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Structure of fully hydrated bilayer dispersions

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A systematic formalism is developed that shows how the results for absolute specific volumes of multilamellar lipid dispersions may be combined with results from diffraction studies to obtain quantitative characterizations of the average structure of fully hydrated lipid bilayers. Quantities obtained are the area per molecule, the thickness and volumes of the bilayer, the water layer, the hydrocarbon chain layer and the headgroup layer, and where appropriate, the tilt angle of the hydrocarbon chains. In the case of the C phase of DPPC this formalism leads to the detection of inconsistencies between three data. Results for the G phases of DPPC and DLPE are in reasonable agreement with, though more comprehensive than, previous work that used fewer data and equations. Various diffraction data for the F phase of DPPC are in disagreement and it is shown how this disagreement affects results for the bilayer structure. A recent method of McIntosh and Simon for obtaining fluid phase structure utilizing gel phase structure is slightly modified to obtain results for the F phase of DLPE. Methods of obtaining the average methylene and methyl volumes in the fluid phases are critically examined.

Introduction

In a previous paper [1] absolute specific volumes were obtained for various phases of fully hydrated DPPC and DLPE multilamellar dispersions. In this paper we will show how these absolute specific volumes can play a key role, together with the results from diffraction measurements, in determining the structural organization of the bilayer. It has long been realized that volumetric measurements usefully supplement diffraction measurements and various ways have been employed for combining volumetric results with diffraction results [2–11]. This paper utilizes some of the preceding concepts and develops a formalism that enables the extraction of more information than previously and that contains consistency/redundancy checks.

The notation and the basic equations are established in the next section. Following that a case by case study is made of DPPC in the C (crystal-L_c), G (gel-$L_{\beta'}$) and F (fluid-L_α) phases and of DLPE in the G and F phases. While the formalism is the same in all cases, the computational path one follows is different in the different cases because different kinds of data are available with different degrees of reliability. In some cases various data are inconsistent or incomplete. Furthermore, the present method does not use the intensities of the low-angle diffraction rings which are the primary data utilized by the Fourier method [12–14] for obtaining bilayer structure; therefore, a synthesis of the two methods should be antic-

Abbreviations; DPPC, dipalmitoylphosphatidylcholine; DLPE, dilaurylphosphatidylethanolamine.

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ipated. Thus, it is unlikely that all the numerical results in this paper will remain unchanged. However, the present paper should serve to clarify where the problem areas are and which experiments might be done to achieve further progress.

Definitions and basic equations

The following definitions, some of which are illustrated in Fig. 1, will be used. It should, of course, be understood that many of the following quantities are average values and that at a molecular level all the straight lines in Fig. 1 will be rough. Nevertheless, the slab picture at the top of Fig. 1 in which space is divided into laterally homogeneous regions of 'pure' components is useful. However, a better representation of the headgroup region may be the one shown at the bottom of Fig. 1, in which the headgroups retain their dimensions, A' and D'_H, as the area per lipid, A, changes; both representations will be employed in this paper. Note that throughout this paper all volumes with a capital V are in cubic ångstroms, all volumes with a small v are in ml/g, all areas are in square ångstroms, all distances are in ångstroms, and angles are in degrees.

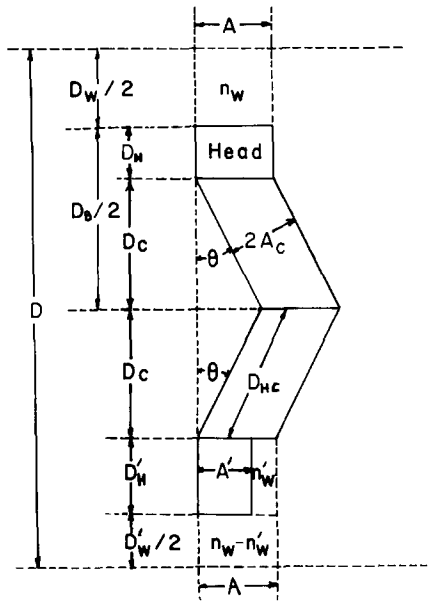

Fig. 1. Illustration of the notation defined in this section. Two lipid molecules are portrayed, one in either monolayer of a single bilayer. One way of portraying the headgroup and its associated water is shown for the molecule in the upper monolayer and a second way is shown for the molecule in the lower monolayer.

n_W, the number of water molecules per lipid molecule between the bilayers;

n'_W, the number of water molecules per lipid between headgroups in the same monolayer (see bottom of Fig. 1); ($n_W - n'_W$, the number of water molecules per lipid between headgroups on adjacent bilayers);

n_{CH_2}, the number of methylene groups per lipid molecule;

v_M, the specific volume measured by the neutral buoyancy technique;

V_L, the volume of a single lipid molecule in the bilayer;

V_X, the volume of a single lipid plus its associated n_W water molecules;

V_H, the volume of the headgroup, including the glycerol backbone;

V_{CH_2}, the average volume of methylenes in the lipid tails;

V_{CH_3}, the volume of the methyls in the lipid tails;

V_C, the total hydrocarbon chain volume per lipid molecule;

V_S, the volume/molecule of the excess solvent;

V_W, the volume/molecule of the solvent between the bilayers;

A, the area/lipid measured parallel to the surface of the bilayer;

A', the area of the headgroup measured parallel to the surface of the bilayer; ($A - A'$, the area of the hydrocarbon region exposed to water at the interface);

A_C, the area/chain measured perpendicular to the chain;

D, the center to center spacing between bilayers in multilamellar dispersions;

D_B, the bilayer thickness;

D_C, the thickness of the hydrocarbon region;

D_{HC}, the effective length of the hydrocarbon tails;

D_H, the headgroup thickness in the slab model at the top of Fig. 1 in which the headgroups are forced to have an area A instead of A';

D'_H, the actual headgroup thickness in the model at the bottom of Fig. 1;

D_W, the interbilayer water thickness in the slab model;

D'_W, the interlamellar water distance between headgroups on opposing monolayers in the model at the bottom of Fig. 1;
θ, the tilt angle of the chains with respect to the bilayer normal;
m_L, the molecular weight of the lipid;
m_S, the molecular weight of the $H_2O/^2H_2O$ excess solvent;
m_W, the molecular weight of the solvent between the bilayers.

There are a number of simple geometric relations between many of the preceding quantities that are useful to list.

$$D = D_B + D_W \tag{1a}$$

$$D_B = 2(D_H + D_C) \tag{1b}$$

$$D_C = D_{HC} \cos\theta \tag{1c}$$

$$2A_C = A \cos\theta \tag{1d}$$

$$V_X = AD/2 \tag{1e}$$

$$V_L = AD_B/2 \tag{1f}$$

$$V_X = n_W V_W + V_L \tag{1g}$$

$$V_L = V_H + V_C \tag{1h}$$

$$V_C = AD_C = 2A_C D_{HC} \tag{1i}$$

$$V_C = n_{CH_2} V_{CH_2} + 2V_{CH_3} \tag{1j}$$

$$V_H = AD_H = A'D'_H \tag{1k}$$

$$n'_W V_W = D'_H(A - A') \tag{1l}$$

$$D'_W/2 = (n_W - n'_W)V_W/A \tag{1m}$$

where Eqn. 1j assumes that there is only one terminal methyl on each hydrocarbon chain and Eqn. 1k assumes that V_H is the same in both representations in Fig. 1.

There are also some important relations involving v_M. By definition of the specific volume of multilamellar liposomes

$$v_M = 0.6023V_X/(m_L + n_W m_W) \tag{2}$$

In Ref. 1 it was established experimentally that $m_W = m_S$ for DPPC dispersions in the G phase and we will assume that this relation holds for all the cases discussed in this paper. Using this result in Eqn. 2 leads directly to

$$v_M = 0.6023[V_X - n_W V_S]/m_L \tag{3}$$

where $V_S = v_M m_S/0.6023$ is known from the measurement of v_M and m_L is, of course known, so Eqn. 3 relates the three experimental quantities V_X, v_M and n_W. As was also discussed in Ref. 1 the relation $V_W = V_S$ is really a convention since V_W is basically not measurable with an accuracy that makes any difference between V_W and V_S meaningful, although with the acceptance of this convention one must be prepared for differences in the headgroup volume V_H of the order of a few cubic ångstroms from one phase to another. Using $V_W = V_S$ together with Eqn. 1g in Eqn. 3 yields

$$V_M = 0.6023V_L/m_L \tag{4}$$

from which V_L is directly determined by measuring the neutral buoyancy specific volume v_M.

Results for some chain-ordered systems

The hydrocarbon chains in the gel (G) phases and the crystal (C) phases of DPPC and DLPE are thought to be conformationally rather well ordered with mostly *trans* rotameric angles. We start our analysis with the C phase of DPPC for which enough information has been reported to enable a consistency check to be made. Numerical results for all cases are summarized in Table I.

A. The C phase of DPPC

Church et al. [15] have analysed diffraction from the low temperature C phase of DPPC to obtain the results $A = 45.8$ and $D = 59.5$, so $V_X = 1363 \pm 5$ at 0°C using Eqn. 1e. At the same temperature we [1] find $v_M = 0.906 \pm 0.001$ ml/g. Ruocco and Shipley [16] have reported n_W to be 11 for this C phase ($T = 4$°C) by measuring the weight percent of water at which the D spacing becomes independent of water content, although from Fig. 10 in their paper we calculate $n_W = 10.2 \pm 0.1$. These three experimental results are inconsistent with Eqn. 3. This is further verified by calculating $m_W = 16.9 \pm 0.3$ from Eqn. 2 (using

TABLE I

SUMMARY OF RESULTS

	DPPC ($m_L = 734$)			DLPE ($m_L = 580$)	
	C	G	F[(1),(2),(3)]	G	F
T (°C0	0	20	50	20	35
V_W	29.9	30.0	30.3	30.0	30.1
v_M	0.906	0.939	1.011	0.896	0.942
V_L	1104	1144	1232	863	907
V_x	1363	1552	1929/1929/2374	1037	1172
V_C	756	796	884	611	655
V_H	348	348	348	252	252
V_{CH_2}	24.0	24.8	27.6	26.0	27.3
V_{CH_3}	42.0	50.8	55.2	45.5	54.6
D	59.5	64.0	60.0/67.0/67.0	50.6	45.8
D_B	48.2	47.2	38.3/42.8/34.8	42.1	35.4
D_{HC}	20.0	20.3	–	15.0	–
D_C	16.5	16.4	13.8/15.4/12.5	15.0	12.9
D_W	11.3	16.8	21.7/24.2/32.2	8.5	10.4
D_H	7.6	7.2	5.4/6.0/4.9	6.1	4.8
D'_W	10.4	15.2	16.5/20.3/26.1	5.3	4.7
D'_H	8.0	8.0	8.0	7.6	7.6
A	45.8	48.5	64.3/57.6/70.9	41.0	51.2
$2A_C$	37.8	39.1	–	41.0	–
A'	43.5	43.5	43.5	33.2	33.2
n_W	8.6	13.6	23.0/23.0/37.7	5.8	8.8
n'_W	0.6	1.3	5.5/3.7/7.2	2.0	4.7
θ	34.4	36.3	–	0	–

$m_L = 734$) and comparing to the measured $m_S = 20.0 \pm 0.1$. This is a very large difference considering that the measurements of preferential partitioning [1] of H vs. D indicate that $|m_W - m_S| \leq 0.04$ [1].

It is, of course, possible that sample preparations were significantly different in the three different labs in which the three aforementioned measurements were performed and future work on the C phase should be coordinated between different labs or at least two of the measurements should be performed on similar samples. However, in an attempt to find which datum is likely to be inconsistent, let us use Eqn. 3 and two of the measured values to calculate the third one. If V_X and n_W are used (and $V_W = 29.9$ at 0 °C), one obtains $v_M = 0.866 \pm 0.012$ ml/g which is well outside the error in our measurements of v_M. If the measured values of n_W and v_M are used, then one obtains $V_X = 1411 \pm 16$ which is outside the error of the results for A and D, assuming, of course, that the diffraction rings were properly indexed. Finally, the measured values of v_M and V_X yield $n_W = 8.6 \pm 0.1$. While this value appears to be outside the value obtainable from Fig. 10 in Ruocco and Shipley [16], direct measurements of n_W appear to be difficult as evidenced by the scatter in n_W values given for the G phase of DPPC (vide infra). If so, determination of n_W might best be accomplished via Eqn. 3 after determining v_M and V_X. We will assume that this is the case for the C phase of DPPC and take $n_W = 8.6$ *. The value of the lipid volume $V_L = 1104$ Å^3 in the C phase of DPPC at 0 °C can now be obtained by subtracting $n_W V_S$ from V_X or, equivalently, using Eqn. 4.

* A rationalization for this value of n_W being lower than that determined by Ruocco and Shipley [16] is that the multilamellar arrays are not perfect so that more water might be required in the imperfect regions. The existence of such imperfect regions would be presumed to add only a rather diffuse scattering that would not affect the determination of V_X.

The wide-angle diffraction for the C phase of DPPC contains two characteristic strong reflections at 4.41 and 3.86 Å [15–17]. Ruocco and Shipley [16] calculate $A_C = 18.9 \pm 0.1$ assuming that the chains are in an orthorhombic array and that the two lines are the (2,0) and (1,2) reflections. Together with the result for A this indicates that the chains are tilted with respect to the bilayer normal and Eqn. 1d yields a tilt angle $\theta = 34.4°$. Next, assuming that the chains are in the nearly straight all-*trans* conformation, the volumes of those methylenes in the straight portions are given by $V_{CH_2} = 1.27A_C = 24.0$. Although the 2-chain is not straight near the glycerol group, it is unlikely that the volumes of those methylene groups could be much different from the others. Also, in chain-ordered crystalline alkanes for which $V_{CH_2} \approx 23$–24, the volumes of methyl groups [18] are about 42, so a similar value appears reasonable here. Adding up 28 CH_2 volumes and two methyl volumes gives $V_C = 756$. Subtracting from V_L yields the volume of the headgroup (which includes the glycerol backbone and the fatty acid carbonyls) to be $V_H = 348$. This value may be compared to the value of 344 obtained by Nagle and Wilkinson [5], 340 used by Luzzati et al. [19] and 324 used by Small [3].

From the previous results it is now straightforward to calculate $D_B = 48.2$ from Eqn. 1f, $D_H = 7.6$ from Eqn. 1k, $D_C = 16.5$ from Eqn. 1b (or 1i), and $D_W = 11.3$ from Eqn. 1a. Also, using the above value of θ yields $D_{HC} = 20.0$ from Eqn. 1c (or 1i). In ordered systems each methylene has a length of 1.27 and each methyl has a length of about 2.2. For DPPC with chains having 14 methylenes and one methyl, one would predict a length of about 20.0, in good agreement with our value of D_{HC}. Our examination of the results of the neutron diffraction studies of Buldt et al. [20,21] leads us to choose an approximate intrinsic head group height $D'_H = 8.0$ A. Then $A' = 43.5$ using Eqn. 1k, $n'_W = 0.6$ using Eqn. 1l and $D'_W = 10.4$ from Eqn. 1m.

B. The G phase of DPPC

For the gel (G) phase of DPPC Ruocco and Shipley [16] report a D spacing of 64.0 at 20 °C from the low angle diffraction. From the wide-angle diffraction and the assumption that the chains pack in an orthorhombic pattern they also report a chain area of $2A_C = 39.1$. Assuming basically all *trans* chains yields $V_{CH_2} = 1.27A_c = 24.8$. The chains are tilted to the normal of the bilayer by an unknown angle, so the area A and the volume V_X are unknown. Our measurements [1] yield $v_M = 0.939$ ml/g at 20 °C which yields $V_L = 1144$ using Eqn. 4. Assuming that the headgroup volume is nearly the same as in the C phase yields $V_C = 796$. This requires $V_{CH_3} = 50.8$ using Eqn. 1j.

In the next part of the analysis, which is independent of the two assumptions in the preceding paragraph, it is necessary to know either the tilt angle θ, the area A or the hydration number n_W. Ruocco and Shipley [16] reported $n_W = 19$, although, as for the C phase, we calculate a smaller $n_W = 17.5 \pm 0.1$ from their raw data. This value differs from an earlier number reported from the same laboratory, namely $n_W = 13.6$, for DPPC at 20 °C [22]. These are both much larger than the earlier value of $n_W = 9$ obtained by a weighing method [23] at 100% relative humidity (at which the D spacing is different than for fully hydrated systems). It may also be of interest to compare two values of n_W for DMPC, namely $n_W = 10$ obtained by a weighing method [24], and $n_W = 15.4$ [22]. In view of the apparent difficulty in obtaining a definitive value for n_W, let us somewhat arbitrarily use the value $n_W = 13.6$ from Ref. 22. From this value of n_W, one obtains $V_X = 1552$ using Eqn. 1g and $V_W = 30.0$ at 20 °C, $A = 48.5$ from D and Eqn. 1e, $\theta = 36.3°$ from Eqn. 1d, $D_H = 7.2$ from Eqn. 1k, $D_B = 47.2$ from Eqn. 1f, $D_C = 16.4$ from Eqn. 1b (or 1i), $D_{HC} = 20.3$ from Eqn. 1c (or 1i) and $D_W = 16.8$ from Eqn. 1a. Taking $D'_H = 8.0$ yields $A' = 43.5$ from Eqn. 1k, $n'_W = 1.3$ from Eqn. 1l and $D'_W = 15.2$ from Eqn. 1m. For comparison it is of interest to see that the larger value of $n_W = 19$ changes these results to $V_X = 1714$, $A = 53.6$, $\theta = 43.1°$, $D_H = 6.5$, $D_B = 42.7$, $D_C = 14.8$, $D_{HC} = 20.3$, and $D_W = 21.3$, with $A' = 43.5$, $n'_w = 2.7$ and $D'_W = 18.2$ for $D'_H = 8.0$.

The most detailed strip model for DPPC was obtained by Buldt et al. [20,21] from neutron diffraction on selectively deuterated lipids. Unfortunately, the maximum hydration state studied had only 25% water which converts to $n_W = 13.6$ which is considerably less than n_W for the F phase in which the lipid is hydrated and danger-

ously close to n_W for the gel phase. That this might be a problem is indicated by the fact that their value of $D = 62.5$ is slightly less than is usually obtained for the fully hydrated gel phase. However, McIntosh and Simon [25] have recently reported that removal of interlamellar water causes little change in bilayer structure in the G phase of DPPC down to $D = 57.8$, so comparison of our results with Buldt et al. are probably appropriate for the chain region. From their mean positions of the C4 and C15 carbons one may compute $\theta = 27.1$ which is a bit smaller than our value of θ. However, the errors in their mean positions are rather large and encompass all angles from $\theta = 0$ to 46, which includes our values of $\theta = 36.3$ or 43.1. Similarly, the mean value of D_C that one obtains from Buldt et al. is a little large than our value, but ours is included within the errors. Unfortunately, they do not report wide angle results and no comparison with area A can be made.

C. The G phase of DLPE

From the measurements for v_M [1] and Eqn. 4 one has $V_L = 863$ for the gel phase at 20 °C. A recent diffraction study [10] for the gel phase of DLPE at 20 °C gives $D = 50.6$ and $2A_C = 41.0$. In an earlier study McIntosh [26] showed that $\theta = 0$ in the gel phases of the saturated phosphatidylethanolamines. This is a very convenient result which makes the phosphatidylethanolamines easier to study than the phosphatidylcholines because when $\theta = 0$, $A = 2A_C$ and the unit cell volume, $V_X = 10.37$, can be obtained using Eqn. 1e without the necessity of indexing lines with weak diffraction intensity.

Using the preceding values of V_X and V_L and Eqn. 1g with $V_W = 30.0$ yields $n_W = 5.8$. Next, $V_{CH_2} = 1.27A_C = 26.0$. In crystal structures of chain-ordered n-alkanes, $V_{CH_3} \approx 1.75V_{CH_2}$ [18]. Adding up 20 methylenes and 2 methyls yields $V_C = 611$. Using Eqn. 1h then yields $V_H = 252$ which may be compared to an earlier estimate [27] of 246. From Eqn. 1f, $D_B = 42.1$, $D_H = 6.1$ from Eqn. 1k, $D_C = 14.9$ from Eqn. 1b (or 1i) and $D_W = 8.5$ from Eqn. 1a.

McIntosh and Simon [10] find the distance between peak electron density, assumed to be the location of the phosphates, to be 37.2. They also define the thickness of the headgroup (D'_H, not D_H) to be 9.0 which is composed of 5.0 ångstroms from the peak in the electron density toward the bilayer center and of 4.0 ångstroms from the peak in the electron density into the interlamellar space. With this convention, they then obtain a thickness of the bilayer to be 45.2 and the thickness of the hydrocarbon region, which they call H, to be 27.2 for the gel phase of DLPE. Their value of H is reasonably close to, but significantly smaller than, our $2D_C = 30.0$. Using our value of $2D_C$ instead of H and accepting their value for the bilayer thickness yields D'_H equal to 7.6 compared to their 9.0. Then, $A' = 33.1$ from Eqn. 1k, $n'_W = 2.0$ using Eqn. 1l, and $D'_W = 5.6$ from Eqn. 1m. (One might argue that $2D_{HC}$ should be a little larger than the actual thickness because it was derived by assuming all-*trans* chains whereas it is known that the 2-chain is definitely not all trans near the glycerol group [20,21]. However, this argument neglects the structure of the glycerol group and the fact that the dividing surface between the hydrocarbon chain region and the glycerol group is not parallel to the membrane. Instead, if each methylene, including those near the glycerol group, have the same volume and if the central carbons of each chain are in the all trans conformation, then the average $D_{HC} = D_C = 15.0$ must be given by the above calculation.)

Results for some chain-disordered systems

Analysis of chain-disordered systems is considerably different than for the chain-ordered systems for several reasons. First, the organization of the lipid molecules within each bilayer is that of a fluid, so there is no possibility of measuring V_X for a unit cell determined by diffraction. Furthermore, each chain is conformationally disordered, so D_{HC} is not well approximated by all-*trans* chains but is a weighted average over many chain conformations where the weights are not a priori obvious. Similarly, A_C is poorly related to the diffuse wide angle X-ray line and the angle θ is not well-defined for disordered chains. Nevertheless, the formalism continues to be useful as indicated for the following cases. Numerical results are summarized in Table I.

A. Fluid phase of DPPC

The measured value $V_M = 1.011$ ml/g at 50 °C [1,5] and Eqn. 4 yields $V_L = 1232$.

Unfortunately, for this phase of DPPC the X-ray results are in poor agreement with each other. The D spacing has been reported to be 67.0 or 67.5 by various groups [15,28–30], but Shipley's group [16,22] has reported the significantly smaller value of 60.0. Also from X-ray measurements the value of n_W is 23.0 from the data of Inoko and Mitsui [29] and of Ruocco and Shipley [16] compared to 37.7 by Lis et al. [30]. One might argue that the smaller D spacing and the smaller n_W values of Ruocco and Shipley [16], when compared to the larger values of Lis et al., indicate less than full hydration. However, the results of Inoko and Mitsui do not fit this pattern. Until this fundamental disagreement in the diffraction results is resolved, let us explicitly consider three combinations for D and n_W, namely, (1) those of Ruocco and Shipley [16], (2) those of Inoko and Mitsui [29] and (3) those of Lis et al. [30]. Superscripts (1), (2) and (3) will be appended to all derived quantities to distinguish which of these three sets of X-ray data was used.

Using $V_W = 30.3$ at 50 °C and Eqn. 1g, $V_X^{(1)} = V_X^{(2)} = 1929$ and $V_X^{(3)} = 2374$. Using Eqn. 1e yields $A^{(1)} = 66.3$, $A^{(2)} = 57.6$ and $A^{(3)} = 70.9$. To proceed further we make the assumption that $V_H = 348$ which is the same as the value obtained in the C and G phases. The justification for this assumption is that, even though the headgroups are not packed together as tightly in the fluid phase, as evidenced by the increase in A, the volume around the headgroups is taken up by water. Further discussion of the effect of headgroup concentration on V_H is given elsewhere [1,5]. With this assumption $V_C = 884$ using Eqn. 1h; no superscript is appended when all cases have the same value.

In contrast to the assumption that V_H changes very little during phase changes under fully hydrated conditions, we do assume that most of the volume expansion in the chain region is not taken up by water. The idea behind this assumption is that the spaces between the chains are not large enough compared to the size of water molecules and the environment is too hydrophobic for many water molecules per molecule of lipid to be present in the hydrocarbon region. In order to test this assumption, consider the permeability P of water across the membrane, which has been measured to be $6.3 \cdot 10^{-4}$ cm/s in the fluid phase of lipid bilayers [31]. To first approximation, $P = pD_{diff}/2D_C$, where p is the partition coefficient of water in the hydrocarbon chain region and D_{diff} is an effective diffusion coefficient for individual water molecules in the hydrocarbon environment [32,33]. Although D_{diff} is not known precisely for lipid bilayers, it would be most surprising if it differed by more than an order of magnitude from the coefficient of diffusion for water molecules in water, which is $2 \cdot 10^{-5}$ cm^2/s. Using this value of D_{diff} yields a value of p less than 10^{-4} ml of water per ml of hydrocarbon chain. Therefore, the maximum volume of water that can enter the chain region, even with the assumption that there is no water in the chain region below the transition, is more than two orders of magnitude less than the measured volume change, 0.038 ml/g, for the main transition of DPPC [1,5].

Continuing with the calculations, $D_H^{(1)} = 5.4$, $D_H^{(2)} = 6.0$ and $D_H^{(3)} = 4.9$ using Eqn. 1k. Using Eqn. 1f, $D_B^{(1)} = 38.3$, $D_B^{(2)} = 42.8$ and $D_B^{(3)} = 34.8$ and $D_C^{(1)} = 13.8$, $D_C^{(2)} = 15.4$ and $D_C^{(3)} = 12.5$ using Eqn. 1b (or 1i) and $D_W^{(1)} = 21.7$, $D_W^{(2)} = 24.2$ and $D_W^{(3)} = 32.2$ using Eqn. 1a. Using $D'_H = 8.0$ yields $A' = 43.5$ by Eqn. 1k, $n'^{(1)}_W = 5.5$, $n'^{(2)}_W = 3.7$ and $n'^{(3)}_W = 7.2$ using Eqn. 1l and $D'^{(1)}_W = 16.5$, $D'^{(2)}_W = 20.3$ and $D'^{(3)}_W = 26.1$ using Eqn. 1m. It may be noted that Lewis and Engelman [6] have analyzed continuous X-ray scattering from unilamellar vesicles of DPPC in the F phase with the results $A = 66.5$ and $D_C = 13$ which are closer to the case (1) results in Table I than to the case (2) or case (3) results.

The volumes V_{CH_2} and V_{CH_3} are also of interest. Since these two quantities are only constrained by Eqn. 1j, it is not possible to separate them rigorously when data are available for only one lipid. However, when data are available for a sequence of lipids with varying chain length, two ways of extracting V_{CH_2} and V_{CH_3} have been proposed in the literature, one by Reiss-Husson and Luzzati [2], utilized in this laboratory [5], and one that has been advocated by Small [18]. Since the differences are of little moment for most researchers, a detailed discussions of this difference in the two

methods will be deferred to the Appendix. Using the former way yields for DPPC the result $V_{CH_2} = 27.6$ and $V_{CH_3} = 55.2$.

B. Fluid phase of DLPE

The measured $v_M = 0.942$ for the F phase of DLPE at 35 °C together with Eq. 4 yields $V_L = 907$. We know of no reports for n_W for this lipid, so V_X and A cannot be determined in the same way as for DPPC. However, a different method has been found by McIntosh and Simon [10] to determine A using the electron densities determined from the interlamellar low-angle diffraction. This method uses the results for the G phase together with measured changes in going to the F phase. The basic formula is

$$\Delta A = A_G[\Delta v/v_G - (H_F - H_G)/H_G][H_G/H_F] \quad (5)$$

where:

$A_G = 41.0$ is the area/molecule in the G phase, measured parallel to the bilayer surface;

Δv is the absolute specific volume change between the F and G phase which we measure to be 0.046 between 20 °C and 35 °C;

v_G is the volume of only the hydrocarbon chains per g of lipid in the G phase,

$v_G = 0.6023\ V_C/m_L = 0.63$;

H_G, H_F are the thicknesses of the bilayer hydrocarbon regions in the G and F phases, respectively.

Instead of using the values $H_G = 27.2$ and $H_F = 23.0$ obtained by McIntosh and Simon [10] from their electron densities, we prefer to use $H_G = 2D_C$ which was found to be 30.0 earlier in this paper when discussing the gel phase of DLPE. However, we expect that the method of McIntosh and Simon gives an accurate value of $H_G - H_F = 4.2$, so we take $H_F = 30.0 - 4.2 = 25.8$. Eqn. 5 now yields $\Delta A = 10.2$ which gives $A_F = A = 51.2$. This agrees well with the corrected value $A_{LC} = 50.6$ reported by McIntosh and Simon [34].

From A and the measured $D = 45.8$ [10] one obtains $V_X = 1172$ using $V_W = 30.1$ at 35 °C and Eqn. 1e and $n_W = 8.8$ from Eqn. 1g which differs from the value of 10.2 given by McIntosh and Simon because of our different values of D_C. Using the same value of $V_H = 252$ as for the G phase yields $V_C = 655$ from Eqn. 1h. Using Eqn. 1f yields $D_B = 35.4$ and Eqn. 1a yields $D_W = 10.4$. Using $2D_C = H_F = 25.8$ in Eqn. 1b yields $D_H = 4.8$. If we assume that $D'_H = 7.6$ is the same as for the G phase, then $A' = 33.2$ by Eqn. 1k, $n'_W = 4.5$ by Eqn. 1l and $D'_W = 5.1$ by Eqn. 1m.

Finally, let us make a rough approximation of V_{CH_2}. Unlike the phosphatidylcholines, a systematic study of a series of the phosphatidylethanolamines has not been performed, so the best we can do is to assume that $V_{CH_3} \sim 2V_{CH_2}$ (see appendix) in the fluid phase of DLPE. With this assumption, $V_{CH_2} = 27.3$ from Eq. 1j, which is in good agreement with V_{CH_2} for the fluid phase of the *n*-alkanes and the phosphatidylcholines at 35 °C.

Discussion

Experimental data from diffraction studies and from dilatometry have been systematically combined, using a new formalism, to determine the structural parameters of lipid bilayers of DPPC in the fully hydrated crystal, gel and fluid phases and for DLPE in the fully hydrated gel and fluid phases. Only in the case of the C phase of DPPC has enough data been presented to allow for a consistency check, which is not satisfied. For the G and F phases of DPPC some of the tabulated results in Table I depend upon experimental values of n_W, which vary widely, so these results will be subject to revision when intensity data from low-angle diffraction are included in the analysis in the future. We have especially emphasized the large experimental disagreement for the F phase of DPPC, which has been the most thoroughly studied of all lipid bilayers with the possible exception of egg PC. This experimental disagreement propagates into unacceptably large uncertainties in the structural parameters, such as the area per molecule. In contrast to DPPC, the results for DLPE would appear to be more certain, owing largely to the chains in the G phase of this lipid having no tilt. This allows the analysis to proceed without having to determine n_W experimentally.

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Appendix

Comparison of two procedures for obtaining V_{CH_2} and V_{CH_3}

The best example to illustrate how V_{CH_2} and V_{CH_3} for chain disordered phases can be obtained from V_C is the *n*-alkanes. When one plots V_C as a function of chain length n at some temperature as in Fig. 2, one obtains a fairly straight line. This has suggested that V_{CH_2} and V_{CH_3} do not change very much as the chain length n changes and that the additional increment (the slope of the line in Fig. 2) gives V_{CH_2} and the intercept at $n = 2$ (C_2H_6) gives $2V_{CH_3}$. For the alkanes this procedure yields $V_{CH_2} = 27.3$ and $V_{CH_3} = 57.6$ at $T = 40\,^\circ C$. These results have suggested that the approximate relation, $V_{CH_3} = 2V_{CH_2}$, might be employed in analyzing lipids [2,4,5] and, for simplicity, we have used this approximation in this paper as shown in Table I. However, use of this approximation is not required if one has lipid data for a series of chain lengths. It is then preferable to repeat the above procedure using the lipid data. This has been done recently [35] for the usual straight chain phosphatidylcholines, for which the above approximate relation is reasonably accurate ($V_{CH_3} \sim 2.1\,V_{CH_2}$), as well as for isobranched lipids,

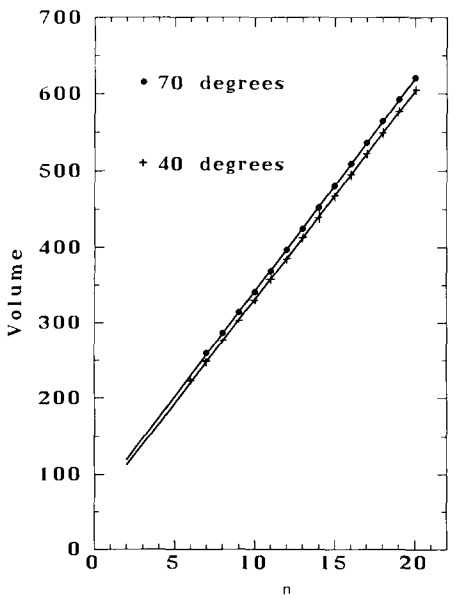

Fig. 2. Volumes (cubic ångstroms per molecule) for the *n*-alkanes C_nH_{2n+2} as a function of n.

where one has more methyls so that the total methyl volume is about 6.4 V_{CH_2}. In both these cases and also in the case of phosphatidylethanolamines V_{CH_2} is about the same (± 0.5) as it is for the *n*-alkanes. This has supported the conclusion that the liquid-crystalline, L_α phase in lipids is similar to the fluid phase of *n*-alkanes.

Small [18] has advocated a procedure for obtaining V_{CH_2} and V_{CH_3} that is very similar to the procedure in the preceding paragraph, but with one difference. Instead of plotting the volumes, V_C, versus chain length n at the same temperature, Small plots the volumes just above the melting temperatures, $T_M(n)$, which are higher for longer chains. For the even *n*-alkanes ($n = 10$–20) the slope of these plots yields $V_{CH_2} = 29.6$ and the intercept at $n = 2$ yields $V_{CH_3} = 35.6$. When lipid data are treated in the same way, similar values of V_{CH_2} are obtained. Therefore, the results from this method also support the conclusion that fluid phase lipids are similar to fluid *n*-alkanes. However, it is clearly incorrect and misleading to say that the value $V_{CH_2} = 27.7$ that has been obtained for the phosphatidylcholines using the first procedure is 'falsely low' (Ref. 18, p. 494) because it does not agree with the value $V_{CH_2} = 29.6$ obtained for the alkanes using the other procedure.

Small's procedure is reminiscent of the law of corresponding states in which the temperature and pressure of one fluid is compared to another at temperatures and pressures that are scaled according to the critical temperatures and pressures. However, the law of corresponding states does not apply to fluid/solid first order coexistence, nor does Small appeal to the law of corresponding states, so this is not a fundamental justification for this procedure. Instead, Small invokes the idea that 'if one plots the value at a phase transition, one eliminates a degree of freedom' (Ref. 18, p. 495). However, one can equally well eliminate a degree of freedom by plotting values at the same temperature as is done in our procedure.

Some fundamental justification for our procedure can be obtained from Flory's theory [36] of the fluid state of flexible chain systems, such as polymers and the *n*-alkanes. Although that theory does not say anything useful about volume, it does yield a result for another property of interest, namely, the degree of flexibility f, usually thought

of as the fraction of gauche bonds. The Flory theory states (Ref. 36, his Eqn. 5) that f is the same for any chain length provided that the temperature is the same, in support of the assumption behind our procedure. Since chains of different length melt at different temperatures in the Flory theory, f is not the same just above the melting point, in contradiction to the assumption behind Small's procedure. While the Flory theory has been criticized as a theory of the melting phase transition [37], its conclusions for the quantity f have not been disproved.

An additional way to decide the relative merits of the two procedures for obtaining V_{CH_2} and V_{CH_3} is to consider the plausibility of the results. It is a straightforward matter to obtain V_{CH_2} and V_{CH_3} in the crystal phase of the alkanes by using the X-ray structural data reproduced in Table 7-1 in Small's book. For $C_{21}H_{44}$, $V_{CH_2} = 23.6$ and $V_{CH_3} = 41.7$. For the fluid state, both procedures indicate that V_{CH_2} increases and our procedure indicates that V_{CH_3} increases to about 55.6. However, Small's procedure yields $V_{CH_3} = 33.6$, which is even smaller than its value in the crystal phase, which is certainly an incongruent result for any substituent group of atoms in a melting transition in which the overall volume increases.

While there is somewhat more theoretical support for our procedure than for Small's, we would not argue that our procedure is compellingly correct. But since our procedure yields more plausible results with regard to the V_{CH_3} groups than Small's, we believe that it is the better of the two.

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