

# A theoretical model for phase transitions in lipid monolayers<sup>a)</sup>

M. J. Zuckermann

*Department of Physics, McGill University, Montreal, Quebec, Canada*

D. A. Pink

*Department of Physics, St. Francis Xavier University, Antigonish, Nova Scotia, Canada*

M. Costas and B. C. Sanctuary<sup>b)</sup>

*Department of Chemistry, McGill University, Montreal, Quebec, Canada*

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The statistics of Guggenheim are used in conjunction with a ten-state conformational model (originally formulated for phase transitions in lipid bilayers) to predict gel–fluid and fluid–vapor phase boundaries for lipid monolayers. The theoretical model is a model of interacting dimers on a two-dimensional lattice with each dimer representing a lipid molecule with two acyl chains and one polar head. The acyl chains are assumed to interact via anisotropic van der Waal's interactions. The interaction between the polar heads is simulated by an attractive isotropic interaction with coupling constant  $K_0$ . The analysis of Costas and Sanctuary is then combined with this model so as to obtain self-consistently the fraction of occupied and vacant lattice sites for the monolayer at different pressures. Surface pressure-area isotherms and coexistence curves are obtained for fixed van der Waals interactions and several values of  $K_0$ . It is shown that a fluid–vapor phase boundary occurs for values of  $K_0$  above a critical value. The method is also applied to the calculation of the change in packing fraction at the main gel–fluid (liquid crystal) transition of lipid bilayers. One of our principal results is that some of the qualitative features of  $\Pi$ - $A$  isotherms for lipid monolayers may now be associated with particular values of  $K_0$ .

## I. INTRODUCTION

Although there has been a considerable amount of work done on constructing mathematical models of lipid monolayers,<sup>1,2</sup> they have tended to fall broadly into two classes. (a) Those that take into account the possible conformational states of the hydrocarbon chains, but do not concern themselves with the excluded volume interactions associated with the glyceride backbone and their effect upon packing and the formation of free volume in the monolayer.<sup>3–13</sup> (b) Those that consider this excluded volume interaction as more important and treat the acyl chain interactions without considering details of their different conformational states.<sup>14,15</sup> A subset of class (a) is that which is not concerned with the formation of free volume at all. A model similar to those of (b) is that of Albrecht *et al.*<sup>16</sup> who make use of the ideas of de Gennes.<sup>17</sup> There are considerable differences in the results obtained by these models concerning the order of the transition between the liquid expanded (LE) and the liquid condensed (LC) states and, if it is first order, whether its end point is a critical or a tricritical point. A recent paper by Cadenhead *et al.*<sup>18</sup> summarizes these points. Here we are not going to concern ourselves with these aspects, but instead present a model which takes into account not only the details of the conformational states of the hydrocarbon chains, but also the excluded volume interaction of the glyceride backbones of the lipids and the formation of free volume.

We make use of a model of lipid hydrocarbon chains which has been used before.<sup>19,20</sup> Two of these chains are attached to the ends of a rigid rod, so forming a dimer with the chains perpendicular to the ends of the rod when they are in their all-*trans* state. These dimers, which represent the glyceride backbone, are then allowed to occupy the bonds of a triangular lattice, with the chains occupying the vertices and directed perpendicular to the lattice. The interaction between two acyl chains is taken to be an anisotropic van der Waal's interaction of the type used by Marcelja<sup>5,19,20</sup> to describe the main gel–fluid (liquid crystal) phase transition of lipid bilayers. The interaction between the polar heads is simulated by a central force between dimers with coupling constant  $K_0$ , which has been previously described in Ref. 20. In Sec. II, it is shown how this model may be combined with a model due to Costas and Sanctuary (CS)<sup>21</sup> in order that the fraction of occupied and vacant lattice sites may be calculated for both lipid monolayers and lipid bilayers. In Sec. II, we describe the models in greater detail and we discuss the approximations used to obtain expressions for the free energy of the system.

In Sec. III, we present the results of numerical calculations for lipid monolayers. In order to describe these phase transitions, we use the following terminology:

- (i) The condensed phase corresponding to areas for lipid chains of the order of  $23 \text{ \AA}^2$  is called the gel phase (G).
- (ii) The condensed liquid crystalline phase corresponding to areas of the order of  $50 \text{ \AA}^2$  per lipid chain is called the fluid phase (F).

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<sup>b)</sup>On leave at the University of York, England.

(iii) The liquid phase with areas of the order  $90 \text{ \AA}^2$  per lipid chain is called the vapor phase (V).

(iv) The monolayer phase with areas of the order of and greater than  $200 \text{ \AA}^2$  per lipid chain will be called the surface gas phase (SG).

Phase (i) is equivalent to the LC phase and phases (ii) and (iii) together form the LE phase. We examine the conditions under which  $G \rightleftharpoons F$  and  $F \rightleftharpoons V$  or  $F \rightleftharpoons \text{SG}$  transitions can occur in the same monolayers by means of a systematic variation of the parameter  $K_0$ . The parameters of the van der Waals interaction are taken to be those appropriate for palmitoyl chains. We do not study the  $V \rightleftharpoons \text{SG}$  phase transition in detail but make qualitative remarks about its relationship to the  $F \rightleftharpoons V$  transition. The  $V \rightleftharpoons \text{SG}$  phase transition is usually referred to as the LE-SG transition. We also show that the  $F \rightleftharpoons V$  transition usually occurs at low pressures. We make comments about the amount of free volume at both transitions and relate our results to observations in Sec. IV. In particular, it is shown that the shape of  $\Pi$ - $A$  isotherms for lipid monolayers in the fluid phase can be related to particular values of  $K_0$ .

It should be noted that the model which we present is one for a monomer-dimer system. The proofs<sup>22</sup> that such a system does not exhibit a phase transition as a function of monomer density (except, possibly, when this is zero), however, do not apply to this model. The model presented here not only has interactions of various kinds between the dimers, but also has an external pressure applied uniformly in the plane of the lattice.

## II. THEORY

As mentioned in the Introduction, the method of calculation involves the combination of two models: the conformational model of Refs. 19 and 20 for phase transitions in lipid monolayers and bilayers and the analysis of fluid-vapor ( $F \rightleftharpoons V$ ) transitions in  $n$ -alkane systems<sup>21</sup> due to CS which uses an equation of state obtained for pure liquids in the Guggenheim<sup>23</sup> approximation. It is, therefore, appropriate to begin this section with a brief description of both methods. The section continues with an analysis of the expression for the free energy obtained combining these models.

The model examined by CS is a three-dimensional lattice with  $N_s$  sites and coordination number  $z$  which contains  $N$  molecules, each of which occupies  $r$  sites. The molecules are flexible in the sense that each link is only restricted so as to occupy one nearest-neighbor bond on the lattice. The Gibbs free energy and the partition function are given, in the isothermic-isobaric ensemble, by

$$F = -kT \ln Z(T, P), \quad (2.1)$$

where

$$Z(T, P) = \sum_{E, V} \Omega(E, V, N) \exp[-(E + PV)/kT]. \quad (2.2)$$

For a system of  $N$   $r$ -mers and  $N_0$  vacant sites or holes the number of configurations available to the system for a given potential energy  $E$  and volume  $V$  is

$$\Omega(E, V, N) = \left(\frac{\omega}{f}\right)^N \left(\frac{1}{f_0}\right)^{N_0} (f_{\text{NB}})^{(z/2)N_0}. \quad (2.3)$$

Here, the packing fraction  $f$  of the lattice is given by

$$f = rN/N_s \quad 0 \leq f \leq 1 \quad (2.4)$$

and the fraction of vacant sites or holes is given by  $f_0 = 1 - f$ . The free volume is then, in this model, explicitly taken into account by assuming the presence of empty sites. In Eq. (2.3),  $(z/2)N_0$  is the total number of nonbonded pairs in the system, while  $f_{\text{NB}}$  is the fraction of nonbonded pairs in the system (that is, the hole-hole, hole-mer, and nonbonded mer-mer pairs). This fraction is given by

$$f_{\text{NB}} = 1 - \phi f \quad (2.5)$$

with

$$\phi = (z/2)[1 - (1/r)] \quad (2.6)$$

being the ratio of the fraction of bonded  $r$ -mer pairs to the fraction of sites occupied by  $r$ -mers. As such,  $f_{\text{NB}}$  gives a measure of the external degrees of freedom of the  $r$ -mers. As  $f_{\text{NB}}$  decreases, the external degrees of freedom of the  $r$ -mers decrease. Finally, in Eq. (2.3)  $\omega$  is, for a given lattice coordination number  $z$  and number of monomers per  $r$ -mer ( $r$ ), a constant given by

$$\omega = z(z-1)^{r-2} r/2. \quad (2.7)$$

CS have shown<sup>21</sup> that the free energy  $F$  can be written as follows:

$$F = F_{\text{mix}} + F_{\text{int}} + F_{\text{ext}}. \quad (2.8)$$

$F_{\text{mix}}$  is the free energy of mixing  $r$ -mers and holes and is given by

$$\frac{F_{\text{mix}}}{rN} = -kT \left[ \frac{1}{r} \ln(\omega/f) - (f^{-1} - 1) \ln(1 - f) + \frac{z}{2} (f^{-1} - \phi) \ln(1 - \phi f) \right], \quad (2.9)$$

$F_{\text{int}}$  is the contribution of nearest-neighbor interactions to the free energy and is given by

$$F_{\text{int}}/rN = -\frac{1}{2} z \epsilon (1 - \phi)^2 f / (1 - \phi f). \quad (2.10)$$

$\epsilon$  is an attractive interaction between two neighboring lattice sites occupied by molecules, but not linked by a molecular bond (the interaction energies hole-hole, hole-mer, and bonded mer-mer are considered equal to zero).  $F_{\text{ext}}$  is the contribution of the external pressure to the free energy. Minimization of the free energy [Eq. (2.8)], with respect to  $f$ , gives a self-consistent equation for  $f$  (equation of state) in the "quasichemical" approximation<sup>23</sup>:

$$f = 1 - \exp \left\{ -\frac{z}{2} \frac{\epsilon}{kT} \left[ \frac{f(1 - \phi)}{1 - \phi f} \right]^2 - \frac{Pv^*}{kT} + \frac{z}{2} \ln(1 - \phi f) \right\}, \quad (2.11)$$

where  $v^*$  is the closed packed volume of a single mer. Equation (2.11) defines the value of  $f$  at a given  $(T, P)$  which minimizes the Gibbs free energy. It has three solutions, the lowest and higher values of  $f$  corresponding to minima on the free energy [Eq. (2.8)] while the intermediate solution corresponds to a maximum in  $F$ . At a given pressure, there is a unique temperature such

that the two free energy minima are equal. This set of temperatures and pressures define the coexistence fluid-vapor line, i. e., the model undergoes a fluid-vapor phase transition.

The model of Refs. 19 and 20 is a ten-state model which describes the conformational states of the acyl chains of the lipid molecules. This model is a variant of the rotational isomeric model in which the conformation of each acyl chain is described by specifying the sequence of *trans* (*t*) and *gauche* (*g*<sup>±</sup>) C-C bonds along the chain. A *gauche* bond has internal energy  $E_g \sim 0.45 \times 10^{-13}$  erg. Since there are three states available to each C-C bond in a saturated chain, a chain with 16 carbon nuclei will have about  $3^{10}$  states available to it. Here we have excluded those states in which the chain intersects itself or doubles back to the glyceride backbone. All the allowable states can be evaluated numerically.<sup>5</sup> However, more insight into the mechanism of, for example, the G $\rightleftharpoons$ F phase transition can be gained by making some simplifications. The simplest approximation is to consider only two states<sup>11,24</sup>: the all-*trans* (ground) state of internal energy  $E_1 = 0$  which projects an area of  $A_1 = 20.4 \text{ \AA}^2$  onto a plane perpendicular to the chain axis and a state of internal energy  $E_{10}$ , area  $A_{10}$ , and degeneracy  $D_{10}$  which approximates the distribution of states with many *gauche* bonds describing the system in the fluid phase. The labeling scheme will become clear below.

In a study of the temperature dependence of the C-C stretch Raman band in saturated phosphatidylcholines,<sup>19</sup> it was found that conformations other than the all-*trans* conformation contributed significantly to the band intensity. These states had energies less than  $E_{10}$  and were classified as "intermediate." Since  $E_{10} \sim 5E_g$ , it was decided to consider as intermediate states those with internal energy  $\leq 3E_g$  since the state 10 was considered to be an average over high energy states and would, therefore, include contributions from those with energy  $4E_g$ . Instead of including all states with energies  $\leq 3E_g$ , however, we adopted the following criterion: In the gel phase, there are hard core interactions which inhibit the chain from adopting conformations which would otherwise be energetically favorable at temperatures appropriate to that phase. Instead of explicitly treating these hard core interactions, which we could do only in a mean field approximation, we chose to select as intermediate states those which have a sufficiently small cross sectional area. This was because such states could be excited in the gel phase without disrupting the lipid packing too much so that their probability of being excited is determined by the van der Waal's interactions and their internal energies. The remaining states of energy less than or equal to  $3E_g$  were ignored. We therefore excluded as intermediate states those with an area greater than  $\sim 26 \text{ \AA}^2$  on the grounds that they would be unlikely to be excited at temperatures below the phase transition and irrelevant above it. The cutoff of  $26 \text{ \AA}^2$  was chosen to be approximately the mean area at the G $\rightleftharpoons$ F transition where the area goes from  $\sim 23 \text{ \AA}^2$  to  $\sim 30 \text{ \AA}^2$  per chain. One test of this model is whether a phase change occurs when the van der Waals interactions are put equal to zero and also whether the order

parameter plateau is maintained along the acyl chains as pointed out by Dill and Flory.<sup>25</sup> This has been tested and it was seen that the ten-state model fulfills both of these requirements.

Accordingly, the ten-state model assumes that each acyl chain can be in one of the following states: (a) the all-*trans* ground state ( $n=1$ ); (b) eight intermediate states possessing internal energy  $E_n$ , area  $A_n$ , and degeneracy  $D_n$  ( $n=2, \dots, 9$ ); and (c) a high energy "melted" state ( $n=10$ ) with parameters  $E_{10}$ ,  $A_{10}$ , and  $D_{10}$ . The details of the excited state are unknown and its parameters must be determined from experiment. These states for saturated chains with 14, 16, or 18 carbon nuclei each are listed in Table I of Ref. 19.

It was found<sup>19</sup> that, in the gel phase, by far the most probable states were the all-*trans* state ( $n=1$ ), states 2 and 3 having single *gauche* bonds at the end of the chain, and state 5 with a kink anywhere along the chain between the third carbon and the methyl group. However, if other intermediate states were omitted from the calculation, the G $\rightleftharpoons$ F phase transition would then become sharper and the calculated Raman band intensities would not be in such good agreement with experiment. A calculation of the latter along these lines has been performed<sup>26</sup> and the expected disagreement with measured and calculated Raman intensities in the gel phase can be clearly seen.

Both lipid monolayers and bilayers are simulated by a two-dimensional triangular lattice with each site representing an acyl chain which is oriented perpendicular to the lattice. The attractive interactions between acyl chains are taken to be quadrupole-quadrupole forces (anisotropic van der Waals interactions) of the type used by Maier and Saupe to describe the phase transition of liquid crystals (see Marcelja<sup>5</sup> for details and references). The expression for these interactions as used in Refs. 19 and 20 depend on the directional (quadrupolar) order parameters<sup>5</sup> between the C-C bonds of different acyl chains and on the distance between chains or nearest-neighbor lattice sites.<sup>19,27</sup> As stated in the introduction, the interaction between polar heads is taken to be a central force and the related order parameter therefore depends on the distance between polar heads. It is shown in Refs. 19 and 20 how both interactions can be replaced by separable expressions. We do not give any explicit form for the Hamiltonian of the ten-state model, but instead refer the reader to Refs. 19 and 20 for details.

The theory of CS is now modified to include the ten-state model for a two-dimensional triangular lattice. The free energy for the combined theory may be written as follows:

$$F/rN = F_{\text{mix}} + F_L \quad (2.12)$$

$F_{\text{mix}}$  is the free energy of mixing of  $r$ -mers and holes given by Eq. (2.9).  $F_L$  is the contribution from the conformational states of the acyl chains and from the interactions between acyl chains and between polar heads

$$F_L = -kT \ln Z_L(f) + [(r-1)/r] J_0 \lambda_w^2 + z/2(K_0 \lambda_p^2 + J_0 \lambda_w^2)(1-\phi)^2 f / (1-\phi f) \quad (2.13)$$

The first term on the right-hand side of Eq. (2.13) represents the energies of the ten conformational states in the molecular field (MF) approximation.  $Z_L(f)$  is the partition function of the lipid chains and is given by

$$Z_L(f) = \sum_{n=1}^{10} D_n \exp[-E_n^{\text{MF}}(f)/kT]. \quad (2.14)$$

$D_n$  and  $E_n^{\text{MF}}$  are the degeneracy and MF energy of the  $n$ th conformational state, respectively, and  $E_n^{\text{MF}}$  is given by

$$E_n^{\text{MF}}(f) = (E_n + \Pi A_n/f) - 2(1 - r^{-1})J_0 I_n^w \lambda_w - z(K_0 I_n^p \lambda_p + J_0 I_n^w \lambda_w)(1 - \phi)^2 f / (1 - \phi f). \quad (2.15)$$

$J_0$  and  $K_0$  are the coupling constants of the van der Waal's and the polar head interactions, respectively, when the chains are in the all-*trans* state ( $n=1$ ).

The first term on the right-hand side of Eq. (2.15) is the energy of the  $n$ th state in the absence of interactions, but in the presence of an external surface pressure  $\Pi$ .  $E_n$  is the conformational energy and  $A_n$  is the cross sectional area of the  $n$ th state. The second term of the right-hand side of Eq. (2.15) is the combination of the van der Waals interactions between acyl chains belonging to the same molecule (taken as an  $r$ -mer in the triangular lattice). The third term on the right-hand side of Eq. (2.15) is the contribution of the interaction between the acyl chains and between the polar heads of neighboring molecules in the spirit of the CS model.  $I_n^w$  and  $I_n^p$  are functions which depend directly on the nature of the anisotropic van der Waal's interaction and the isotropic central force, respectively (see Ref. 20 for details).  $\lambda_w$  and  $\lambda_p$  are "order parameters" for the acyl chains and the polar heads, respectively,<sup>20</sup> and are given by the self-consistent equation

$$\lambda_{w,p} = \sum_{n=1}^{10} I_n^{w,p} D_n \exp[-E_n^{\text{MF}}(f)/kT] / Z_L(f). \quad (2.16)$$

The second and third terms on the right-hand side of Eq. (2.13) are included to compensate for overcounting of the interactions in the first term. The expression for  $E_L$  of Eq. (2.13) has two important limits. In the limit in which the lipid monolayer is assumed to be composed of independent acyl chains in the absence of holes ( $r=1$ ,  $f=1$ ), Eq. (2.13) reduces to the expression for  $F_L$  given in Ref. 20. In the limit in which each acyl chain is assumed to have only one conformational state with zero energy,  $\lambda_w = \lambda_p = 1$  from Eqs. (2.15) and (2.16). If we then take  $J_0 = 0$ ,  $F_L$  of Eq. (2.13) reduces to  $F_{\text{int}}$  of Eq. (2.10) provided that  $\epsilon$  is replaced by  $K_0$ . The combined model therefore reduces to the model of CS in the limit of a single conformational state per acyl chain and the absence of anisotropic interactions.

The equation for  $f$  is found by minimizing the total free energy  $F$  of Eq. (2.12) with respect to the reduced volume  $f^{-1}$ . The result is

$$f = 1 - \exp\left\{-\frac{(z/kT)(K_0 \lambda_p^2 + J_0 \lambda_w^2)[(1 - \phi)f / (1 - \phi f)]^2}{\Pi A_{av}/kT + (z/2) \ln(1 - \phi f)}\right\}. \quad (2.17)$$

Equation (2.17) reduces to the two-dimensional version of expression (2.11) of CS in the limit  $\lambda_p = \lambda_w = 1$ .  $A_{av}$  is the average area per acyl chain and is given by

$$A_{av} = \sum_{n=1}^{10} A_n D_n \exp[-E_n^{\text{MF}}(f)/kT] / Z_L(f). \quad (2.18)$$

Equations (2.14)–(2.18) form a set of coupled self-consistent equations for  $\lambda_w$ ,  $\lambda_p$ , and  $f$  for fixed  $\Pi$  and  $T$ . These equations were solved numerically for parameters appropriate to monolayers of lipids with acyl chains composed of 16 carbon atoms (palmitoyl chains). Since nearly all lipid molecules have two acyl chains, they become dimers in the two-dimensional triangular lattice model ( $r=2$ ,  $z=6$ ) in the above equations. The results are described in the next section for the case when the interaction  $K_0$  between neighboring polar heads is taken to be Coulomblike (see Ref. 20).

The above formalism is applicable to bilayers if  $\Pi$  is taken to be the internal pressure introduced by Marcelja<sup>26</sup> to describe the lipid environment relative to the monolayer. Numerical calculations for phase diagrams for monolayers and for the free volume in bilayers are presented and analyzed in the next section.

### III. NUMERICAL RESULTS FOR LIPID MONO- AND BILAYERS

The next step is to use the equations of Sec. II to obtain surface pressure–area ( $\Pi$ - $A$ ) isotherms for lipid monolayers. This requires a knowledge of the numerical values of the conformational and interaction parameters of the ten-state model. Such parameters have already been obtained for phospholipids with chain lengths of 12 to 22 carbon atoms by fitting to experimental data for lipid bilayers. Their numerical values are given in Ref. 20. In this section, we use the parameters pertaining to palmitoyl chains which have 16 carbon atoms. These are  $J_0 = 0.736 \times 10^{-13}$  erg,  $E_{10} = 2.78 \times 10^{-13}$  erg, and  $D_{10} = 6.3$  – the relevant energies of the intermediate chain conformations and the interactions parameters  $I_n^{p,w}$  can be evaluated using Eqs. (3.2)–(3.6) and Table II all of Ref. 20. Several values were chosen for the coupling constant of the polar head interaction.

The coexistence curve and the  $\Pi$ - $A$  isotherms are shown in Figs. 1 and 2, respectively, for the case when there is no direct interaction between the polar heads, i. e.,  $K_0 = 0$ . In this case, the  $\Pi$ - $A$  phase diagram only shows the  $G \rightleftharpoons F$  phase transition. Isotherms for the packing fraction  $f$  as a function of pressure  $\Pi$  are displayed in Fig. 3. However, for low enough temperatures and transition pressures ( $\Pi \cong 2.5$  dyn/cm) the phase transition continuously becomes a  $G \rightleftharpoons V$  phase transition, since the packing fraction  $f$  changes from 0.98 in the solid or gel phase to 0.35. This implies that the isotherms of Fig. 2 lying to the right of the tieline represent a continuous transition from a dimer fluid (F) to a dimer vapor (V) as the area increases and the lateral pressure decreases, i. e., no first order  $F \rightleftharpoons V$  phase transition occurs. Figure 4 shows the variation of the transition pressure  $\Pi_c$  and transition enthalpy  $\Delta H$  with temperature  $T$  for the  $G \rightleftharpoons F$  transition. The curve of  $\Pi_c$  vs  $T$  is linear except at low temperatures and  $\Delta H$  decreases with increasing temperature as expected.

These results show that an additional attractive inter-

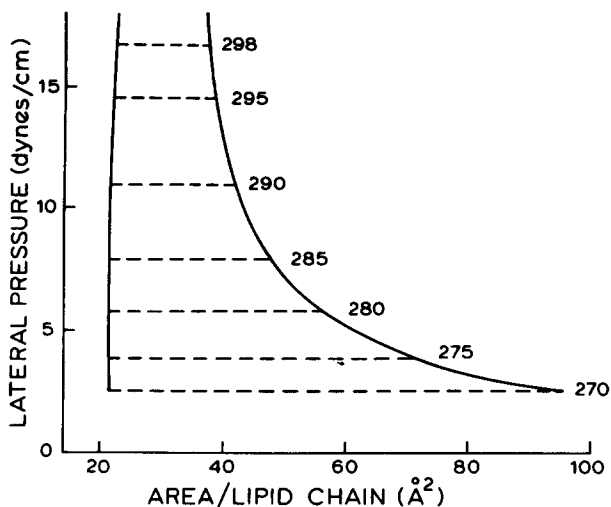


FIG. 1. Coexistence curve for the  $G \rightleftharpoons F$  phase transition for monolayers of lipids with palmitoyl chains when  $K_0 = 0$ . The numbers on the tie lines give the temperature in degrees Kelvin.

action between the lipid molecules is required for an  $F \rightleftharpoons V$  phase transition to occur. In consequence, the calculations were repeated with the same chain parameters as above and with serial nonzero values of  $K_0$ . It was found that  $F \rightleftharpoons V$  phase transitions did not occur for values of  $K_0$  less than a threshold value of  $K_0^c = 0.2385 \times 10^{-13}$  erg. The first value of  $K_0$  used was, therefore, a value of  $K_0$  close to  $K_0^c$  ( $K_0 = 0.245 \times 10^{-13}$  erg). This leads to the system of self-consistent equations displaying two separate van der Waals loops or two separate phase transitions. The results are shown in Figs. 5-7. Figure 5 shows the  $\Pi$ - $A$  isotherm in the range of lateral pressures  $0 \leq \Pi \leq 10$  dyn/cm for  $K_0 = 0.245 \times 10^{-13}$  erg. Two phase transitions now occur. These are (i) the  $G \rightleftharpoons F$  phase transition found for  $K_0 = 0$  and (ii) an  $F \rightleftharpoons V$  phase transition between a dimer fluid and a dimer vapor at low lateral pressures. The coexistence curve and  $\Pi$ - $A$  isotherms for this pressure range are given in detail in Fig. 6. The critical pressure  $\Pi_{cv}$  and critical area per per lipid chains  $A_{cv}$  for the fluid-vapor transition are

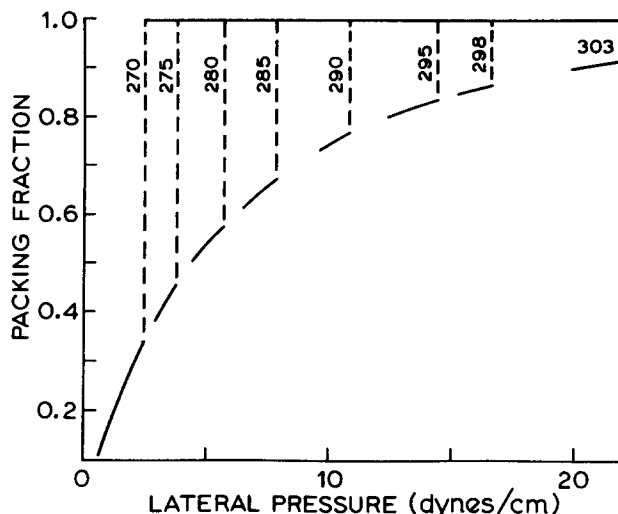


FIG. 3. The packing fraction  $f$  as a function of lateral pressure for different temperatures given in degrees Kelvin (corresponding to Figs. 1 and 2).

$$\begin{aligned} \Pi_{cv} &= 1.3 \text{ dyn/cm,} \\ A_{cv} &= 68 \text{ \AA}^2. \end{aligned} \quad (3.1)$$

Figure 6 shows that both a  $G \rightleftharpoons F$  phase transition and a  $F \rightleftharpoons V$  phase transition occur in the pressure range  $1.16 \text{ dyn/cm} \leq \Pi \leq 1.3 \text{ dyn/cm}$ . For  $\Pi \leq 1.16 \text{ dyn/cm}$ , a direct  $G \rightleftharpoons V$  transition takes place. Figure 7 shows the isotherm of the packing fraction  $f$  as a function of lateral pressure. The presence of two transitions can be seen quite clearly in the behavior of  $f$  at low pressures.

Figure 8 shows the coexistence curves for the  $G \rightleftharpoons F$  and  $F \rightleftharpoons SG$  phase transitions when  $K_0 = 0.4 \times 10^{-13}$  erg. The  $F \rightleftharpoons SG$  phase transition is now considerably broader

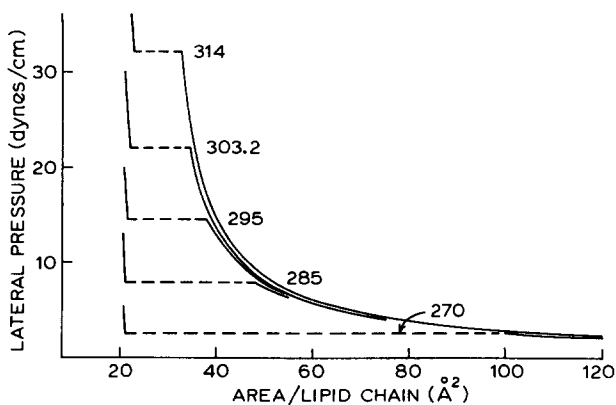


FIG. 2.  $\Pi$ - $A$  isotherms for monolayers of lipids with palmitoyl chains with  $K_0 = 0$ . The temperatures are given in degrees Kelvin.

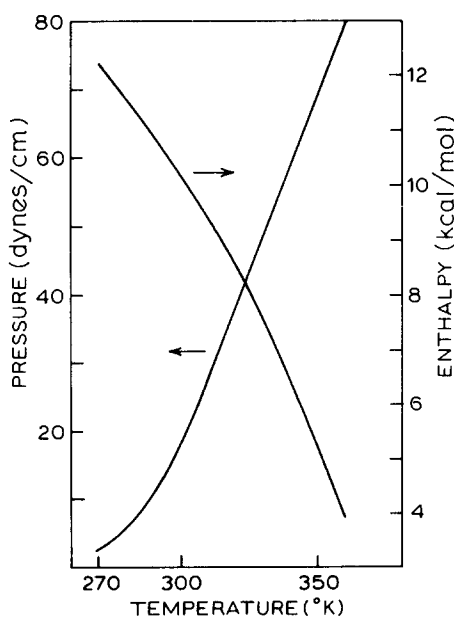


FIG. 4. The transition pressure and transition enthalpy as functions of temperature for the  $G \rightleftharpoons F$  phase transition when  $K_0 = 0$ .

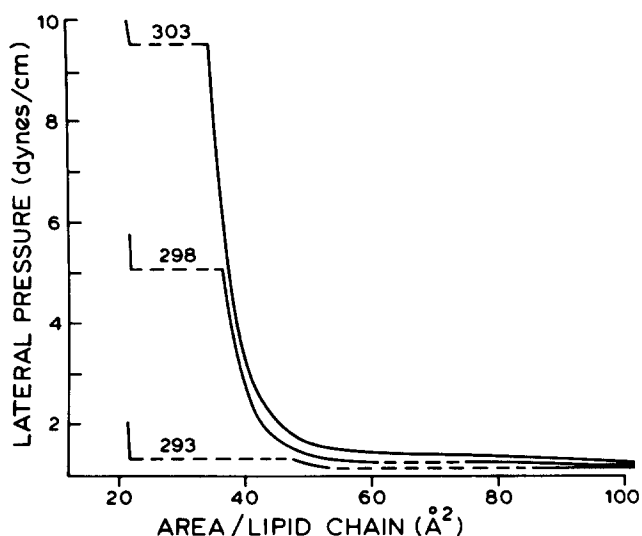


FIG. 5.  $\Pi$ - $A$  isotherms for monolayers of lipids with palmitoyl chains when  $K_0 = 0.245 \times 10^{-13}$  erg. An  $F \rightleftharpoons V$  phase transition is now predicted at higher areas per polar head in addition to the  $G \rightleftharpoons F$  phase transition. The numbers give the temperature in degrees Kelvin.

than the  $F \rightleftharpoons V$  transition of Fig. 6. For example, the areas per lipid chain change from 35 to  $645 \text{ \AA}^2$  when  $T = 305 \text{ K}$  and  $\Pi = 0.27 \text{ dyn/cm}$ . The critical point of this transition occurs at  $440 \text{ K}$ . The critical pressure  $\Pi_{cv} = 1.78 \text{ dyn/cm}$  and the critical area per lipid chain  $A_{cv} = 70.8 \text{ \AA}^2$ . It can be seen from Fig. 8 that sublimation ( $G \rightleftharpoons SG$ ) occurs below  $301 \text{ K}$ .

The next value used for  $K_0$  was:  $K_0 = 0.675 \times 10^{-13}$  erg. This value of  $K_0$  gives the correct temperature  $T_f = 65^\circ \text{C}$  ( $338 \text{ K}$ ) for the main phase transition of dipalmitoyl-phosphatidyl ethanolamine (DPPE) bilayers assuming that the difference between the values of  $T_f$  for DPPE and dipalmitoyl-phosphatidyl choline (DPPC) are due to a strong attractive interaction between the

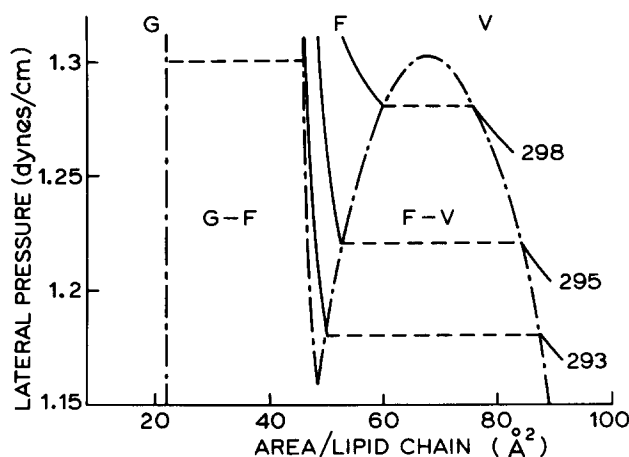


FIG. 6.  $\Pi$ - $A$  isotherms for  $K_0 = 0.245 \times 10^{-13}$  erg at low pressures. This shows the coexistence curve of the  $F \rightleftharpoons V$  phase transition in detail in relation to the  $G \rightleftharpoons F$  phase transition.

PE polar heads. The  $\Pi$ - $A$  isotherms for this case are shown in Fig. 9. Again, there are two transitions with the isotherms shown in Fig. 9 *decreasing dramatically* with lipid chain area in the fluid phase until an extremely low lateral pressure is reached ( $\Pi \sim 0.1 \text{ dyn/cm}$ ). An  $F \rightleftharpoons SG$  phase transition then occurs as can be seen from the behavior of the packing fraction  $f$ . Figure 10 shows that the value  $K_0 = 0.675 \times 10^{-13}$  erg is sufficiently high to prevent the formation of holes in the gel ( $G$ ) or fluid ( $F$ ) phase, i. e.,  $f = 0.985$  in both phases. Then, at the  $F \rightleftharpoons SG$  transition, the fluid monolayer becomes a rarified surface gas ( $SG$ ) for which  $f = 0.015$ . Isotherms were only calculated for temperatures below  $370 \text{ K}$  since isotherms for higher temperatures lie above the coexistence curve for the  $G \rightleftharpoons F$  phase transition.

The coexistence curve of the  $F \rightleftharpoons SG$  phase transition is shown in Fig. 11 for  $K_0 = 1.0 \times 10^{-13}$  erg. It can be seen that a very broad  $F \rightleftharpoons SG$  phase transition occurs

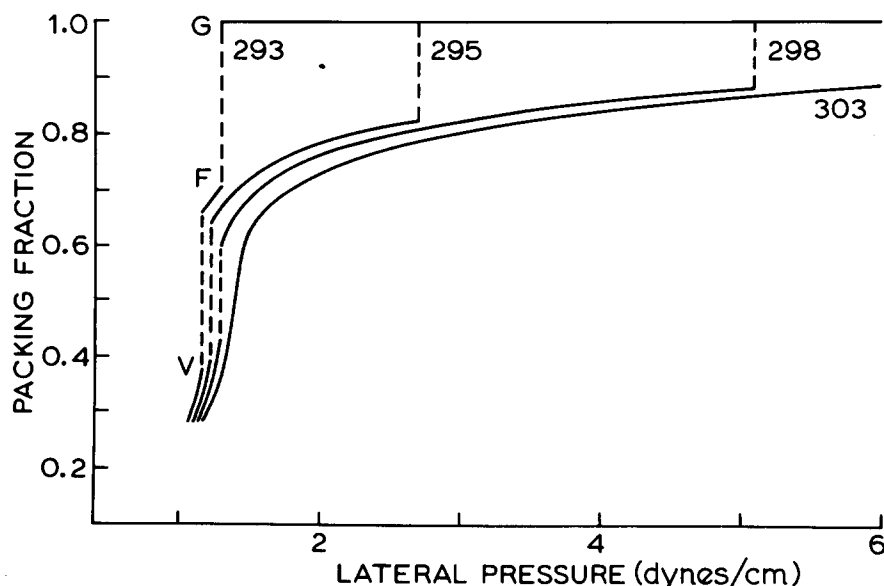


FIG. 7. The packing fraction  $f$  as a function of lateral pressure for different temperatures when  $K_0 = 0.245 \times 10^{-13}$  erg (corresponding to Figs. 5 and 6).

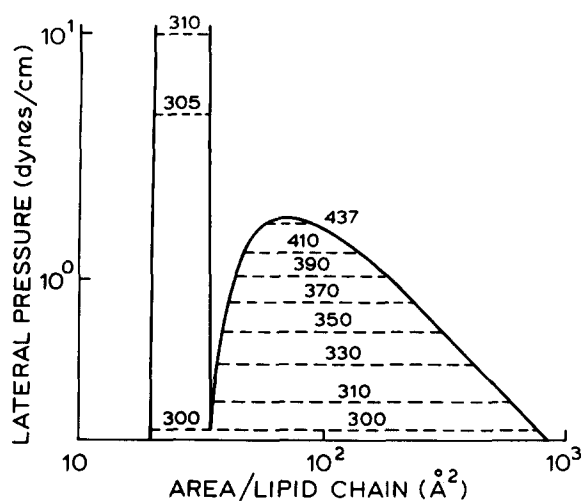


FIG. 8. Coexistence curves for the  $G \rightleftharpoons F$  and  $F \rightleftharpoons SG$  phase transitions when  $K_0 = 0.4 \times 10^{-13}$  erg.

at 350 K and  $4.7 \times 10^{-3}$  dyn/cm. Figure 11 also shows that all  $F \rightleftharpoons SG$  phase transitions taking place at pressures  $\Pi \geq 0.01$  dyn/cm correspond to unrealistic temperatures for monolayers spread on water surfaces ( $T > 370$  K). This implies that the strong interactions between the polar heads have stabilized the monolayer in the gel phase down to very low surface pressures for  $T < 370$  K. The coexistence curve for the  $G \rightleftharpoons F$  phase

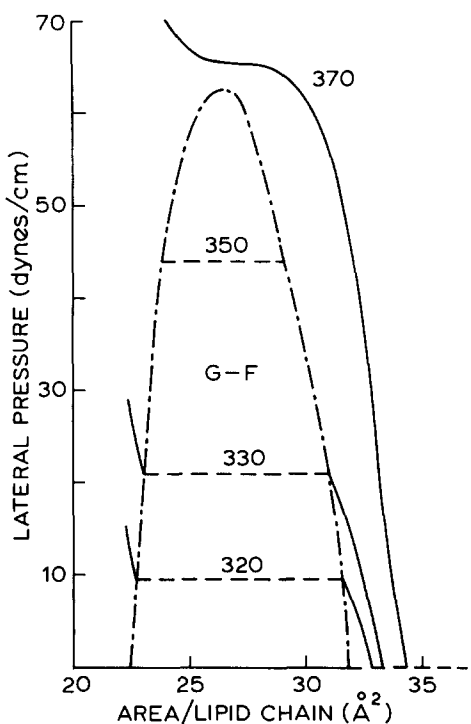


FIG. 9.  $\Pi$ - $A$  isotherms for monolayers of lipids with palmitoyl chains when  $K_0 = 0.675 \times 10^{-13}$  erg. The numbers give the temperature in degrees Kelvin. The coexistence curve for the  $G \rightleftharpoons F$  phase transition is shown on the left-hand side of the figure. The dashed line close to zero lateral pressure at the bottom right-hand side of the figure represents the  $F \rightleftharpoons SG$  phase transition.

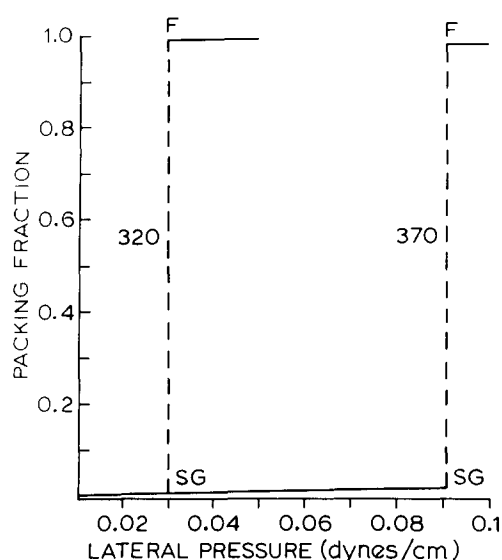


FIG. 10. The packing fraction  $f$  as a function of lateral pressure for different temperatures showing clearly the  $F \rightleftharpoons SG$  phase transition of Fig. 8.

transition and the  $\Pi$ - $A$  isotherms are not shown for this value of  $K_0$  since they are similar to those shown in Fig. 9. The critical pressure  $\Pi_c$  for the  $G \rightleftharpoons F$  phase transition is 31 dyn/cm for  $K_0 = 1.0 \times 10^{-13}$  erg, whereas  $\Pi_c = 62$  dyn/cm for  $K_0 = 0.675 \times 10^{-13}$  erg (see Fig. 9).

We obtained  $\Pi$ - $A$  isotherms for two greater values of  $K_0$  in order to examine the disappearance of the  $G \rightleftharpoons F$  phase transition as  $K_0$  increases. Figure 12 displays the  $\Pi$ - $A$  isotherms and the coexistence curve for the  $G \rightleftharpoons F$  phase transition when  $K_0 = 2.8 \times 10^{-13}$  erg. This figure shows that the critical pressure  $\Pi_c$  has decreased to a value of 4.8 dyn/cm. An  $F \rightleftharpoons SG$  phase transition does not occur for any of the isotherms shown in Fig. 12. In fact, the monolayer remains in the gel or fluid phase for all surface pressures for temperatures  $T$

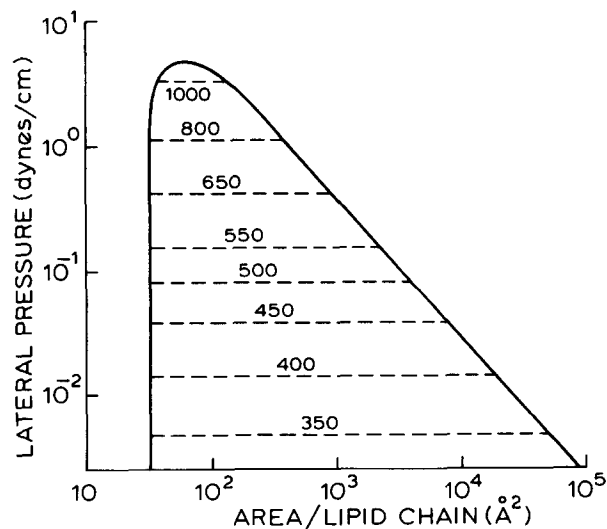


FIG. 11. Coexistence curve for the  $F \rightleftharpoons SG$  phase transition when  $K_0 = 1.0 \times 10^{-13}$  erg.

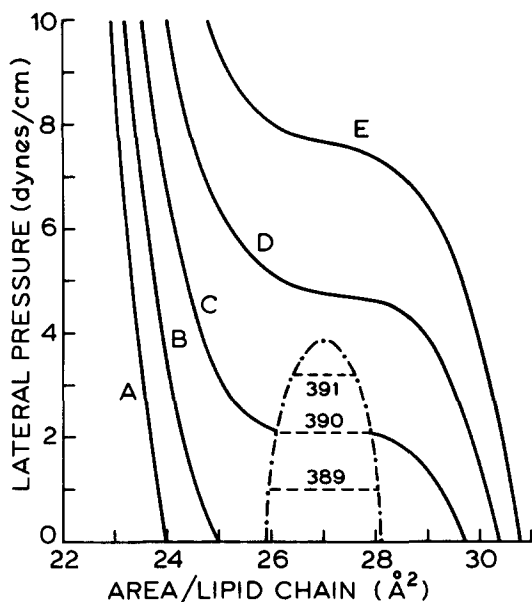


FIG. 12.  $\Pi$ - $A$  isotherms and coexistence curve for the  $G \rightleftharpoons F$  phase transitions when  $K_0 = 2.8 \times 10^{-13}$  erg. A = 385 K, B = 387.5 K; C = 390 K; D = 392.5 K; E = 395 K.

$\leq 900$  K [see Fig. 14(c) below]. The critical value of  $K_0$  above which the  $G \rightleftharpoons F$  transition is absent is  $K_0 = 3.1 \times 10^{-13}$  erg. This can be seen in Fig. 13 which shows the  $\Pi$ - $A$  isotherm for  $K_0 = 5 \times 10^{-13}$  erg. No  $F \rightleftharpoons SG$  phase transitions occur for the isotherms of Fig. 13. This will be discussed further in Sec. IV.

It is important to note that the temperature corresponding to the isotherms of Figs. 12 and 13 are, therefore, unrealistic for the case monolayers spread on a water surface. This implies that the monolayer remains stable in the gel phase at all experimentally attainable surface pressures and realistic temperatures if the value of  $K_0$  is large. The isotherms and coexistence curves for the  $F \rightleftharpoons SG$  phase transitions were not shown for the two large values of  $K_0$  used since they occur at unrealistic temperatures for all surface pressures.

The formalism of Sec. II can also be used to obtain the change in packing fraction or free volume at the main gel-liquid crystal phase transition of lipid bilayers. The original fits to experimental data for lecithin bilayers were performed using the ten-state model together with a value of 30 dyn/cm for the internal lateral pressure. We obtain the following values of  $f$  for DPPC at the main phase transition ( $T = 314$  K or  $41^\circ\text{C}$ ):

$$\text{gel phase: } f_G = 0.9954; \text{ fluid phase: } f_F = 0.9518. \quad (3.2)$$

Our calculations, therefore, predict free volumes of 0.46% in the gel phase and 4.8% in the fluid phase, respectively, i. e., a change of 4.34% across the main phase transition. This value is in good agreement with the volume change of 4% found experimentally for DPPC bilayers.<sup>28</sup>

#### IV. DISCUSSION

In Sec. III, we predicted both a gel-fluid phase boundary and a fluid-vapor phase boundary for monolayers composed of lipids with short-range attractive polar head interactions of coupling constant  $K_0$ . The  $\Pi$ - $T$  phase diagrams for this situation are shown in Fig. 14 for different values of  $K_0$ . Figure 14 also shows that both phase boundaries meet at a triplet point  $P_2$ . The critical pressure at  $P_2$  decreases with increasing  $K_0$ . When  $K_0 = 0.245 \times 10^{-13}$  erg, the area per lipid chain  $A_{av}$  increases from 50–88  $\text{\AA}^2$  across the fluid-vapor phase boundary for  $T = 20^\circ\text{C}$  and  $\Pi = 1.18$  dyn/cm. The transition enthalpy is 0.8 kcal/mol. As  $K_0$  increases, the  $F \rightleftharpoons V$  transition becomes broader and becomes a  $F \rightleftharpoons SG$  transition (see Fig. 8). For example, for  $K_0 = 0.675 \times 10^{-13}$  erg, the phase transition is a  $F \rightleftharpoons SG$  transition for which  $A$  increases from 33  $\text{\AA}^2$  to 7300  $\text{\AA}^2$  across the phase boundary when  $T = 37^\circ\text{C}$  and  $\Pi = 0.02$  dyn/cm. The transition enthalpy is 5 kcal/mol. Note that fluid-vapor phase boundaries of this type do not occur in our model for  $K_0 = 0$ . We reiterate that the  $F \rightleftharpoons V$  and  $F \rightleftharpoons SG$  phase transitions analyzed in Sec. III are all transitions to a dimer vapor or gas.

Vapor-surface gas phase boundaries have been found for pure lipid monolayers by Gershfeld and Pagano<sup>29</sup> and Gershfeld and Tajima.<sup>30</sup> In particular, Gershfeld and Tajima observe  $LE \rightleftharpoons SG$  phase transitions for DMPC monolayers in the pressure range  $0.012 \text{ dyn/cm} \leq \Pi \leq 0.022 \text{ dyn/cm}$ . The corresponding temperature range is  $25.5^\circ\text{C} \leq T \leq 31^\circ\text{C}$ . The area per lipid chain increases from 115  $\text{\AA}^2$  across the  $LE \rightleftharpoons SG$  transition for  $\Pi = 0.012$  dyn/cm and  $T = 25.5^\circ\text{C}$ . Gershfeld and Tajima<sup>30</sup> make a detailed analysis of the enthalpy of transition and this shows that interactions between the PC polar heads are repulsive. Also, the maximum area per lipid chain in the LE state of the monolayer (115  $\text{\AA}^2$ ) is much higher than in the case of the  $F \rightleftharpoons V$  and  $F \rightleftharpoons SG$  phase transitions analyzed above. In consequence, the  $LE \rightleftharpoons SG$  transition found for DMPC is not

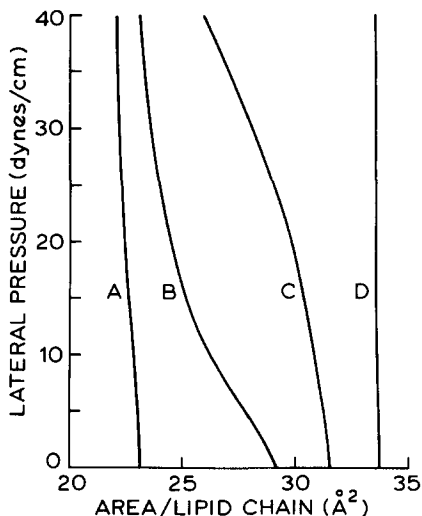


FIG. 13.  $\Pi$ - $A$  isotherms for  $K_0 = 5.0 \times 10^{-13}$  erg — A = 450 K; B = 475 K; C = 500 K; D = 700 K.



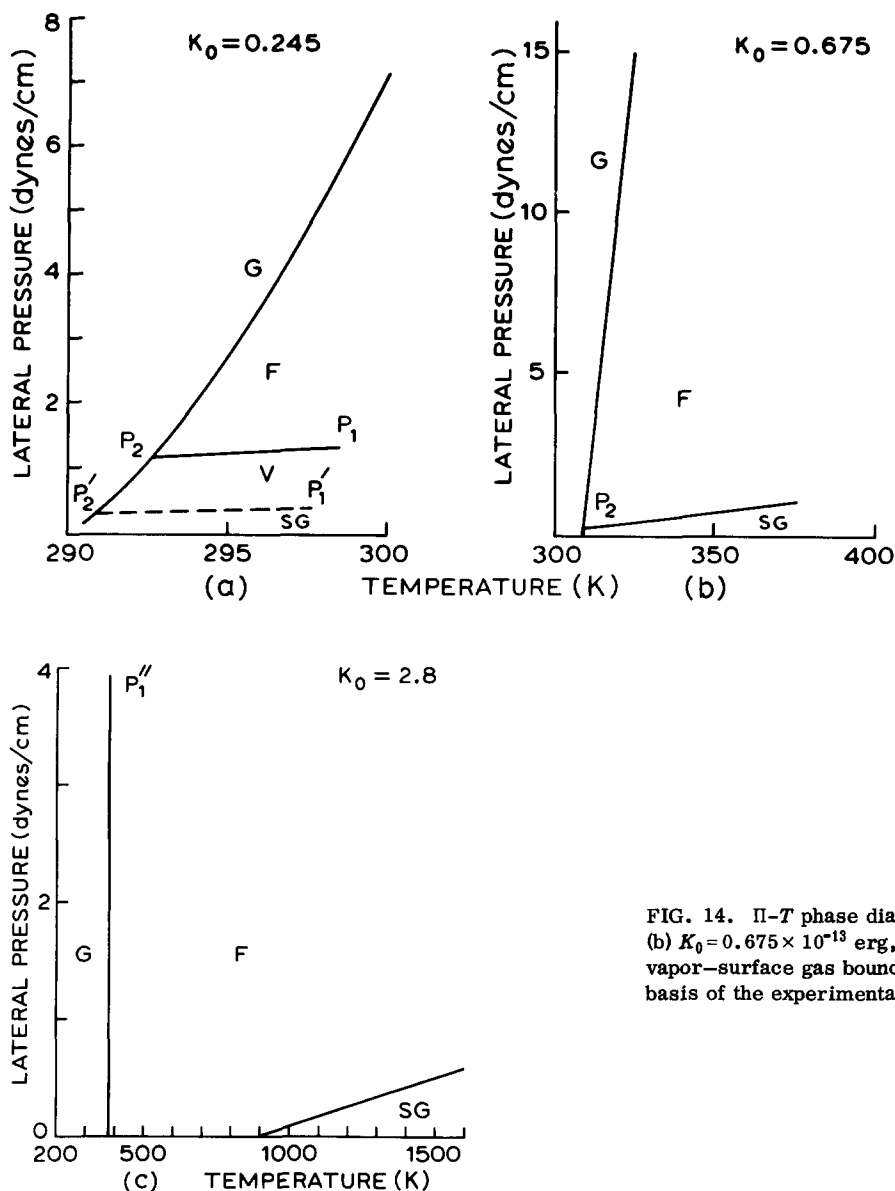


FIG. 14.  $\Pi$ - $T$  phase diagrams for (a)  $K_0 = 0.245 \times 10^{-13}$  erg, (b)  $K_0 = 0.675 \times 10^{-13}$  erg, and (c)  $K_0 = 2.8 \times 10^{-13}$  erg. The vapor-surface gas boundary ( $P'_2 P'_1$ ) in (a) is conjectured on the basis of the experimental results of Ref. 28.

the  $F \rightleftharpoons V$  transition of Sec. III. Instead, it is probably a  $V \rightleftharpoons SG$  transition in which the lipid molecules can "collapse" onto the air-water interface. Such a transition has been described by Firpo *et al.*<sup>31</sup> using the Landau theory.

We may therefore summarize the behavior of lipid monolayers as follows using the calculations of Sec. III together with the experimental results of Gershfeld and Tajima<sup>30</sup> for  $V \rightleftharpoons SG$  phase transitions in DMPC monolayers.

(i) When  $K_0$  is repulsive or attractive and less than  $0.2385 \times 10^{-13}$  erg, three phase boundaries occur; a gel-fluid phase boundary, a vapor-surface gas phase boundary, and a gel-surface gas boundary. This situation corresponds to the  $\Pi$ - $A$  diagram of Fig. 2 up to the vapor phase theoretically and to monolayers of lipids with PC polar heads experimentally.

(ii) When  $K_0$  is attractive and greater than, but close

in value to,  $0.2385 \times 10^{-13}$  erg, several phase boundaries are predicted to exist for the monolayer as shown in the  $\Pi$ - $T$  diagram of Fig. 14(a), in particular, a gel-fluid phase boundary, a fluid-vapor phase boundary at low pressures ( $\Pi \sim 1$  dyn/cm), and a vapor-surface gas phase boundary at very low pressures ( $\Pi \sim 0.01$  dyn/cm). This implies the presence of two triple points at low pressures, a  $G$ - $F$ - $V$  triple point, and a  $G$ - $F$ - $SG$  triple point.

(iii) Figure 8 shows that the  $F \rightleftharpoons V$  phase transition becomes quite broad for  $K_0 = 0.4 \times 10^{-13}$  erg. This implies that the  $F \rightleftharpoons SG$  phase transition and the  $V \rightleftharpoons SG$  transition of Ref. 30 may overlap in such a way that only a  $F \rightleftharpoons SG$  phase transition is seen.

(iv) When  $1.5 \times 10^{-13}$  erg  $\geq K_0 \geq 0.6 \times 10^{-13}$  erg, three phase boundaries are predicted, a gel-fluid phase boundary, a gel-surface gas phase boundary, and a fluid-surface gas phase boundary. These boundaries all meet at a  $G$ - $F$ - $SG$  triple point. This situation is

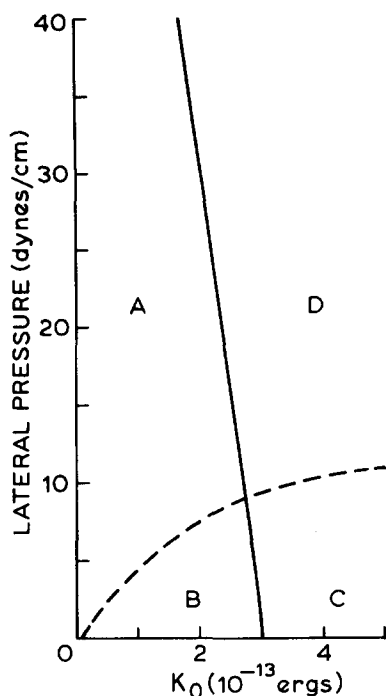


FIG. 15.  $\Pi$ - $K_0$  phase diagram: A—one phase transition ( $G \rightleftharpoons F$ ); B—two phase transitions [ $G \rightleftharpoons F$  and  $F \rightleftharpoons SG(V)$ ]; C—one phase transition ( $F \rightleftharpoons SG$ ); D—no phase transitions. The solid line and dashed lines show the variation of  $\Pi_c$  and  $\Pi_{av}$ , respectively, with  $K_0$ .

shown in the  $\Pi$ - $T$  phase diagram of Fig. 14(b) for  $K_0 = 0.675 \times 10^{-13}$  erg. The experimentally observed  $V \rightleftharpoons SG$  of Ref. 28 should, however, broaden the  $F \rightleftharpoons SG$  transition.

(v) When  $3.1 \times 10^{-13} \geq K_0 \geq 1.5 \times 10^{-13}$  erg, the  $G$ - $F$ - $SG$  triple point is absent and only two phase boundaries remain—a gel–fluid boundary and a fluid–surface gas boundary which do not meet. This situation is shown in Fig. 14(c) for  $K_0 = 2.8 \times 10^{-13}$  erg. Figure 14(c) also shows that the critical pressure  $\Pi_c$  for the  $G \rightleftharpoons F$  phase transition is now quite small.

(vi) When  $K_0 \geq 3.1 \times 10^{-13}$  erg, the  $G \rightleftharpoons F$  phase transition no longer occurs and only one phase boundary remains—the fluid–surface gas phase boundary.

(vii) It is important to note that the phase transition obtained for the two greater values of  $K_0$  used in this analysis are all predicted to occur at temperatures which are unrealistic for monolayers composed of lipids with palmitoyl chains. This situation may, however, be somewhat different for lipids with shorter acyl chains.

Figure 15 summarizes the phase behavior in terms of a  $\Pi$ - $K_0$  phase diagram which explicitly shows the region of  $K_0$  space in which  $F \rightleftharpoons G$  and  $F \rightleftharpoons V$  ( $SG$ ) phase transitions occur in the same monolayer.

Recent work by Albinet and Caillé<sup>32</sup> uses a spin-1 Ising model to obtain  $\Pi$ - $A$  isotherms for monolayers composed of amphiphilic molecules with one or two polar heads. The three states of the spin represent the

gel state of the molecule, the excited state of the molecule, and a vacancy, respectively. The authors obtain results for  $\Pi$ - $A$  isotherms which are similar to those shown in Fig. 2 in the case of molecules with a single polar head.

In a recent review article, Boggs<sup>33</sup> examines the nature of the interaction between polar heads of phospholipids in detail and reaches the following conclusions.

(i) Lipids with phosphatidyl choline (PC) polar heads at  $pH$  7 behave as if they were negatively charged, the positive charge on the choline group having no effect on the main bilayer phase transition temperature  $T_f$ .

(ii) Lipids with dipolar PE polar heads at  $pH$  7 and with dipolar PS (phosphatidyl serine) polar heads at  $pH$  2 each form bilayers with a higher value of  $T_f$  than for the same lipids with PC polar heads. Boggs<sup>33</sup> states that the most likely explanation is that the polar heads interact via intermolecular hydrogen bonding.

(iii) PA (phosphatidic acid) polar heads have two ionizable hydroxyl groups and can be electrically neutral at  $pH$  2, singly charged ( $PA^-$ ) at  $pH$  7 or doubly charged ( $PA^{2-}$ ) at  $pH$  11. The experimental results of Eibl and Blume<sup>34</sup> indicate that  $PA^-$  polar heads form hydrogen bonds, whereas  $PA^{2-}$  polar heads repel each other via Coulomb forces.

Lipids with dipolar PE or PS polar heads or with  $PA^-$  polar heads should therefore be candidates for the case  $K_0 > 0$  of Sec. III. However, since no experimental data are available for monolayers of such lipids at very low lateral pressures, we can, at best, examine the behavior of the  $\Pi$ - $A$  isotherms as the interaction between polar heads changes from repulsive to strongly attractive. The obvious systems are monolayers composed of lipids with PA polar heads [see (iii) above]. The interaction between the polar heads is repulsive at high  $pH$  ( $\geq 11$ ) and maximally attractive at  $pH$  4.<sup>34</sup> The results of Sec. III then predict that the  $\Pi$ - $A$  isotherms at high  $pH$  should have low pressure tails which spread to high areas per lipid chain,  $A_{av}$ , in this case (see Fig. 2). However, for  $pH \geq 4$ , the  $\Pi$ - $A$  isotherms should decrease very abruptly to values of  $A_{av}$  of the order of  $40 \text{ \AA}^2$  at low lateral pressures. The data of Albrecht *et al.*<sup>16</sup> show that this does indeed occur for DMPA monolayers (see Figs. 4 and 5 of Ref. 16). The  $\Pi$ - $A$  isotherms for DMPA monolayers at  $pH$  11.5 have the same qualitative behavior as shown in Fig. 2. In contrast, the  $\Pi$ - $A$  isotherms for DMPA monolayers at  $pH$  5.5 fall abruptly to values of  $A$  between  $36$  and  $42 \text{ \AA}^2$  at low lateral pressures in a similar manner to the isotherms in Fig. 8. A detailed experimental study of the behavior of  $\Pi$ - $A$  isotherms for DMPA monolayers at low lateral pressures as a function of  $pH$  would therefore be of great interest to us.

We have not examined the behavior of  $\Pi$ - $A$  isotherms of lipid monolayers as a function of acyl chain length. Qualitatively, the attractive van der Waal's interactions between acyl chains decrease in magnitude as  $M$  decreases. This would be equivalent to making the interactions between lipids more repulsive.

The formalism of Sec. II is sufficiently general to permit the analysis of several problems. We intend to analyze the effect of repulsive electrostatic interactions on the properties of lipid monolayers, the  $G \rightleftharpoons SG$  and  $V \rightleftharpoons SG$  transitions of Ref. 29 and mixed monolayers using this formalism.

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