concomitant increase in its contact with water (increase in a'_m) which severely limits its solubility.

(*4*) *Solubilization of Alcohols in Potassium Oleate/Water Bilayers.* Solubilization of the aliphatic alcohols decanol and octanol in potassium oleate bilayers would be expected to be very similar to that of benzyl alcohol. The –OH groups are likely to interact with the carboxylate groups at the apolar/polar interface, and this will govern their distribution in the bilayers. Thus, the effect on the order profiles of the aliphatic chains ought to be nearly identical for decanol, octanol, and benzyl alcohol. In practice, however, the behavior appears to be far more complex.

Figure 10 summarizes the effects on the order profile when decanol and octanol are incorporated into potassium oleate bilayers containing 9% perdeuteriated potassium stearate as a probe. The aliphatic slcohols are seen to enhance the ordering of the entire surfactant chain. This is unlike the behavior of benzyl alcohol, where the order increases only for the eight uppermost methylene segments but decreases for the remaining segments (Figure 5). In fact, the results for decanol and octanol Figure 10 show a very close resemblance with those of the hydrocarbons. For instance, the relative order profile for the potassium oleate/octanol system at R_s = 0.29 is seen (Figure 10) to be very similar to the one for the potassium oleate/benzene system at R_s = 0.58. Furthermore, the relative CD bond order parameters increase roughly monotonically with concentration as for the hydrocarbons. Figures 11 and 12 show that a'_m increases linearly with $R_{s/l}$ while a_{ch} decreases, behavior reminiscent of that of benzene (Figure 7). The origin of this behavior is, however, quite different. This can be seen from Figure 11b, which shows that for decanol $2l_{ch} > d_b$, indicative of substantial chain interdigitation. This would be consistent with the shapes of the order profiles in Figure 10. For $R_{s/l}$ = 1.0, the difference (1.15 nm) between d_b (3.00 nm) and l_{ch} (1.85 nm) is about the length expected for a decanol molecule constrained to a surface area of 0.26 nm^2 [volume of the $CH_3(CH_2)_9$ chain is 0.297 nm^3]. This is a quantitative verification of a fully interdigitated decanol/oleate (1:1) bilayer. We can also calculate the average area a_s occupied by a decanol molecule at the apolar/polar interface from

$$a_s = (a'_m - a_{ch})/R_{s/l}$$

The results plotted in Figure 11a show that a_s behaves very much like a_{ch} and has, to within 0.02 nm^2, simlar values. The density of packing is, thus, essentially the same for both "host" and "guest" molecules.

The behavior of octanol appears to be qualitatively similar to that of decanol. But quantitative analysis of the results reveals a distinctly different behavior. While $2l_{ch} > d_b$ (Figure 12b), as for decanol, the difference is substantially smaller. In particular, for $R_{s/l}$ = 1.0, the difference (1.27 nm) between d_b (2.90 nm) and l_{ch} (1.63 nm) is far too large to be identified with the average length of an octanol chain (about 0.9 nm). Thus, the results are not consistent with a simple chain interdigitation model: the behavior is more complex than for decanol. A clue to the origin of this behavior is obtained by calculating the average area per surfactant headgroup from

$$a_{hg} = a'_m - R_{s/l}a_{ch}(\text{octanol})$$

where the a_{ch}(octanol) are obtained from use of the deuterium quadrupole splittings of octanol-d_{15} in eq 1. The resulting values of a_{hg} (Figure 12a) are seen to vary with $R_{s/l}$ very similarly to those of a_{ch}(octanol) and to be of a comparable magnitude. Indeed, the variation of a'_m, a_{ch}(octanol), and a_{hg} (Figure 12a) with $R_{s/l}$ for octanol closely parallels that of a'_m, a_s(decanol), and a_{ch} (Figure 11a) for decanol at $R_{s/l}$ = 1.0: a_{hg} = 2.70 nm^2 and a'_m = 5.40 nm^2 for octanol as compared with a_{ch} = 2.65 nm^2 and a'_m = 5.50 nm^2 for decanol. Thus, although the density of packing of both alcohols at the interface is identical, the packing of the hydrocarbon chains inside the bilayer is quite different. For the octanol system we see that $a_{hg} \approx a_{ch}$(stearate) until $R_{s/l}$ reaches a value of 0.3. This is characteristic of the behavior of decanol and chain

interdigitation. However, for $R_{s/l} > 0.3$, $a_{hg} < a_{ch}$(stearate), behavior reminiscent to that of benzyl alcohol and indicative of enhanced disordering of the chain terminus so as to take up the free volume created by intercalation of the octanol into the bilayer interface. Thus, interdigitation of the chains of apposing monolayers is favored at low concentrations of octanol but not at high concentrations.

To summarize, the three alcohols which have been studied are solely intercalated into the apolar/polar interface; there is no evidence that any is incorporated into the center of the bilayer. The free volume so created in the center of the bilayer is taken up by disordering of the chains in the case of benzyl alcohol and by interdigitation of the chains in apposing monolyaers in the case of decanol. Clearly, these represent the two extrema of possible behavior. Octanol exhibits aspects of both:chain interdigitation at low concentrations switching over to chain disordering at high concentrations. These results suggest the upper limit to chain interdigitation is about half of the length of the surfactant chain. Clearly the free energy price to pay for extensive interdigitation of conformationally disordered hydrocarbon chains becomes too great. It becomes more favorable to have excessively disordered chains.

Implications for Theoretical Models of Chain Ordering in Bilayers. It is beyond the scope of this paper to develop a theoretical framework which could account for the empirical correlation between $\bar{S}_{CD}$ and a_{ch}. It is, nevertheless, instructive to compare the observed behavior (eq 1) with the predictions of existing theories of chain ordering in bilayers.

The simplest possible model for the motion of a chain assumes that the chain reorients as a rigid rod. Petersen and Chan (1977) have analyzed this model using a square-well form for the distribution function $g(\beta)$, where β is the angle between the bilayer normal and the long axis of the chain. It is easy to show that this treatment yields the following expression, relating $\bar{S}_{CD}$ and $\langle\cos\beta\rangle$: $\bar{S}_{CD} = \langle\cos\beta\rangle^2 - 1/2\langle\cos\beta\rangle$. Assuming liquid alkane density, the cross-sectional area of an all-trans paraffinic chain is 0.213 nm^2 and $a_{ch} = (0.213\ \text{nm}^2)\langle\cos\beta\rangle$. In this way it is possible to derive a dependence of $\bar{S}_{CD}$ on a_{ch}. The result is shown in Figure 13. It is not surprising to find that the rigid-rod model is incapable of predicting a realistic behavior. The motion of a real amphiphile is, of course, far more complex. The occurrence of internal rotation about the C–C bonds gives rise to a distribution of molecular conformations, and the reorientation of each of these is described in terms of its own orientational ordering tensor (Boden et al., 1981).

A similar $\bar{S}_{CD}$ versus a_{ch} dependence to the rigid-rod model is predicted by an expression, which was derived by Seelig and Seelig (1974b), for the average length l_{ch} of a hydrocarbon chain in a bilayer:

$$l_{ch} = 0.125\left(n - 1/2\sum_{i=1}^{n}\frac{1 + 2S^{i}_{CD}}{1.125}\right)$$

This equation can be rewritten in terms of the variables $\bar{S}_{CD}$ and a_{ch} as follows:

$$-\bar{S}_{CD} = (0.24\ \text{nm}^2/a_{ch}) - 0.625$$

where, again, liquid alkane density has been assumed. This model, like the rigid-rod model, predicts that $\bar{S}_{CD}$ is only a function of a_{ch} and is independent of the length of the chain. Interestingly, it also predicts a linear dependence of $\bar{S}_{CD}$ on $1/a_{ch}$. Since the relation between l_{ch} and the segmental CD bond order parameters S^{i}_{CD} has actually been used in the literature for calculations of bilayer thickness, (Oldfield et al., 1978; Davis & Jeffrey 1977), it is important to recognize that,

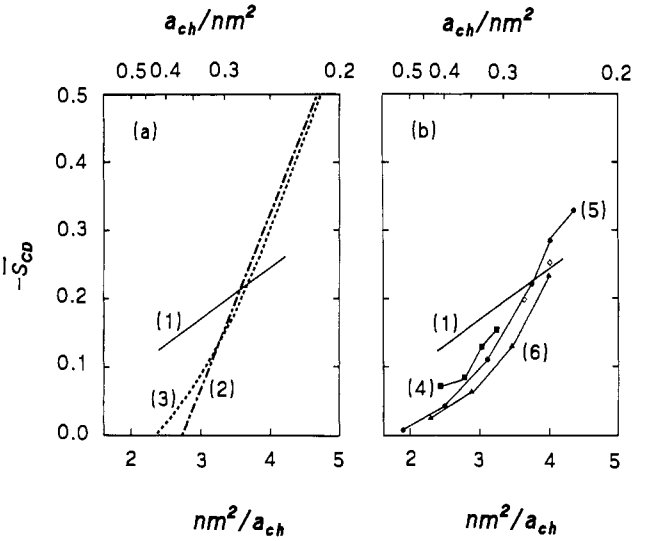

FIGURE 13: Comparison of (1) the empirical correlation of eq 1 with theoretical models. In (a): (2) Seelig and Seelig (1974b); (3) Petersen and Chan (1977). In (b): (4) mean-field calculation for a C_8 chain (Gruen, 1985b); (5 and 6) mean-field calculations, respectively, for C_9 and C_{11} chains (Gelbart, personal communication); (◇) molecular dynamics simulation for C_{10} chains (Ploeg & Berendsen, 1981, 1983).

except for values of a_{ch} close to 0.28 nm^2, there is little agreement with the empirical correlation (see Figure 13). The discrepancy at smaller chain areas is mostly due to the fact that both the rigid-rod and Seelig's models have been developed for chains which are fixed to a perfectly planar surface and which are immersed into a crystalline hexagonal lattice; i.e., there is no positional disorder. This latter assumption gives rise to an internal inconsistency, concerning the choice of a value for the density. The density of liquid hydrocarbons is the more realistic one, but the value which would be most appropriate to the model assumptions is the density of crystalline hydrocarbons. The maximum value of $-1/2$ for $\bar{S}_{CD}$ would then be attained when the chain area becomes 0.204 nm^2 (Tardieu et al., 1973). In addition, headgroup fluctuations perpendicular to the bilayer/water interface would, for a given value of a_{ch}, also result in values of $\bar{S}_{CD}$ which are smaller than calculated from either of the two above models.

Headgroup fluctuations are taken into account by more realistic statistical mechanical (mean-field) models (Gruen, 1985a,b; Szleifer et al., 1986; Ben-Shaul & Gelbart, 1985). Furthermore, the constraint of a fixed orientation for the first methylene group (Seelig & Seelig, 1974b) is relaxed. Figure 13 shows that simulations for C_9 and C_{11} (Gelbart, personal communication) and C_8 chains (Gruen, 1985b) yield and improved representation of the real behavior for small values of a_{ch} ($\leq$0.28 nm^2). However, for more expanded bilayers there is a characteristic deviation common to the results of all the theoretical models, in that for a given chain area the predicted average order parameter $\bar{S}_{CD}$ is significantly smaller than the measured one. This means that there must be constraints on the motion of the chains in addition to those implicit in the models. One such constraint is the interdigitation of chains from apposing monolayers, which is not allowed in either the rigid-rod or Seelig's model. Some of the improvement of the mean-field models is very likely due to the inclusion of chain interdigitation. A possible cause for the lack of a quantitative agreement at high values of a_{ch} between the empirical correlation and the mean-field models is that the latter are single-chain theories. Nearest-neighbor interactions are taken into account only in an average (mean) field. This simplification might be too severe, and a more realistic approach

would have to consider cooperative motions of the chains including fluctuations.

All the above difficulties are avoided in many chain molecular dynamics simulations, but problems may arise because of, for instance, the complexity of the interactions between lipid and water molecules. Ploeg and Berendsen (1981, 1983) have performed molecular dynamics simulations for C_{10} chains in bilayers which were constrained to average areas of 0.250 and 0.276 nm^2. The results (Figure 13) show very good agreement with eq 1. However, the range of a_{ch} values is rather limited, and an extension of these studies to more expanded bilayers is needed.

It is, of course, always possible that the experimental a_{ch} used in the construction of the empirical correlation in Figure 3 could be systematically in error, becoming progressively too large with increasing a_{ch}. However, for the reasons presented under Results, we are of the opinion that such a scenario is very unlikely in view of the wide variety of systems embraced by the correlations. This assertion is further supported by the apparent applicability of the correlation to bilayers into which various solutes have been incorporated. The possible involvement of intrinsic structural defects which would themselves be a similar function of the thermodynamic state of the bilayer as the chain ordering cannot, of course, be excluded, though curved edges associated with any defects in these classical bilayer systems are expected to have a high free energy and to be quite rare and also to be affected by the incorporation of solutes such as benzyl alcohol.

Acknowledgments

We are grateful to Dr. B. Mann of the SERC high-field NMR service, University of Sheffield, for recording the ^{2}H NMR spectra.

Registry No. DMPC, 18194-24-6; potassium oleate, 143-18-0; potassium laurate, 10124-65-9; potassium palmitate, 2624-31-9; potassium stearate, 593-29-3; rubidium stearate, 26121-36-8; benzyl alcohol, 100-51-6; benzene, 71-43-2; cyclohexane, 110-82-7; tetradecane, 629-59-4; decanol, 112-30-1; octanol, 111-87-5.

References

Ben-Shaul, A., & Gelbart, W. M. (1985) *Annu. Rev. Phys. Chem. 36*, 179.

Boden, N., Clark, L. D., Bushby, R. J., Emsley, J. W., Luckhurst, G. R., & Stochley, C. P. (1981) *Mol. Phys. 42*, 565.

Boden, N., Jones, S. A., & Sixl, F. (1987) *J. Phys. Chem. 91*, 137.

Boden, N., Bushby, R. J., Knowles, P. F., & Sixl, F. (1988) *Chem. Phys. Lett. 145*, 315.

Boulanger, Y., Schreier, S., & Smith, I. C. P. (1981) *Biochemistry 20*, 6824.

Colley, C. U., & Metcalfe, J. C. (1972) *FEBS Lett. 24*, 241.

Davis, J. H., & Jeffrey, K. R. (1977) *Chem. Phys. Lipids 20*, 87.

Davis, J. H., Jeffrey, K. R., Bloom, M., Valic, M. I., & Higgs, T. P. (1976) *Chem. Phys. Lett. 42*, 390.

Ekwall, P., Eikrou, H., & Mandell, L. (1963) *Acta Chem. Scand. 17*, 111.

Franks, N. P. (1976) *J. Mol. Biol. 100*, 345.

Füldner, H. H. (1980) Ph.D. Thesis, University of Ulm.

Gallott, B., & Skoulios, A. (1965) *Kolloid Z. Z. Polym. 208*, 37.

Gally, H. U., Pluschke, G., Overath, P., & Seelig, J. (1981) *Biochemistry 20*, 1826.

Gruen, D. W. R. (1985a) *J. Phys. Chem. 89*, 146.

Gruen, D. W. R. (1985b) *J. Phys. Chem. 89*, 153.

Guppa, C. M., Radhakrishnan, R., & Khorona, H. G. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 4315.

Hauser, H., Pascher, I., Pearson, R. H., & Sundell, S. (1981) *Biochim. Biophys. Acta 650*, 21.

Inoko, Y., & Mitsui, T. (1978) *J. Phys. Soc. Jpn. 44*, 1918.

Janiak, M. J., Small, D. M., & Shipley, G. G. (1976) *Biochemistry 15*, 4575.

Janiak, M. J., Small, D. M., & Shipley, G. G. (1979) *J. Biol. Chem. 254*, 6068.

Jeffrey, K. R., Wong, T. C., & Tulloch, A. P. (1984) *Mol. Phys. 62*, 283.

Jones, S. A. (1982) Ph.D. Thesis, University of Leeds.

Kuroda, Y., & Fujiwara, Y. (1987) *Biochim. Biophys. Acta 903*, 395.

Laggner, P., & Stabinger, H. (1976) *J. Colloid Interface Sci. 5*, 91.

Lewis, B. A., & Engelman, D. M. (1983) *J. Mol. Biol. 166*, 211.

Lis, L., McAlister, M., Fuller, N., Rand, R. P., & Parsegian, V. A. (1982) *Biophys. J. 37*, 657.

Luzzati, V. (1969) in *Biological Membranes* (Chapman, D. Ed.) Vol. 1, p 71, Academic Press, London.

Mandell, L., Fontell, K., Lehtiuen, N., & Ekwall, P. (1968) *Acta Polytech. Scand., Chem. Incl. Metall. Ser. 74*, II.

McIntosh, T. J., & Castello, M. J. (1981) *Biochim. Biophys. Acta 645*, 318.

McIntosh, T. J., & Simon, S. A. (1986) *Biochemistry 25*, 4058.

McIntosh, T. J., Simon, S. A., & McDonald, R. G. (1980) *Biochim. Biophys. Acta 597*, 445.

Mely, B., Charvolin, J., & Keller, P. (1975) *Chem. Phys. Lipids 15*, 161.

Nagle, J. F., & Wilkinson, D. A. (1978) *Biophys. J. 23*, 159.

Oldfield, E., Meadows, M., Rice, D., & Jacobs, R. (1978) *Biochemistry 17*, 2727.

Pauls, K. P., MacKay, A. L., & Bloom, M. (1983) *Biochemistry 22*, 6101.

Petersen, N. O., & Chan, S. I. (1977) *Biochemistry 16*, 2657.

Pope, J. M., & Dubro, D. W. (1986) *Biochim. Biophys. Acta 858*, 243.

Pope, J. M., Dubro, D., Doane, J. W., & Westerman, J. (1986) *J. Am. Chem. Soc. 108*, 5426.

Seelig, J., & Niederberger, W. (1974) *Biochemistry 13*, 1585.

Seelig, A., & Seelig, J. (1974b) *Biochemistry 13*, 4839.

Seelig, J., & Seelig, A. (1974a) *Biochem. Biophys. Res. Commun. 57*, 406.

Seelig, J., & Seelig, A. (1980) *Q. Rev. Biophys. 13*, 19.

Sixl, F., & Watts, A. (1982) *Biochemistry 21*, 6446.

Sixl, F., & Watts, A. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 1613.

Szleifer, J., Ben-Shaul, A., & Gelbart, W. M. (1986) *J. Chem. Phys. 85*, 5345.

Tardieu, A., Luzzati, V., & Reman, F. C. (1973) *J. Mol. Biol. 75*, 711.

Turner, G. L., & Oldfield, E. (1979) *Nature 277*, 669.

van der Ploeg, P., & Berendsen, H. J. C. (1981) *J. Chem. Phys. 76*, 3271.

van der Ploeg, P., & Berendsen, H. J. C. (1983) *Mol. Phys. 49*, 233.

White, S. H., Jacobs, R. E., & King, G. I. (1987) *Biophys. J. 52*, 663.