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## **Continuous Phase Transitions and Orientational Ordering in Monolayers of Zwitterionic Amphiphilic Molecules at Fluid Interfaces**

By

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Experimental and theoretical evidence is presented which shows that orientational ordering of highly polar head groups in monolayers of amphiphilic molecules (zwitterionic 2 amino-tetradecanoic acid) at fluid interfaces produces a smooth continuous phase transition. Hence this supports the view that models which interpret the sharp 'kink' point phase transitions exhibited by phospholipid monolayers at oil/water interfaces as being purely due to reorientation are deficient in important respects and suggests that the model which is most likely to be applicable to lipids of biological interest at oil/water interfaces is a blend of chain melting and collective molecular orientational ordering.

### **1. Introduction**

Monolayers of amphiphilic molecules at fluid interfaces have long been studied, a motivation for which is that they provide a simplified model of the membranes of living cells [1]. However, despite much effort the molecular origin of the phase transitions which occur in monolayers of amphiphilic molecules at fluid interfaces is still uncertain. Spread monolayers of biologically important lipid molecules provide detailed physicochemical information in which surface pressure/area ( $\Pi/A$ ) isotherms may exhibit phase transitions. For an extensive review see [2]. There have been numerous proposals for the interpretation of such monolayer behaviour which are supported to varying degrees by approximate statistical mechanical treatments [2 to 7].

In this paper we make a detailed study of orientational ordering models and conclude that reorientation effects in themselves cannot provide an adequate description of lipid monolayer phase behaviour. In particular orientational ordering models have been invoked [3] to explain the 'kink point' continuous phase transitions (see Fig. 1) such as those which occur in monolayers of phospholipids at the oil/water interface. In disagreement with much earlier experimental evidence it now appears [8] that the phase transition in lipid monolayers formed at an air/water interface are simply first order while those which occur at an oil/water

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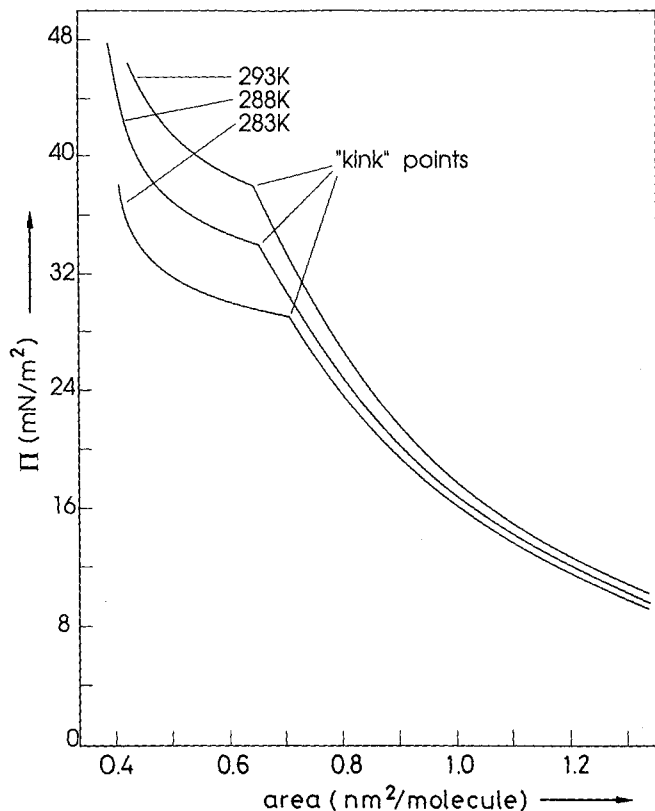


Fig. 1. Typical lipid monolayer surface pressure/area behaviour at oil/water interface (lecithin monolayers, sketched from [3]). Note that the isotherms are not flat above the "kink" point

interface may be continuous transitions. These later transitions exhibit the 'kink points' shown in Fig. 1 and hence our discussion will be focussed on the appropriateness of reorientation models for describing the higher order transitions which occur in phospholipid monolayers at an oil/water interface.

The essence of reorientation models is that the anisotropy of the intermolecular potential between pairs of amphiphilic molecules, largely due to the dipolar nature of the polar head group, causes collective alignment of the molecules in the monolayer as compression occurs analogous to the alignment of spins in a ferromagnetic crystal below the transition temperature. A detailed discussion of this is given in [9]. The first notable model of molecular reorientation in monolayers was presented long ago by Kirkwood [10]. Some time ago Bell, Mingins, and Taylor [3] (BMT) proposed a molecular reorientation model which was developed further by Dunne and Combs [9] and which appeared to account for the "kink" points (see Fig. 1) in the surface pressure/area isotherms of monolayers in molecular terms. The "kink" in reorientational models signifies the onset of a macroscopic molecular orientational alignment.

The apparent success of simple orientation models was questioned by Lavis et al. [11] using a real space renormalization group (RSRG) method applied to the model of BMT [3]. They concluded that the "kink" points present in the surface pressure/area isotherms

calculated using the mean-field method of BMT [3] are artefacts of the approximate statistical mechanical treatment since these features essentially disappeared when more accurate real space renormalization group calculations were undertaken. However, the question of the appropriateness of simple reorientation models for the “kink” points in the  $\Pi/A$  curves of monolayers remains since the RSRG calculations are approximate lattice model calculations. It has been known for a long time that lattice models of fluids [12] are crude because fluids do not have the discrete positional order appropriate to a lattice but rather have a continuous nature and so more accurate treatments are not feasible. Hence an experimental study of this question is desirable.

Reorientation effects are likely to dominate when the intermolecular potential is highly anisotropic and where the number of conformational degrees of freedom of the molecule is minimal. This situation is most nearly satisfied in the zwitterionic form of those short chain 2-amino aliphatic acids which form monolayers. The driving force for chain isomerization is the entropy of conformational disorder, which is reduced as far as possible in the shortest possible molecules which form monolayers. For these reasons we have investigated monolayer formation in the 2-amino aliphatic acids on aqueous substrates close to their isoelectric point. The shortest chain length compound which we found to form stable monolayers is 2-amino tetradecanoic acid,  $\text{CH}_3(\text{CH}_2)_{11}\text{CHCO}_2\text{H}\text{NH}_2$  which, as far as we can ascertain, most nearly satisfies the criteria of short chain length which minimizes conformational disorder, while the zwitterionic head group gives a very substantial anisotropy to the intermolecular potential. We have measured  $\Pi/A$  curves, estimated the intermolecular potential and its orientation dependence and undertaken mean field and RSRG statistical mechanical calculations of the surface pressure behaviour.

It is of considerable interest that neither the experimental results nor the RSRG calculations show evidence of “kinks” in the  $\Pi/A$  curve due to strong orientational ordering. We will conclude that an even-handed blend of orientational and isomerization effects is the most likely origin of the “kink” points at the liquid expanded/liquid condensed transition of phospholipid monolayers at oil/water interfaces.

## 2. Experimental

High purity samples of the amino acids  $\text{CH}_3(\text{CH}_2)_n\text{CHCO}_2\text{H}\text{NH}_2$ ,  $n = 2, 3, 6, 11$  were synthesized by standard methods [13] and the structures confirmed by high resolution NMR and mass spectral analyses. Attempts were made to produce monolayers of these compounds from freshly prepared benzene solutions spread on an aqueous subphase contained in a decontaminated Joyce-Loebl-Langmuir minitrough in a dust free environment. The only compound to form a stable monolayer was  $\text{CH}_3(\text{CH}_2)_{11}\text{CHCO}_2\text{H}\text{NH}_2$ .

The isoelectric point of the aliphatic amino acids is very close to pH 6 and hence the subphase pH was adjusted to this value [14]. As expected, the monolayers were the most condensed at the isoelectric point. Raising or lowering the acidity of the subphase causes expansion of the monolayer due to increased electrostatic repulsion between the polar head groups which, when the subphase pH is moved away from the isoelectric point, are not electrically neutral. Fig. 2 shows the measured surface pressure/area isotherm at the isoelectric point when the head groups are zwitterionic. It can be seen from the experimental  $\Pi/A$  curves that there is no evidence of distinct “kink” points or a liquid expanded/liquid condensed transition. The films collapse at the highest surface pressure which prevents investigation of the  $\Pi/A$  behaviour at high compression.

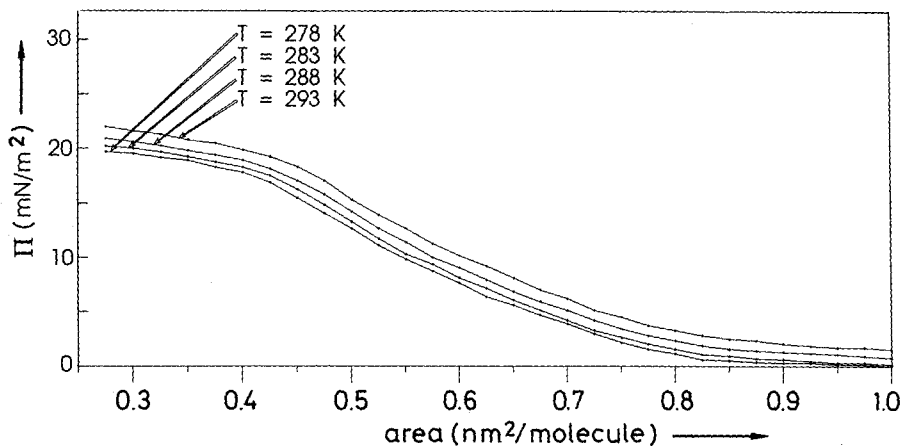


Fig. 2. Experimental surface pressure/area isotherms for a monolayer of zwitterionic 2-amino tetradecanoic acid on aqueous subphase at isoelectric point (pH 6). Note that the isotherms do not exhibit a sharp kink point

### 3. Reorientational Statistical Mechanical Calculations

Computations of surface pressure/area were made using the RSRG and BMT methods which has been previously described [3, 9, 11]. The essence of the model is as follows. Consider a monolayer of area  $A$  spread on an aqueous surface and having  $M$  aliphatic amino acid molecules. The surface pressure  $\Pi$  is the two-dimensional pressure of a plane lattice fluid in which the amino acid head group of each molecule is situated at one of the lattice sites. If  $N_s$  is the number of lattice sites and  $a_0$  the cross-sectional area of a molecule,

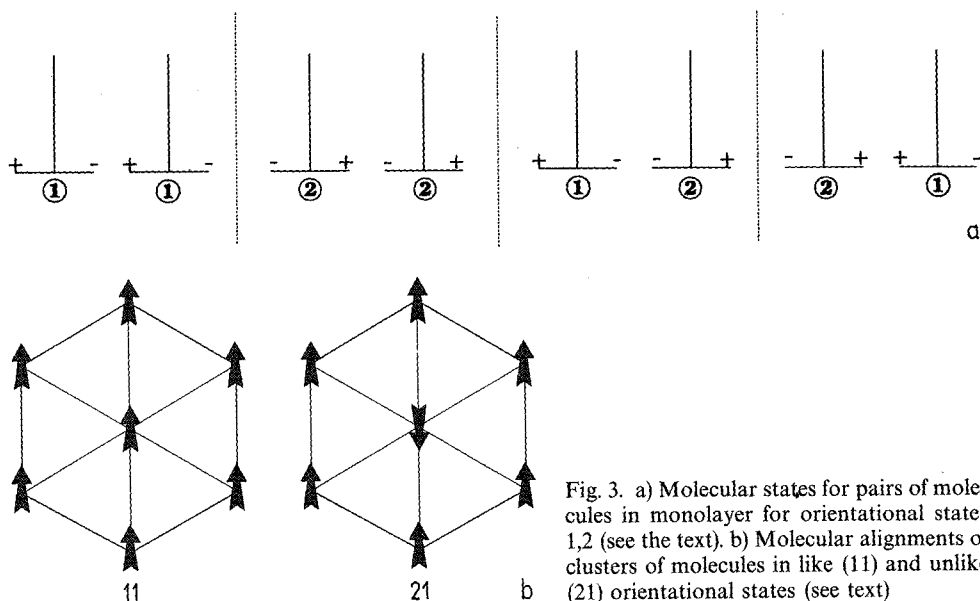


Fig. 3. a) Molecular states for pairs of molecules in monolayer for orientational states 1,2 (see the text). b) Molecular alignments of clusters of molecules in like (11) and unlike (21) orientational states (see text)

the monolayer area is  $N_s a_0$ . Assuming some lattice sites are vacant, then the area,  $a$ , per molecule is given by  $a_0 N_s / M$ . For a monolayer of fixed  $M$ ,  $N_s$  decreases as the density increases and at closest packing  $a = a_0$ .

The molecular states of the monolayer are dependent on the configurations shown in Fig. 3. Consider nearest-neighbour pairs of molecules each occupying one lattice site. Owing to steric and electrostatic interactions between the molecules, the interaction energy of pairs will depend on orientation.

Labeling the two orientations as 1 and 2, respectively, we see from Fig. 3a that 11 and 22 pairs are likely to be energetically favourable while 12 and 21 are energetically unfavourable. The energy is calculated by averaging over the interactions of the chain pairs in both 11 and 12 states; for the calculation an average of six positions on the triangular lattice is calculated as shown in the 11 and 21 energy states, Fig. 3b. The triangular lattice is the most favourable since it leads to the closest packing of the cylindrically shaped 2-amino tetradecanoic acid molecule.

RSRG and BMT (mean field) calculations of the  $\Pi/A$  behaviour were performed. The intermolecular interaction parameters in the lattice model were adjusted with a view to make the theoretical and experimental surface pressure isotherms coincide as closely as possible with the intention of comparing the intermolecular energies with theoretical values. The interaction parameters are shown in Table 1.

The cross-sectional area was taken to be  $0.30 \text{ nm}^2$ . For the reasons indicated below the  $\Pi/A$  curves have kink points for the mean field method but not for the RSRG calculations. Fig. 4 shows the calculated theoretical  $\Pi/A$  curves calculated using the RSRG method for the interaction parameters shown in Table 1. The general shape of the curves appears to be well reproduced by the RSRG calculations but not the less accurate mean-field method. In the RSRG case the behaviour of the isotherm at the transition is determined by the exponent  $y$  (see [11]) and the amplitude associated with the singular part of the compressibility. For a two-dimensional system, unless the amplitude is zero, the compressibility diverges at the transition, giving a zero slope to the isotherm, if  $y > 1$ . If  $y = 1$ , as is the case for any mean-field calculation like that of BMT [3], there is a discontinuity of slope in the isotherm. If  $y < 1$  the compressibility is dominated by its non-singular part and the isotherms will not necessarily indicate the presence of the phase transition by an anomaly of slope. In the present calculations (see [11] for details)  $y = 0.638$  and the amplitude of the singular part of the compressibility is also zero. This latter effect is a consequence of the weight function employed and can be removed by a change of weight function. It was, however, reported by Lavis et al. [11] that changes in weight function in no case yielded a

Table 1  
Interaction parameters for the 11 and 12 energy states (in  $10^3 \text{ eV}$ )

$T(\text{K})$	parameters	
	like (11)	unlike (12)
278	-6.42	6.23
283	-4.80	8.99
288	-4.10	10.12
293	-3.06	11.94
mean	-4.6	9.32

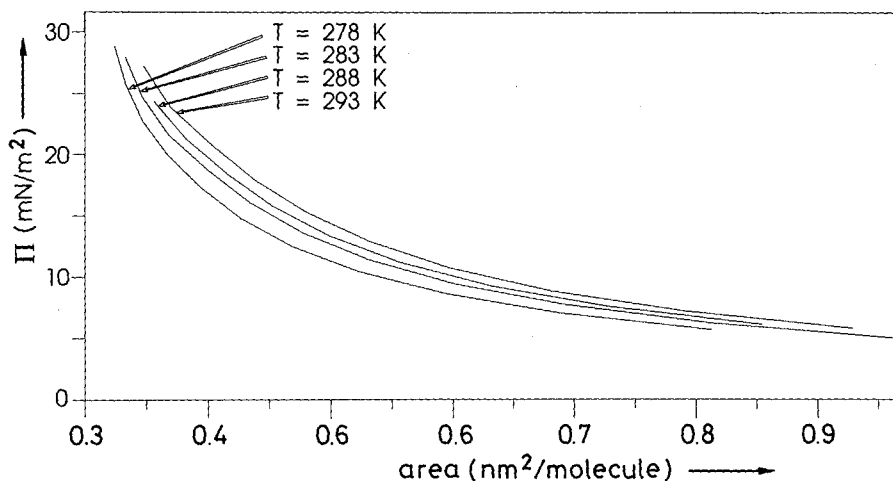


Fig. 4. Theoretical surface pressure/area isotherms calculated using real space renormalization group method (RSRG) for parameters discussed in the text. Note that the isotherms do not exhibit sharp kink points. However, (despite this) the calculations indicate the onset of collective molecular orientation at an area less than  $0.4 \text{ nm}^2/\text{molecule}$  in all cases

value of  $y > 1$ . Despite the fact that the theoretical isotherms do not exhibit sharp kink points the calculations indicate the onset of collective molecular orientation at an area less than  $0.4 \text{ nm}^2/\text{molecule}$  in all cases.

#### 4. Intermolecular Energies

The energies used to parameterize the real space renormalization group calculations were compared with estimates of intermolecular energies calculated with the extended atom-atom potential method of Gelin and Karplus [15] using standard geometries as a function of intermolecular separation with parallel chains. Accurate calculations for such complex systems are prohibitively difficult at present. The potential is given by

$$U(\{r_{ij}\}) = \sum_{i>j} \left[ \frac{A_{ij}}{r_{ij}^{12}} - \frac{C_{ij}}{r_{ij}^6} + \frac{q_i q_j}{r_{ij}} \right],$$

where  $r_{ij}$  is the interatomic separation and  $A_{ij}$ ,  $C_{ij}$  are the parameter values which are defined and given in [15].  $q_i$ ,  $q_j$  are the formal charges on the atoms of the zwitterionic head group which we take as  $+1$  for the  $\text{NH}_3^+$  and  $-0.5$  for each oxygen atom in  $\text{CO}_2^-$ .

Fig. 5 shows the calculated intermolecular potential energies for like and unlike pairs as a function of interchain separation. The substrate molecules which support the monolayer will solvate the head group region of the lipid molecules thereby providing a screening mechanism which will modify the intermolecular potential. We do not know of any simple and accurate way to account for such solvation effects. However, if the aqueous substrate is viewed as a continuous dielectric medium, then the role of the substrate will be to screen all interactions. There is considerable uncertainty in the literature as to what is an appropriate value for the short-range dielectric constant in aqueous media. Even less is known about

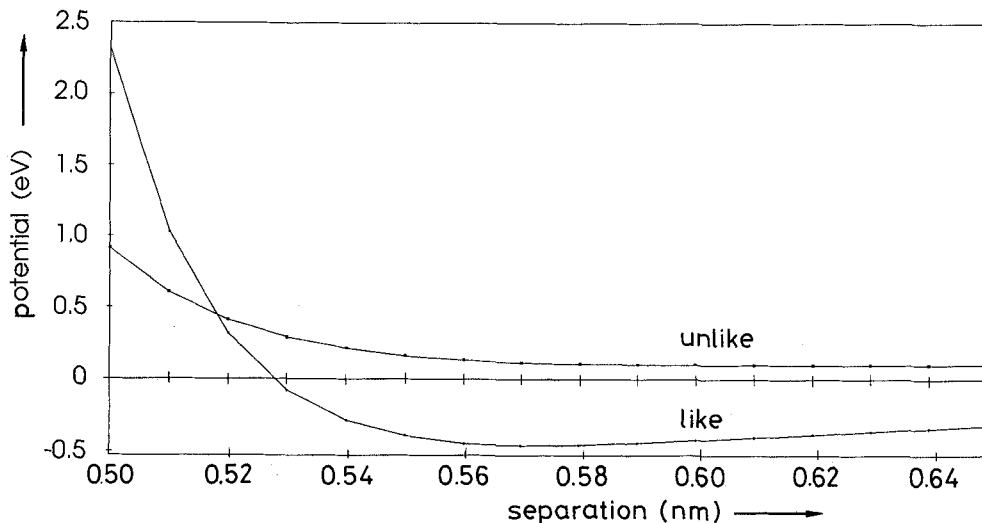


Fig. 5. Calculated intermolecular potential energies for like and unlike pairs of zwitterionic 2-amino tetradecanoic acid as a function of interchain separation

screening effects at interfaces. However, we can confidently expect a reduction of the unscreened interaction between charge distributions of one or two orders of magnitude.

The unscreened potential shown in Fig. 5 is strongly anisotropic and for this reason we believe that the experimental system consisting of monolayers of 2-amino tetradecanoic acid at the fluid interface is likely to be the best feasible test of reorientational models. A comparison of the potential energies shown in Fig. 5 at a separation of about 0.56 nm with those in Table 1 suggests that the substrate screening reduces the bare interaction by two orders of magnitude which is close to that expected since the static dielectric constant of liquid water is approximately 80.

## 5. Conclusion

The objective was to examine the appropriateness of simple reorientational models for describing the liquid expanded/liquid condensed 'kink' point transition in phospholipid monolayers. We must conclude that neither experimentally nor theoretically is there much justification for the view that collective molecular reorientation alone is the origin of such transitions in monolayers of biological interest such as lecithin as shown in Fig. 1. There is some reason to believe that reorientational effects and chain melting [2, 16, 17] may provide a satisfactory description of such a phospholipid monolayer. The reason for this optimism follows from our previous studies of the statistical mechanics of the orientable monomer-dimer model [2, 16, 17] which is a blend of chain melting and molecular orientation models. In particular the model exhibits very strong "kink" point transitions. However, no accurate results are available but mean-field theory studies indicate very promising qualitatively correct behaviour over a wide regime [16 to 18].

It may be possible to make some further progress with molecular dynamical studies although this would be a major undertaking. There is an urgent need for an accurate statistical mechanical treatment of such a model and work is under way to achieve this goal.

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