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SURFACE CHEMISTRY OF L-α-DIPALMITOYL LECITHIN AT THE AIR–WATER INTERFACE

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SUMMARY

1. The surface pressure–area curves of L-α-dipalmitoyl lecithin at the air–water interface were determined at 25°, 35° and 45°; the analysis of the curves and the thermodynamic parameters of the compression process suggest the existence of a phase transition in the monolayer, which could be explained through the action of Van der Waals forces acting between the methylene groups of the hydrocarbon chains of neighbour molecules.

2. The surface potential–area curves, as well as the magnitude of the surface dipole moment per molecule and its temperature dependence, are in accord with the molecular models previously suggested for lecithin monolayers with the positive and negative charges coplanar, in a plane parallel to the interface.

3. Surface viscosity and its temperature dependence were determined with a floating canal viscometer at 25° and 35°. The change in the slope of the plot of the logarithm of surface viscosity against surface pressure suggests the existence of different flow units below and above 12 dyne·cm^{-1}. The energy, and the free energy of viscous flow calculated for the interval 14–30 dyne·cm^{-1}, are lower than those calculated for the interval 1 to 12 dyne·cm^{-1}; the entropy of viscous flow at lower surface pressures is less negative than that corresponding to the interval 14–30 dyne·cm^{-1}, in accord with the suggestion of two different flow units.

4. The equilibrium spreading surface pressure shows a peculiar behaviour. No spreading occurs below 40°; at 45° spreading occurs attaining the equilibrium value after 2 h.

INTRODUCTION

Surface properties that can give information on the structure of lecithin monolayers and its interaction with the components of the subphase are chiefly the surface pressure, the surface potential, the surface dipole moment per molecule and the surface viscosity.

Previous studies on monolayers of synthetic dipalmitoyl lecithin spread at the air–water interface reported that surface pressure– and surface potential–area curves obtained in the presence of 0.02 M Na^+, K^+, Li^+, SO_4^{2-}, NO_3^- or Cl^- were identical[1]. Similar results have been reported for 0.01–0.1 M NaCl and KCl (see ref. 2), though

SHAH AND SCHULMAN[3] reported a slight expansion (5 $Å^2$ $mole^{-1}$) of the monolayer spread at the air–0.1 M NaCl or $CaCl_2$ interface.

The basic parameters for comparison, *i.e.* the properties of L-α-dipalmitoyl lecithin spread at the air–water interface, are not available in the literature though some properties of the dimyristoyl[4] and distearoyl[5] homologs in this interface have been described. The work reported here examines the surface pressure–area curves and the thermodynamic parameters for the compression process, the surface potential, the surface viscosity and the equilibrium spreading pressure of the synthetic lecithin: L-α-dipalmitoyl lecithin.

MATERIALS AND METHODS

Reagents

Chromatographic standard, L-α-dipalmitoyl lecithin was purchased from Mann Research Laboratories (New York). During the course of the investigation the sample was analyzed several times by thin-layer chromatography on Silicagel (Merck), with the solvent system chloroform–methanol–acetic acid–water to check its purity and stability: it gave consistently a single spot. The fatty acid composition was determined by gas–liquid chromatography which showed that palmitic acid comprised more than 96.4 % of the total fatty acids; the rest (up to 99.99 %) was formed by saturated C_{12} and C_{15} fatty acids in the proportions of 2.74 and 0.85, respectively.

The solvent used in the preparation of the spreading solutions was a mixture (2:8, v/v) of absolute ethanol and purified light petroleum ether (b.p. 35–65°); 0.2 ml of this solvent spread on 550 cm^2 of water produces a surface pressure less than 0.2 $dyne \cdot cm^{-1}$ when the surface was reduced to 15 cm^2. To minimize the influence of the solvent on the film properties, the concentration of the spreading solutions was such as to maintain the volume to be spread below 0.1 ml. This was measured with a Gilmont micropipet (± 0.1 μl). Deionized water, treated with Norit A, was distilled with alkaline permanganate and redistilled in all-glass apparatus. After air equilibration it gave consistently a pH value of 5.6–5.8 and a conductivity of $0.2 \cdot 10^{-6}\ \Omega^{-1} \cdot cm^{-1}$; Na^+ concentration, determined with a flame microspectrophotometer (Hitachi-Perkin Elmer M 139-0400-0251) was below 0.002 ppm.

Instruments

Surface pressure (Π)–area (A) isotherms were measured with an automatic recording film balance as previously described[6]. The balance could be operated at constant area (± 0.14 cm^2) or at constant surface pressure (± 0.1 $dyne \cdot cm^{-1}$); it was located in an air-conditioned room. Temperatures were maintained constant (± 0.2°) by circulating water from a constant-temperature bath and were measured with two thermometers located just below the water surface.

Surface potentials were measured with a General Radio DC amplifier and electrometer (1230 A) and an ^{241}Am air electrode located at 2.5 cm from the float and 2 mm on the water surface; the water in the trough was earthed with a Pt electrode.

Monolayer viscosities were determined by the method described by TRURNIT AND LAUER[7]. A teflon compression barrier with a transverse slit (0.16 cm × 2.56 cm) was substituted for the standard barrier. Monolayers were compressed to a particular surface pressure with the slit closed; the slit was opened and the monolayer was allowed to flow on the clean surface behind the barrier; the automatic constant pressure device was connected and the rate of compression was adjusted to maintain the surface pressures constant ($\pm$ 0.2 dyne·cm^{-1}). Thus, rates of monolayer flow were obtained at different constant surface pressures.

Equilibrium surface pressures were obtained by dropping the finely divided sample on 200 cm^2 of water surface. The procedure was to record the surface pressures as a function of time until Π became constant; the equilibrium spreading pressure (Π_e) was taken as that value of Π which remained constant ($\pm$ 0.2 dyne·cm^{-1}) for at least 90 min. Duplicate runs agreed to within 0.5 dyne·cm^{-1}.

Insolubility of the dipalmitoyl lecithin monolayer and effect of the rate of compression on Π–A curves

In order to check the insolubility of the dipalmitoyl lecithin monolayers at the experimental temperatures chosen (25–45°) the following tests were performed. The films were spread at large film areas ($\Pi = 0$) and a standard time of 5 min between spreading and compression was fixed arbitrarily. Compression curves were also obtained after 30 min of spreading. In both cases the compression curves coincided within $\pm$ 0.4 Å^2·molecule^{-1} indicating no dissolution of the dipalmitoyl lecithin monolayer at $\Pi = 0$.

A dipalmitoyl lecithin monolayer compressed in the standard conditions was decompressed and recompressed 3 times successively allowing 10 min to elapse between each complete cycle of compression and recompression. The coincidence of the values of each curve within $\pm$ 0.4 Å^2·molecule^{-1} was accepted as evidence of no dissolution of the monolayer. Finally, a monolayer was compressed under standard conditions to 20 dyne·cm^{-1} and left at this surface pressure for 15 min; no significant drop of surface pressure ($<$ 0.4 dyne·cm^{-1}) was observed indicating no dissolution at this constant surface pressure.

The rate of compression can affect some details in the Π–A curves[8]; monolayers of dipalmitoyl lecithin were compressed at 4, 8 and 12 Å^2·molecule^{-1}·min^{-1}. The general features of the curves obtained were similar; it was observed that at 8 Å^2·molecule^{-1}·min^{-1} the minimum dispersion ($\pm$ 0.1 Å^2·molecule^{-1}) was obtained. For that reason this rate of compression was adopted which allows one to obtain a Π–A curve in 8–9 min.

RESULTS

Π–A isotherms

Fig. 1 represents the Π–A isotherms obtained at 25°, 35° and 45°. Each curve was drawn on the basis of the average of eight isolated experiments. The standard error of the mean represented by the small horizontal segments every 5 dyne·cm^{-1}, was always between 0.25 and 0.50 Å^2·molecule^{-1}. Fig. 2 represents the compressibility $C_s = -(1/A)\,(\partial A/\partial \Pi)_T$ as a function of the surface pressure; the best straight lines were drawn through the experimental points. Table I represents the thermodynamic parameters calculated from the Π–A curves for the process:

$$\text{Initial state}_{(\Pi = 0,\, p,\, T)} \longrightarrow \text{Final state}_{(\Pi = x,\, p,\, T)}$$

Calculations were performed using the following equations[6,9,10]:

$$(\Delta G)_{pT} = \int_{\Pi=0}^{\Pi=x} A\,\mathrm{d}\Pi;\ \Delta H = \Delta G - T\Delta S$$

$$\Delta S_{p\Pi} = \left(\frac{\partial\gamma_{\mathrm{w}}}{\partial T}\right)_{p\Pi}(A_0 - A_x) - \left(\frac{\partial\Delta G}{\partial T}\right)_{p\Pi}$$

Here, γ_{w} is the surface tension of pure water, A_0 is the area per mole at $\Pi = 0$ and A_x the area per mole at $\Pi = x$, G and H are defined[11] by the equations $G = E - TS + pV - \gamma A$ and $H = E + pV - \gamma A$. The integrals $\int_{\Pi=0}^{\Pi=x} A\,\mathrm{d}\Pi$ were separately evaluated from the corresponding compression curves; the standard error of the mean of the

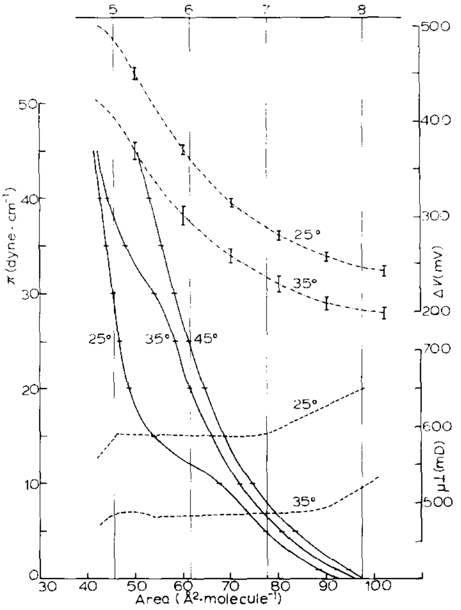

Fig. 1. Surface pressure–area (———), surface potential–area (–·–·–·) and surface dipole moment per molecule–area (-------) curves for L-α-dipalmitoyl lecithin at the air–water interface. Upper abscissa represents the hypothetical distance in Å between hydrocarbon chains of neighbour molecules.

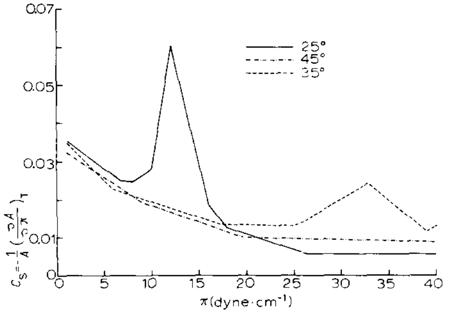

Fig. 2. Compressibility as a function of the surface pressure of L-α-dipalmitoyl lecithin at the air–water interface at 25°, 35° and 45°.

TABLE I

THERMODYNAMIC PARAMETERS FOR THE COMPRESSION PROCESS OF L-α-DIPALMITOYL LECITHIN AT THE AIR–WATER INTERFACE

Process: Initial state$(\Pi = 0, p, T) \longrightarrow$ Final state$(\Pi = x, p, T)$.

Interval (dyne·cm⁻¹)	*Temperature*	*ΔG (kcal·mole⁻¹)*	*−ΔH (kcal·mole⁻¹)*	*−ΔS (cal·degree⁻¹·mole⁻¹)*
0–40	25–35°	3.43	12.57	52.8
0–40	35–45°	3.74	6.47	32.6
0–10	25–35°	1.20	2.04	10.7
0–10	35–45°	1.24	1.67	9.3

ΔG values was always between 0.016 and 0.020 kcal·mole^{-1}. Values for $(\partial \gamma_w/\partial T)_p$ were obtained using the complete equation[12]

$$\gamma_w = 75.680 - 0.138\ t - 3{,}56 \cdot 10^{-4}\ t^2 + 4.7 \cdot 10^{-7}\ t^3$$

The findings may be summarized by the following: (a) Expansion of the monolayer by the effect of temperature produces quasi-parallel compression curves between 0 and 10 dyne·cm^{-1} for 25°, 35° and 45° and between 0 and 25 dyne·cm^{-1} for 35° and 45°. (b) Compression curves obtained at 25° and 35° show inflections at 11.6 and 32.6 dyne·cm^{-1}; no inflections were observed for the compression curves obtained at 45° between 0 and 45 dyne·cm^{-1}. (c) The plot of compressibility as a function of surface pressure exhibits sudden discontinuities at 25° and 35°: no discontinuity was observed at 45°. (d) For the compression process from $\Pi = 0$ to $\Pi = 40$ dyne·cm^{-1}, at a mean temperature of 30°, the dipalmitoyl lecithin monolayer absorbs (per mole) twice the heat necessary for the same process at a mean temperature of 40°. (e) The negative entropy change for this process at a mean temperature of 30° was approximately 1.5 times the entropy change for the process carried out at a mean temperature of 40°.

ΔV–A curves

In Fig. 1 are represented the ΔV values obtained at 25 and 35°; the standard error of the mean represented by the vertical segments every 10 Å^2·molecule^{-1} was always between ± 4 and ± 6 mV for the 25° curve. For the curves obtained at 35° and 45°, the maximum standard error was ± 18 mV because of the irregular water condensation on the electrode surface; for that reason only the 35° curve has been represented. Surface potential and surface pressure rise in an approximately parallel manner up to 46–48 Å^2·molecule^{-1} at 25° and up to 50–52 Å^2·molecule^{-1} at 35°; above these points there is a tendency for the surface potential curve to attain a plateau.

Surface dipole moment per molecule is defined by the equation[13]: $\mu_\perp = (A \Delta V)/(12 \cdot 3.14)$, in which A is the area per molecule in Å^2 and ΔV the surface potential in mV. Fig. 1 shows $\mu_\perp$ in milliDebye units (mD) as a function of the area per molecule (1 Debye unit = 10^{-18} esu·cm).

Surface viscosity

Fig. 3a represents the viscosity and Fig. 3b the rate of flow through the slit for dipalmitoyl lecithin monolayers at various surface pressure gradients at 25 and 35° obtained with the average of at least four separate experiments. Monolayer viscosities were calculated using the full formula of JOLY[14]:

$$Q = \frac{\Delta \Pi}{l(c\eta_0)} \left[w - 2\left(\frac{\eta_0}{c\eta_0}\right)^{\frac{1}{2}} \left\{ \tanh\left(\frac{c\eta_0}{\eta_s}\right)^{\frac{1}{2}} \cdot \frac{w}{2} \right\} \right]$$

in which Q is the rate of flow in cm^2·sec^{-1} of a monolayer through a slit of l cm length and w cm width by the action of a surface pressure gradient $\Delta\Pi$ dyne·cm^{-1}. The values of $(c\ \eta_0)$, in which c is a constant (cm^{-1}), and η_0 (the viscosity of water) were interpolated from the table given by JOLY. The expansion of the hyperbolic tangent term was carried to the x^9 term to check the validity of the approximation $(1 - x)^{-1} = 1 - x + x^2 - x^3 \ldots$

A factor (F) thus calculated corrects for the lack of slippage of the monolayer on the underlying water and was subtracted from the uncorrected viscosity. The final equation used was: $\eta_s = (\Delta\Pi w^3)/(12\,Ql) - F$ in which η_s is the viscosity of the monolayer in surface poises (g·sec^{-1}), w and l the width and length, respectively, of the slit (in cm), and Q the rate of flow of the monolayer (in cm^2·sec^{-1}); $\Delta\Pi$ is the surface pressure gradient in g·sec^{-2}. The order of magnitude of the correction factor F was 10^{-4} surface Poises, *i.e.* from one-third to one half of the uncorrected values[15]. Assuming that the same relationship between viscosity and temperature that applied to liquids in bulk also applies to monolayers, the energy of activation of viscous flow was calculated using the equation[16]: $\eta_s = C\cdot\exp(\Delta E_{\text{vis}}/RT)$ where C is a numerical constant, ΔE_{vis} the energy of activation for viscous flow, R the gas constant and T the absolute temperature. Free energy of activation of viscous flow was calculated using the equation of MOORE AND EYRING[17]: $\eta_s = h/A\cdot\exp\,(\Delta G/RT)$ where h is Planck's constant, A the cross-sectional area of the flow unit and ΔG the free energy

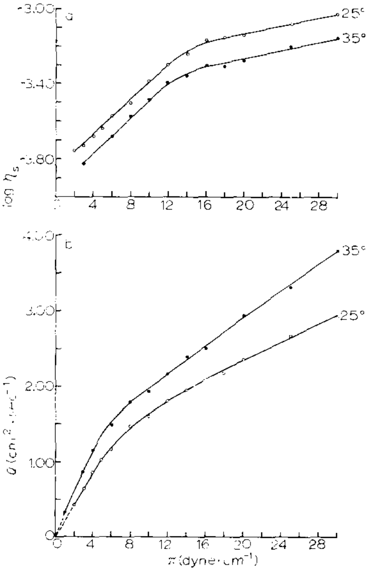

Fig. 3. Logarithm of the surface viscosity (a) and the rate of flow (b) as a function of the surface pressure for monolayers of L-α-dipalmitoyl lecithin at the air–water interface.

TABLE II

MEAN VALUES OF THE ENERGY FUNCTIONS OF VISCOUS FLOW OF L-α-DIPALMITOYL LECITHIN AT THE AIR–WATER INTERFACE

Temperature, 30°.

Interval (*dyne·cm*$^{-1}$)	ΔE_{vis} (*kcal·mole*$^{-1}$)	ΔG (*kcal·mole*$^{-1}$)	ΔH (*kcal·mole*$^{-1}$)	$-\Delta S$ (*cal·degree*$^{-1}$·*mole*$^{-1}$)
14–30	5.28 (± 0.07)	12.12 (± 0.02)	1.31 (± 0.19)	36 (± 0.6)
1–12	4.31 (± 0.07)	11.70 (± 0.06)	3.42 (± 0.25)	27 (± 1.0)

of activation for viscous flow. This equation is applicable to both Newtonian and non-Newtonian films; in the last case ΔG varies with the shear rate. Table II shows the values of the energy functions of viscous flow of dipalmitoyl lecithin for 14 to 30 and for 1 to 12 dyne·cm^{-1}.

The findings may be summarized by the following: (a) The low-pressure points on the plot of rate of flow of monolayer as a function of surface pressure extrapolate to the origin, indicating that monolayers of dipalmitoyl lecithin are liquid films[18] at 25°, and 35°. (b) Between 0 and 30 dyne·cm^{-1} the rate of flow increases with temperature. (c) From the lowest surface pressures investigated up to 30 dyne·cm^{-1} the logarithm of the surface viscosity is a linear function of the surface pressure, obeying the equation: $\log \eta_s = \log \eta_0 + k\Pi$ in which k is a constant (dyne^{-1}·cm). A net change in the slope of this function was observed between 12 and 14 dyne·cm^{-1}.

Equilibrium spreading pressure

At 25°, 35° and 40° no increment of the surface pressure was observed up to 4 h after dropping the finely divided sample on the water surface; experiments were discontinued after this period had elapsed.

At 42.5°, after an initial period of 10–12 min in which the surface pressure remains at zero value, there is an abrupt increase to 6–7 dyne·cm^{-1}. The surface pressure increases after that slowly until the equilibrium spreading pressure is attained at approximately 3 h. For 45° the surface pressure attains 25 dyne·cm^{-1} in the first 2 min, increasing after that slowly until the equilibrium spreading pressure is attained at approx. 2 h.

DISCUSSION

Various models have been proposed on theoretical and experimental grounds[3,19–21] for close-packed monolayers of saturated and unsaturated cephalins and lecithins considered as fully ionized charged pairs, as is the case at biological pH values or at pure water interfaces. For saturated lecithins the common characteristic of these models is a regular array with a lattice spacing[21] of 4.8 Å with each molecule occupying two lattice points; the most significant difference seen by considering the positive and negative charge of the polar moiety of the lecithin molecule in the same plane, which could be perpendicular[3] or parallel[19–21] to the interface.

The surface dipole moment per molecule of lecithin[21], and the results obtained by comparing the measured electrokinetic potentials[19,20] with those to be expected from these two orientations, strongly suggest that the positive and negative charges are coplanar in a plane parallel to the interface. The distance between the electric charges may be as large as 5 Å or as small as 2.5 Å depending on the degree of polarization of charge centers towards each other; in both cases the choline groups are readily accommodated beneath the hydrocarbon chains. Fig. 4 shows a schematic representation of the state of a dipalmitoyl lecithin monolayer at 9 dyne·cm^{-1} at 25° on this basis.

Van der Waals forces between methylene groups of long-chain hydrocarbons can be calculated using SALEM's[22] equation:

$$W_{\text{disp}} = A\,\frac{3\Pi}{8l}\cdot\frac{n}{d^5}$$

in which W_{disp} is the total interaction in kcal·mole^{-1} per pair of methylene groups; A is a constant (—1340·kcal mole^{-1}); l is the effective C–C- distance along the chain (1.253 Å), n the number of methylene pairs and D the distance between the axes of the hydrocarbon chains: the equation being valid for $d \geqslant 4$ Å. The energy of interaction becomes appreciable when d decreases below 7 Å and increases exponentially as the methylene chains approach each other.

Taking as 15 the number of methylene pairs that can be in close contact between the hydrocarbon chains of an isolated dipalmitoyl lecithin molecule and 4.17 Å the minimum distance between the axes of the chains, a total energy of interaction of —15 kcal·mole^{-1} is easily calculated. Since this is the energy per mole necessary for the formation of a stable complex, from 10 $\boldsymbol{k}T$ to 15 $\boldsymbol{k}T$ (ref. 23) (from —5.9 to —9.5 kcal·mole^{-1} for the interval 25–45°), it can be assumed that for a monolayer of dipalmitoyl lecithin the most energetically favoured configuration for each molecule would be that represented in Fig. 4.

Dipalmitoyl lecithin molecules at the air–water interface can also interact with each other; Table III shows the magnitude of the total interaction between neighbour molecules as a function of the distance d between two hydrocarbon chains of neigh-

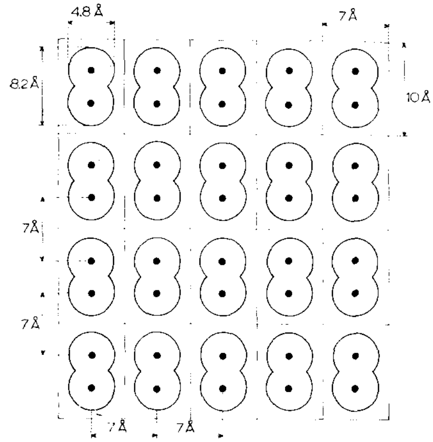

Fig. 4. Schematic representation of a L-α-dipalmitoyl lecithin monolayer at 9 dyne·cm^{-1} and 25°.

TABLE III

ENERGY OF INTERACTION BETWEEN HYDROCARBON CHAINS OF NEIGHBOUR MOLECULES OF L-α-DIPALMITOYL LECITHIN

d (distance between axes of hydrocarbon chains in Å)	*W_{disp} (for 15 CH_2 pairs in kcal·mole^{-1})*	*Area per molecule (Å^2)*
8	0.58	97.6
7	1.12	78.4
6	2.43	61.2
5	6.05	46.0
4.8	7.04	42.0
4.2	15.00	35.3

bour molecules of dipalmitoyl lecithin; on the basis of the repeating unit, the area per molecule at the monolayer has also been calculated for each d value.

The thermal energy for 25 to 45° is in the range of 0.59–0.63 kcal·mole^{-1}. It can be seen from Table III and Fig. 1 that when the distance between the axes of the hydrocarbon chains of neighbour molecules is reduced from 8 to 6 Å the energy of interaction, which was in the range of thermic energy at room temperatures, becomes 4 times this value. It is precisely in that part of the Π–A-curves that the inflection of the 25° curve was observed. The surface dipole moment per molecule which was slowly decreasing then became practically constant up to a d value of 5 Å.

It is interesting to point out here that the L-1,2-dimyristoyl-3-lecithin at the air–water interface exhibits a transition point for the temperature interval 1–12.3° which does not exist at 23.5° (ref. 4). The lower temperature at which this transition point disappears could be related to the shortest hydrocarbon chains existing in this substance compared with the dipalmitoyl lecithin; bearing in mind that the distearoyl homolog does not show any transition point in the Π–A curve at 25° (see ref. 5), the conclusion could then be drawn that other forces may also be operative in this interaction.

Thermodynamic parameters in Table I show that the compression of dipalmitoyl lecithin monolayer from 0 to 40 dyne·cm^{-1} at a temperature between 25 and 35° is associated with a decrease of entropy of 52.8 cal·degree^{-1}·mole^{-1}. For the same process carried out at a temperature between 35 and 45°, the entropy decrement is 32.6 cal·degree^{-1}·mole^{-1}. It can also be seen that for the same temperature interval, the compression from 0 to 10 dyne·cm^{-1} (*i.e.* from 100 Å^2·molecule^{-1} to 68 Å^2·molecule^{-1}, $d = 8$ to 6.5) the ΔS values differ by only 1.4 cal·degree^{-1}·mole^{-1} which is equivalent to saying that for the compression between 10 to 40 dyne·cm^{-1} the ΔS value at 25–35° is practically twice the corresponding value at 35–45°.

These observations seem to indicate that, in the interval 25–35° and for a d value between 6 and 7, there is an additional ordering factor besides the reduction in area per molecule. Similar reasoning can be adopted on the basis of ΔH values if we consider the spontaneous expansion process (at T and p constant) from 40 to 0 dyne·cm^{-1}; it can be seen that this process, at 25–35°, produces twice the heat evolved by the same process carried out at 35–45°. There is a striking coincidence between the difference of ΔH values ($12.57 - 6.47 = 6.10$ kcal·mole^{-1}) and the energy per mole released when Van der Waals forces—because of the increase of the distance between the axes of hydrocarbon chains—are no longer operative.

Surface potential is defined as the difference in potential between a "clean" aqueous surface and one covered with the monolayer; it is a complex quantity[24] representing the sum of the individual dipoles of the monolayer molecules and the water subphase dipoles. Insertion of a molecule at the air–water interface produces a displacement of water molecules and its reorientation about the dipoles of the molecules forming the monolayer.

Several factors can account for the variation of the surface dipole moment per molecule by the compression of a monolayer: first, the modification of the angle of tilt of the molecule; second, the modification of its intrinsic dipole moment; and third, any variation in the number, orientation or distribution of the water molecules oriented by the dipoles of the monolayer molecules. The magnitude of the isothermic variation of the surface dipole moment (Fig. 1) and its strong temperature dependence seem to point to these latter factors, though, being to some degree interdependent, the individual contributions cannot be separately evaluated.

It can be seen from Fig. 1 that at 25°, $\mu_\perp$ becomes constant ($\mu_\perp$ is approx. 590 mD) from 78 to 46 Å^2·molecule^{-1} ($\Delta d = 7$–5 Å) indicating no modification of the intrinsic surface dipole moment per molecule or of its interaction with the water molecules of the subphase; at 35° $\mu_\perp$ becomes constant ($\mu_\perp$ is approx. 480 mD) from 88–52 Å^2·molecule^{-1}. The decrement in its $\mu_\perp$ value and the shift towards lower surface molecular concentration could be interpreted in terms of the interaction of the dipalmitoyl lecithin molecule dipoles with water dipoles being diminished because of the effect of temperature on the dipolar distribution of water molecules.

Studies on palmitic acid spread on a 0.01 M acid subphase[25–28] have established the order of magnitude of its surface viscosity between 10^{-3} and 10^{-4} surface poises, and shown that the logarithm of the surface viscosity is a linear function of the surface pressure between 2 and 25 dyne·cm^{-1}; this monolayer acts as a two-dimensional Newtonian liquid (*i.e.* surface viscosity is independent of the rate of flow). On the other hand, the order of magnitude of the surface viscosity of cetyl alcohol monolayers[29,30] is between 10^{-1} (for surface pressures above 12 dyne·cm^{-1}) and 10^{-3} (for Π values of 2–4 dyne·cm^{-1}). For this substance the logarithm of the surface viscosity is not a linear function of the surface pressure; it varies rapidly between 2 and 12 dyne·cm^{-1}, becoming independent of the surface pressure from 12 to 30 dyne·cm^{-1}. At low values these films are Newtonian but when the surface pressure is increased above 6 dyne·cm^{-1} the viscosity shows a marked dependence upon the rate of shear.

The flow unit cannot, in either case, strictly be identified with the cross-sectional area of a single molecule of palmitic acid or cetyl alcohol. It may in fact be a complex of dimers, trimers *etc.*, or a complex formed by solvation of the polar groups[17]; the linearity of the plot of log η_s *vs.* Π could be interpreted as corresponding to a flow unit of constant composition flowing through the slit.

Fig. 3b shows that monolayers of dipalmitoyl lecithin at the air–water interface are liquid films[18], at low surface pressures, at 25 and 35°. In Fig. 3a it can be seen that the surface viscosity of dipalmitoyl lecithin monolayers is of the order of magnitude of 10^{-3}–10^{-4} surface poises and that the logarithm of the surface viscosity is in general a linear function of the surface pressure with a distinct change in the slope at 12 dyne·cm^{-1}, approximately, suggesting a possible change in the structure (and/or composition) of the flow unit at this surface pressure.

Each molecule in a monolayer can be considered as being in a potential well, each of these corresponds to a possible equilibrium position for the molecules. The energy of activation of viscous flow (ΔE_{vis}) is the energy that a molecule must acquire to pass over the potential barrier which separates it from another equilibrium condition. Energies of activation of viscous flow calculated for an average temperature of 30° gave two distinct values: for the interval 1–12 dyne·cm^{-1} the value of ΔE_{vis} is 4.31 ($\pm$ 0.07) kcal·mole^{-1} and for the interval 12–30 dyne·cm^{-1} the value of ΔE_{vis} is 5.28 ($\pm$ 0.07) kcal·mole^{-1}. This difference (approx. 1 kcal·mole^{-1}) is in accord with the suggestion, mentioned before, of a change in the characteristics of the flow unit.

L-α-Dipalmitoyl lecithin monolayers at the air–water interface can be considered, for given Π and T values, as a system of fixed charges, uniformly distributed. The fact that the molecular unit component of the monolayer varies its structure or composition at certain values of the surface pressure, *i.e.* for a certain value of the distance between the fixed charges, could be of significance with respect to the interaction of the molecular unit of the monolayer with the components of the subphase.

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REFERENCES

1 D. O. Shah and J. H. Schulman, *J. Lipid Res.*, 6 (1965) 341.
2 E. Rojas and J. M. Tobias, *Biochim. Biophys. Acta*, 94 (1965) 394.
3 D. O. Shah and J. H. Schulman, *J. Lipid Res.*, 8 (1967) 227.
4 D. A. Cadenhead, R. J. Demchak and M. C. Phillips, *Kolloid Z.*, 220 (1967) 59.
5 R. A. Demel, L. L. M. Van Deenen and B. A. Pethica, *Biochim. Biophys. Acta*, 135 (1967) 11.
6 F. Vilallonga, R. Altschul and M. S. Fernández, *Biochim. Biophys. Acta*, 135 (1967) 406.
7 H. Trurnit and W. Lauer, *Rev. Sci. Instr.*, 30 (1959) 975.
8 G. C. Nutting and W. D. Harkins, *J. Am. Chem. Soc.*, 61 (1939) 1180.
9 F. C. Goodrich, *Proc. 2nd Intern. Congr. Surface Activity, London, 1956*, Vol. I, Butterworths, London, 1957, p. 85.
10 F. Vilallonga, R. Altschul and M. S. Fernández, *Biochim. Biophys. Acta*, 135 (1967) 557.
11 E. A. Guggenheim, *Thermodynamics*, North-Holland, Amsterdam, 3rd Ed., 1957, p. 48.
12 W. D. Harkins and E. Alexander, in A. Weisberger, *Physical Methods of Organic Chemistry*, Vol. I, Part I, Interscience, New York, 1959, p. 772.
13 G. L. Gaines, Jr., *Insoluble Monolayers at Liquid–Gas Interfaces*, Interscience, New York, 1966, p. 190.
14 M. Joly, *Kolloid Z.*, 63 (1939) 26.
15 G. L. Gaines, Jr., *Insoluble Monolayers at Liquid–Gas Interfaces*, Interscience, New York, 1966, p. 95.
16 W. J. Moore, *Physical Chemistry*, Longmans, New York, 4th Ed., 1963, p. 190.
17 W. J. Moore and M. Eyring, *J. Chem. Phys.*, 6 (1938) 391.
18 R. J. Myers and W. D. Harkins, *J. Chem. Phys.*, 5 (1937) 601.
19 T. Hanai, D. A. Haydon and J. Taylor, *J. Gen. Physiol.*, 48 (1965) 59.
20 T. Hanai, D. A. Haydon and J. Taylor, *J. Theoret. Biol.*, 9 (1965) 278.
21 B. A. Pethica, *Soc. Chem. Ind. London, Monograph*, 19 (1965) 85.
22 L. Salem, *Can. J. Biochem. Physiol.*, 40 (1962) 1287.
23 R. B. Setlow and E. C. Pollard, *Molecular Biophysics*, Addison Westley, Reading, Mass., 1962, p. 157.
24 J. T. Davies and E. K. Rideal, *Interfacial Phenomena*, Academic Press, New York, 2nd Ed., 1963, p. 71.
25 G. C. Nutting and W. D. Harkins, *J. Am. Chem. Soc.*, 62 (1940) 3155.
26 M. Joly, *J. Chim. Phys.*, 44 (1947) 206.
27 M. Joly, *J. Colloid Sci.*, 11 (1956) 519.
28 E. Boyd and W. D. Harkins, *J. Am. Chem. Soc.*, 61 (1939) 1188.
29 N. L. Jarvis, *J. Phys. Chem.*, 69 (1965) 1789.
30 L. Fourt and W. D. Harkins, *J. Phys. Chem.*, 42 (1938) 897.