lecularly adsorbed D_2O was observed. Our results for the adsorption of D_2O on gadolinium are consistent with the conclusion that at 300 K water undergoes dissociative adsorption followed by oxide and surface hydroxyl formation.

Conclusions

At 300 K O_2, NO, N_2O, and D_2O were found to adsorb dissociatively on polycrystalline gadolinium. For O_2, NO, and N_2O, the oxide O(1s) peak was observed at a binding energy of around 530.5 eV. For O_2 and NO, high binding energy components attributed to OH were found near 530 and 533 eV, respectively. No hydroxyl peak was observed for exposures to N_2O. For D_2O,

peaks at 530.4 and 532.6 eV were found. The lower binding energy peak was assigned to the oxygen in Gd_2O_3, while the higher binding energy peak was attributed to hydroxyl formation. Further studies using UPS and programmed thermal desorption to study small oxygen-containing molecules on gadolinium and other lanthanide rare earths are in progress.

Spontaneous Assembly of a Phospholipid Bilayer as a Critical Phenomenon: Influence of Temperature, Composition, and Physical State

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Experimental conditions for the spontaneous assembly of isolated phospholipid bilayers have been obtained by a systematic thermodynamic study of aqueous lipid dispersions. The analysis utilizes the equilibrium air–water surface film to monitor the physical state of the dispersed lipid phase. At a critical temperature T^* the surface film and bulk dispersion simultaneously form the unilamellar state; this state forms only at T^*. The critical point is generally higher than T_m, the gel–liquid crystal transition temperature. Thus, the unilamellar state is always liquid crystalline. Examples are presented to show how T^* varies in multicomponent and multiphase systems in accordance with the phase rule. The relevance of the critical unilamellar state to membrane bilayer assembly is examined within the context that T^* for total lipid extracts of cell membranes is identical with the temperature at which the cells were grown.

Introduction

The lipids of plasma membranes are generally considered to be organized in a single bimolecular leaflet or "bilayer" with a radius of curvature comparable in magnitude to the size of a cell.[1] Although attempts to reconstruct this extraordinary biological structure indicate that a purely unilamellar state with a radius of curvature of cellular dimensions rarely forms spontaneously,[2] equilibrium conditions for the formation of this state have been found.[8-11] The conditions were obtained largely from studies in

which the properties of phospholipid dispersions in water were monitored by the equilibrium lipid films that form at the air–water surface.[12]

These studies have yielded a number of surprising results: Phospholipid dispersions consisting only of unilamellar vesicles with large radii $(1-10\ \mu m)$[8] and films of bilayer density in the air–water surface[9,10] with water permeabilities commensurate with a single bilayer[11] form spontaneously at a singularity in temperature, T^*. The unilamellar structure appears simultaneously in the dispersion and in the surface film but only at T^*.[8-11] At temperatures above and below T^* the properties of the film and the dispersion differ widely; multilamellar structures are observed in the dispersion,[8] and lipid concentration in the films falls below the bilayer density.[9,10] Thus, T^* exhibits the characteristics of a critical point where all the phospholipid in the dispersed phase and in the surface film spontaneously self-assembles to the unilamellar or single bilayer state. In the case of the surface film, transformation to the unilamellar state at T^* is manifested as a lambda point.[15]

(1) Gorter, E.; Grendel, F. *J. Exp. Med.* **1925**, *41*, 439. Danielli, J. F.; Davson, H. *J. Cell. Comp. Physiol.* **1935**, *5*, 495. Green, D. E. *Science* **1971**, *174*, 863. Singer, S. J.; Nicholson, G. L. *Science* **1972**, *175*, 720.

(2) Phospholipid dispersions generally contain multilamellar structures or "liposomes";[3] there is a distribution of the number of lamellae or bilayers per liposome particle including single bilayers as unilamellar vesicles.[4] Dispersions containing unilamellar vesicles may also be prepared by use of either sonication[5] or organic solvents[6] or with other lipids or detergents not normally present in cells;[7] these states form under unnatural conditions, and therefore their significance for understanding bilayer assembly processes in cells is obscure.

(3) Bangham, A. D. *Prog. Biophys. Mol. Biol.* **1968**, *18*, 29. Pagano, R. E.; Weinstein, J. N. *Annu. Rev. Biophys. Bioeng.* **1978**, *7*, 435.

(4) Servuss, R. M.; Harich, W.; Helfrich, W. *Biochim. Biophys. Acta* **1976**, *43*, 900.

(5) Huang, C. *Biochemistry* **1969**, *8*, 344.

(6) Batzri, S.; Korn, E. D. *Biochim. Biophys. Acta* **1973**, *298*, 1015.

(7) Gabriel, N. E.; Roberts, M. F. *Biochemistry* **1985**, *23*, 4011.

(8) Gershfeld, N. L.; Stevens, Jr., W. F.; Nossal, R. J. *Faraday Discuss. Chem. Soc.* **1986**, *81*, 19.

(9) Gershfeld, N. L.; Tajima, K. *Nature (London)* **1979**, *279*, 708.

(10) Tajima, K.; Gershfeld, N. L. *Biophys. J.* **1985**, *47*, 203.

(11) Ginsberg, L.; Gershfeld, N. L. *Biophys. J.* **1985**, *47*, 211.

(12) Membrane phospholipids are generally poorly soluble in water, and it is not feasible to develop a thermodynamic model of the lipid dispersions based on conventional solution properties. In principle, the thermodynamic properties of the dispersion may be rigorously expressed in terms of the more easily measured properties of the equilibrium surface film.[13-15]

(13) Defay, R.; Prigogine, I.; Bellemans, A.; Everett, D. H. *Surface Tension and Adsorption*; Wiley: New York, 1966.

(14) Gershfeld, N. L. *Annu. Rev. Phys. Chem.* **1976**, *27*, 349.

(15) Gershfeld, N. L. In *Cell Surface Dynamics*; Perelson, A. S., DeLisi, C., Weigel, F. W., Eds.; Marcel Dekker: New York, 1984; Chapter 4, pp 111-141.

The formation of the unilamellar state in the bulk dispersion also appears as a higher order transformation, as exemplified by our observations with dispersions of an ionized phospholipid:[8] At temperatures below T^* a three-dimensional jellylike state forms which appears to consist of a matrix of extended bilayer sheets; at T^* the jelly phase transforms to unilamellar vesicles, and at temperatures above T^* typical multilamellar liposomes are observed. The reversible transformations are of higher order because they do not exhibit a latent heat.[8] Aqueous dispersions of neutral phospholipids such as the lecithins also exhibit a critical temperature for the formation of the unilamellar state,[9-11] although the morphological transformations are not as obvious as those observed with ionized lipids.

The initial studies of these systems were devoted largely to establishing that dispersions containing only large unilamellar vesicles can form spontaneously under equilibrium conditions.[8-11] These studies are now extended to show how the assembly of the unilamellar state at T^* generally may be identified from the surface film properties. Included in the study is a thermodynamic analysis of the equilibrium conditions for the assembly of the unilamellar state and examples of how T^*, in accordance with the phase rule, depends on lipid composition in multiphase and multicomponent systems. A concluding discussion analyzes the properties of the critical unilamellar state as they relate to the conditions for assembly of the lipid bilayer in cell membranes; in growing cells T^* is identical with the growth temperature.[16]

Materials and Methods

Dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), and dioleoylphosphatidylcholine (DOPC) (Avanti Polar Lipids, Birmingham, AL) with a purity of >99% by thin-layer chromatography were used without further purification.

A Perkin-Elmer DSC Model II scanning calorimeter was used to obtain bulk-phase diagrams of the lipids dispersed in water.

The method employed in this study utilizes the principle that the thermodynamic properties of the dispersions may be rigorously expressed in terms of the more easily measured properties of the equilibrium air–water surface film.[13-15] Surface pressures were measured by a horizontal-float film balance enclosed in a constant-temperature chamber described previously.[9,10,16] Precision of surface pressures was ±0.3 mN/m; temperatures were maintained ±0.1 °C.

Results and Discussion

Critical Conditions for the Formation of the Unilamellar State. When phospholipids are gently dispersed in a large excess volume of water, the bulk of the lipid imbibes a limited amount of the water to form a mesomorphic state consisting of a stacked array of bilayers. These arrays may exist either as crystalline (gel) or liquid crystalline phases; the transformation between the two phases exhibits a first-order transition temperature T_m and a latent heat.[17] Some of the lipid also adsorbs to the air–water surface, and some dissolves in the continuous aqueous phase. Since bilayer-forming phospholipids are generally poorly soluble in water, this section examines only the equilibrium between the dispersed phase and the air–water surface. The phase diagrams of phospholipid dispersions are described by the temperature dependence of the surface pressure Π measured at the equilibrium air–water surface. The thermodynamic relationships that define the critical conditions for assembly of the unilamellar state are examined first in an aqueous dispersion that contains two lipids, DMPC and DOPC; this is followed by an extension of this analysis to multiple components and phases.

Typical Π–T phase diagrams for mixtures of DMPC and DOPC (open symbols) and for each pure component (closed symbols) as dispersions in water are shown in Figure 1.   A maximum in the surface pressure is found at a characteristic
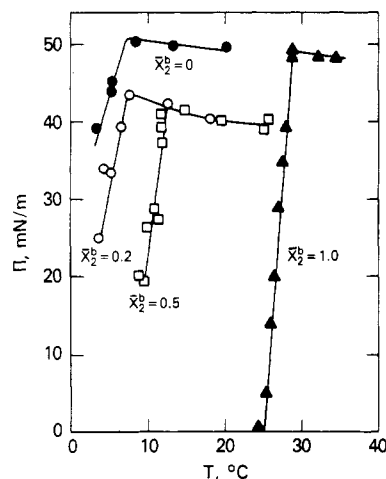

Figure 1. Surface pressure (Π)–temperature phase diagrams for DMPC, DOPC, and their mixtures. $\bar{X}_2^b$ refers to DMPC in the dispersion. $\bar{X}_2^b + \bar{X}_3^b = 1$ (see ref 18).
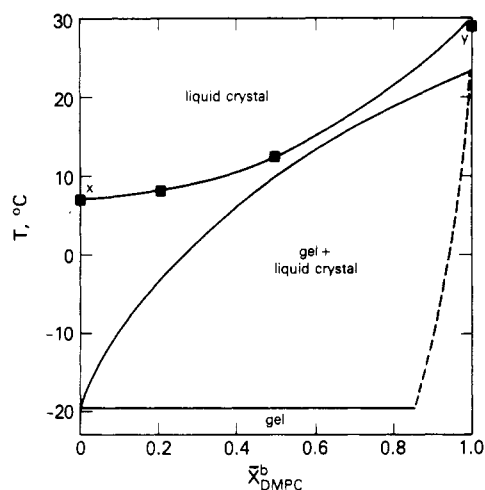

Figure 2. Temperature–composition[18] phase diagram for aqueous dispersions of DMPC–DOPC mixtures. Line xy is the T^*–composition relation with points obtained from the surface pressure maxima in Figure 1.

temperature T^* for each composition.[18] The temperature–composition bulk-phase diagram obtained by differential scanning calorimetry for the dispersions is given in Figure 2, where the domains representing the gel and liquid crystal phases are identified. Values of T^* obtained from Figure 1 fall along the line xy in Figure 2.

The surface pressure maxima in Figure 1 occur at temperatures where the bulk phases are liquid crystalline (Figure 2). The following analysis shows that T^* represents the critical temperature for the formation of the unilamellar state. We begin with the expressions for describing the Π–T phase diagram.

For a liquid crystalline aqueous dispersion containing two phospholipids, the Gibbs equation relates the surface pressure Π to the intensive variables T and μ_i

$$d\Pi = S^\sigma \, dT + \Gamma_1 \, d\mu_1 + \Gamma_2 \, d\mu_2 + \Gamma_3 \, d\mu_3 \qquad (1)$$

where μ_i is the chemical potential of component i with the subscripts 1, 2, and 3 referring to water and to the two lipids, respectively, in the dispersion, S^σ is the entropy per square centimeter of the surface phase, T is the absolute temperature, and Γ_i is the

(16) Gershfeld, N. L. *Biophys. J.* **1986**, *50*, 457.

(17) Small, D. M. *Handbook of Lipid Research*; Plenum Press: New York, 1986; Vol. 4.

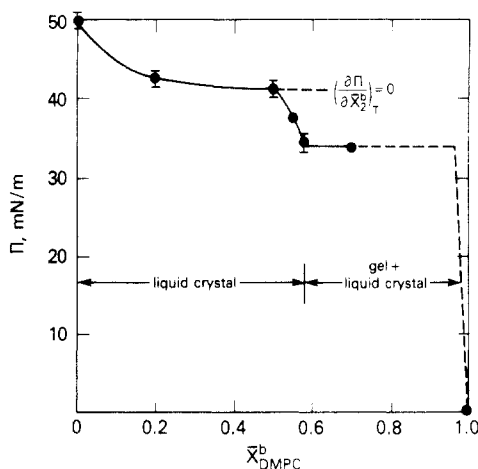(18) Lipid compositions of bilayers in dispersions are generally represented as mole fractions X_i^b where $\sum X_i^b = 1$. However, the water content (X_1^b) of liposomes and bilayers will vary with lipid composition. To simplify representation of the phase diagrams, a new variable $\bar{X}_i^b$ is defined which expresses the bilayer composition in terms of only the lipid components. Thus, for two lipids dispersed in water $\bar{X}_2^b + \bar{X}_3^b = 1$. Usage of this new composition variable does not influence the general conclusions of this study; see ref 20.

Figure 3. Dependence of surface pressure on phospholipid composition[18] in dispersions containing mixtures of DMPC and DOPC, at 12 °C. Error bars indicate the range for duplicate runs. The dispersions are homogeneous liquid crystals up to $\bar{X}_{DMPC}^{b} = 0.58$; in this concentration range the curve is monotonic and shows a horizontal inflection with zero slope at $\bar{X}_{DMPC}^{b} = 0.5$. When $\bar{X}_{DMPC}^{b} > 0.58$, the dispersion contains both gel and liquid crystal; see Figure 2 for complete phase diagram.

surface concentration (mol/cm^2). The Gibbs–Duhem equation gives the relationship among the intensive variables that define the equilibrium bulk lipid phase; thus, at constant (atmospheric) pressure

$$S^b \, dT + c_1 \, d\mu_1 + c_2 \, d\mu_2 + c_3 \, d\mu_3 = 0 \qquad (2)$$

where S^b is the entropy per cubic centimeter of the bulk lipid phase and c_i is the concentration (mol/cm^3) of component i in this bulk liquid crystalline phase. Since the chemical potential is the same in each equilibrium phase, $d\mu_1$ may be eliminated from eq 1 and 2, yielding

$$d\Pi = (S^\sigma - S^b\Gamma_1/c_1) \, dT + (\Gamma_2 - \Gamma_1 c_2/c_1) \, d\mu_2 +$$
$$(\Gamma_3 - \Gamma_1 c_3/c_1) \, d\mu_3 \quad (3)$$

We show now that at T^*, when $(\partial\Pi/\partial T)_{\mu_i} = 0$, a unique condition exists where all the coefficients of the variables in eq 3 equal zero. Consider, for example, the equimolar mixture which shows a surface pressure maximum at $T^* = 12$ °C (Figure 1). The dependence of the surface pressure on lipid composition at this temperature is presented in Figure 3; two significant features of the phase relations are noteworthy. For $\bar{X}_2^b > 0.58$ the phase diagram indicates that both gel and liquid crystal phases coexist, characterized by a discontinuity in $(\partial\Pi/\partial\bar{X}_2^b)_T$. The constant value for Π at $\bar{X}_2^b > 0.58$ signifies that the composition of each phase remains constant, although the relative amounts of gel and liquid crystal will vary along this line.[19]

For compositions of $\bar{X}_2^b < 0.58$ only the liquid crystal phase is present in the dispersion, and therefore $(\partial\Pi/\partial\bar{X}_2^b)_T$ must be continuous throughout this composition range. At $\bar{X}_2^b = 0.5$ the Π–$\bar{X}_2^b$ relation exhibits a point of inflection with zero slope. At this point $(\partial\Pi/\partial\mu_2)_T = (\partial\Pi/\partial\mu_3)_T = (\partial\Pi/\partial T)_{\mu_i} = 0$,[20] and all the coefficients of the variables in eq 3 are zero, yielding

$$S^\sigma/S^b = \Gamma_1/c_1 = \Gamma_2/c_2 = \Gamma_3/c_3 \qquad (4)$$

Writing the surface and bulk compositions in terms of the mole fractions of each component in the surface X_i^σ and liquid crystal X_i^b phases,[18]

$$X_i^\sigma = X_i^b \qquad i = 1, 2, 3 \qquad (5)$$

Introducing partial molar entropies $S^\sigma = \sum\Gamma_i\bar{S}_i^\sigma$ and $S^b = \sum c_i\bar{S}_i^b$ for the surface and liquid crystalline phases, respectively, in eq 4, i.e., $S^\sigma/S^b = \Gamma_i/c_i$, yields

$$\bar{S}_i^\sigma = \bar{S}_i^b \qquad i = 1, 2, 3 \qquad (6)$$

Equation 5 has been verified with radiotracer studies of the surface film.[10] A radiotracer for each lipid component was used to obtain the individual concentrations of each lipid in the surface film at T^*. For the equimolar dispersion of DMPC and DOPC the surface film was shown to contain equimolar amounts of the phospholipids; at temperatures above and below T^* the surface film composition was found to be significantly different than in the dispersion.

The surface film and dispersed phases are at equilibrium; therefore, the chemical potential for each component is uniform throughout the entire system. With partial molar entropies, and hence partial molar heat contents, also identical (eq 6), the surface film and bulk lipid must be in identical states.

The radiotracer studies also show that at T^* the density of the surface film is the same as an isolated, single bilayer which we have called the "surface bilayer".[9,10] Since the states contained in the surface and bulk phases are identical, the bilayer in the dispersion must also be unilamellar; this deduction has been verified experimentally.[8] Thus, T^* represents a critical point where the entire lipid system, surface and bulk, becomes uniform and forms the unilamellar state throughout.

For multicomponent systems such as biomembrane lipid mixtures containing C components, at the surface pressure maximum

$$(\partial\Pi/\partial T)_{\mu_i} = (S^\sigma/\Gamma_1 - S^b/c_1) = 0 \qquad i = 2, ..., C \quad (7)$$

When the critical point is attained, $\bar{S}_i^\sigma = \bar{S}_i^b$, $X_i^\sigma = X_i^b$, and the surface pressure maximum (eq 7) will denote T^*, independent of the number of components in the dispersion.

Surface pressure maxima have indeed been observed for dispersions of membrane lipid extracts which contain several hundred lipid components; for each of these natural lipid mixtures T^* has been directly associated with the assembly of the unilamellar state in biomembranes.[16] On the basis of these studies with single, binary, and biomembrane lipid dispersions, we use the surface pressure maximum to identify the critical point.

In dispersions it is often found that the lipids form two coexisting phases, the crystalline or gel as well as the liquid crystalline state. How to find T^* in the case of two coexisting lipid phases is readily specified by application of the phase rule. A discussion of the phase rule as it applies to the unilamellar critical state and some examples to illustrate principles of particular importance for understanding phase relations in membranes are presented in the following section.

The Critical Temperature T^* in Multicomponent and Multiphase Systems. We now examine the general case of multicomponent lipid dispersions in water in which the lipid exists either in a single homogeneous phase or in two coexisting phases, gel and liquid crystal. The number of independent intensive variables F for a system containing P phases and C components is $F = C - P + 2$. However, there are $C - 1$ additional conditions that must be satisfied at T^*, namely, the relationships between the surface and bulk compositions given in eq 5. These $C - 1$ conditions reduce the number of intensive variables that must be specified to describe the dispersion at T^*. The phase rule then becomes

$$F^* = 3 - P \qquad (8)$$

This form of the phase rule indicates that, among the F independent variables, at T^* only $3 - P$ may be chosen arbitrarily, regardless of the number of components $(C > 3)$ present. Only two conditions need consideration: when $P = 2$, with coexisting phases liquid crystal (or gel) and water, or when $P = 3$, with coexisting phases gel, liquid crystal, and water. The two situations are illustrated by the following examples.

(A) Homogeneous Liquid Crystalline Phase at $T = T^*$; $P = 2$, $F^* = 1$. According to the phase rule, one intensive variable
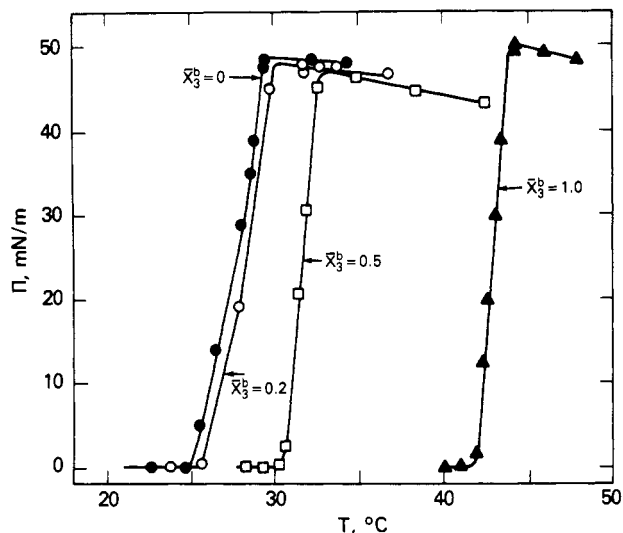
(19) For other examples of two coexisting lipid phases exhibiting a "flat" discontinuity in the surface pressure–composition phase diagram, see ref 15. In each example, with constant temperature and atmospheric pressure the phase rule indicates that there are no independent variables; hence, Π does not vary with composition when a "flat" discontinuity is seen.

(20) Using the identity $(\partial\Pi/\partial\bar{X}_2^b) = -(\partial\Pi/\partial\bar{X}_3^b) = (\partial\Pi/\partial\mu_2)(\partial\mu_2/\partial X_2^b) \cdot (\partial X_2^b/\partial\bar{X}_2^b)$; by inspection, $(\partial\Pi/\partial\mu_2) = 0$, when $(\partial\Pi/\partial\bar{X}_2^b) = 0$. See ref 18 for distinction between X_2^σ and $\bar{X}_2^b$.

(21) Mabrey, S.; Sturtevant, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 3862.

(22) Lentz, B.; Barenholz, Y.; Thompson, T. E. *Biochemistry* **1976**, *15*, 4529.

Figure 4. Surface pressure–temperature relations for DMPC and DPPC mixtures dispersed in water. $\bar{X}_3^b$ refers to DPPC in the dispersion.[18]
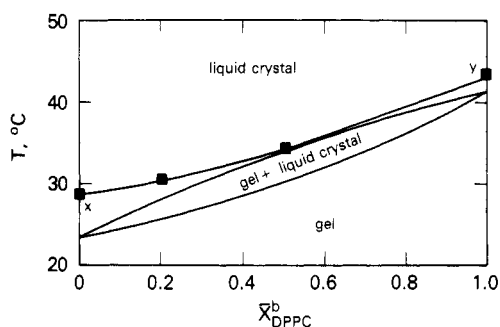

Figure 5. Temperature–composition phase diagram for mixtures of DMPC and DPPC.[21] Line xy gives the T^*–composition[18] relation; the points were obtained from the surface pressure maxima in Figure 4.

Figure 6. Surface pressure–temperature relations for DPPC and DOPC mixtures dispersed in water. $\bar{X}_2^b$ refers to DPPC in the dispersion.[18]

Figure 7. Temperature–composition phase diagram for mixtures of DOPC–DPPC dispersions.[22] The lines AB and CD indicate the T^*–composition[18] relation. The points were obtained from the surface pressure maxima in Figure 6. Location of point C is approximate, assuming that the T^*–$\bar{X}_2^b$ relation would be monotonic as in Figures 2 and 5. See text for full description of phase diagram.

may be arbitrarily selected to fix the conditions for the unilamellar critical state. If temperature is chosen, with composition of the dispersion kept constant, T^* may then be represented as a function of this composition by isolated points that fall on a line. As an example consider the binary lipid dispersions containing mixtures of DOPC and DMPC. The phases formed by these components in the dispersion are shown in the temperature–composition diagram in Figure 2. T^* taken from the maxima in Figure 1 as a function of composition is shown as the line xy in Figure 2. Since the T^*–composition phase line lies in the domain where the lipids are always in the liquid crystalline state, the critical unilamellar state along xy is therefore also liquid crystalline. For this example, once $\bar{X}_i^b$ is chosen T^* is fixed according to the relationship that exists along line xy. Similar phase behavior is observed with dispersions of DMPC–DPPC mixtures shown in Figures 4 and 5.

If composition of the dispersion is chosen as the independent variable, then for a given temperature there may be one or more states in which $(\partial \Pi / \partial \bar{X}_i^b)_T = 0$. For binary lipid dispersions, as in the examples given for DMPC–DOPC and for DMPC–DPPC mixtures, only one composition is found for each temperature. For multicomponent lipid mixtures ($C > 3$), we shall illustrate shortly that more than one composition may form the bilayer at each critical temperature.

(B) Coexisting Phases: Gel, Liquid, Crystal, and Water. $F^* = 0$. For a heterogeneous dispersion containing both gel and liquid crystal phases there are no independent variables, and T^* is fixed at a value characteristic of the composition of the coexisting lipid phases. This situation presents an interesting dilemma because the compositions of these bulk lipid phases are generally different. But the critical unilamellar state requires that the composition of the surface film and one of the bulk phases become identical (eq 5). Therefore, only one of the bulk phases can form the
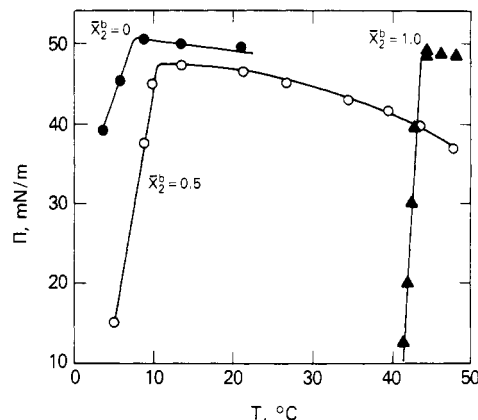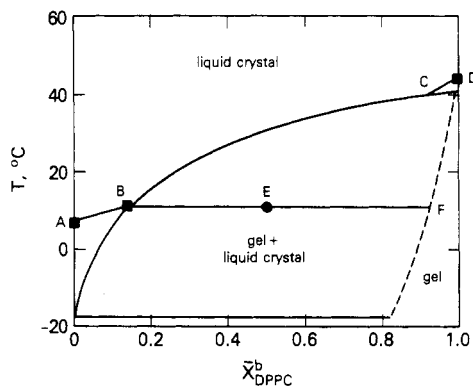
unilamellar state at T^*. Since the phospholipids examined have always formed the unilamellar state as liquid crystals, it is assumed that this state will be the one that forms rather than the gel. But what becomes of the gel state in this instance? Since the gel phase cannot conform to the required concentration relationship of eq 5 for the unilamellar critical state, it will coexist as a separate (multilamellar) phase in equilibrium with the liquid crystalline unilamellar state.

To illustrate this situation, consider binary lipid mixtures of DOPC and DPPC. The surface pressure–temperature relationships for 1:0, 1:1, and 0:1 mole ratio dispersions are given in Figure 6, and T^* for each composition is superimposed on the bulk phase relations in Figure 7. All three compositions exhibit a surface pressure maximum, and it is noteworthy that T^* for the equimolar mixture is just a few degrees above that for pure DOPC. This temperature falls within the gel–liquid crystal two-phase region of the phase diagram, point E in Figure 7. Thus, at T^* for the equimolar mixture both gel and liquid crystal phases coexist with a composition of $\bar{X}_{\mathrm{DPPC}}^b = 0.9$ (point F) for the former and 0.15 (point B) for the latter. From the lever rule the proportion of each phase present is determined by the ratio of the lengths of the lines EF and BE. Although the overall composition in the dispersion is 1:1 at T^*, the unilamellar state composition is approximately 1:6 (DPPC:DOPC). The relative amounts of gel and liquid crystalline phases and the overall composition of the lipid in the dispersion may vary along line BF, but tht composition of the unilamellar state is fixed at point B. Although T^* will vary with composition along the lines AB and CD where only the liquid crystal phase is present, there will be a temperature range between points B and C where no critical unilamellar state can form.

For membrane systems that contain numerous lipid components, the phase rule at T^* (eq 8) simplifies the analysis because the number of independent variables is determined solely by the
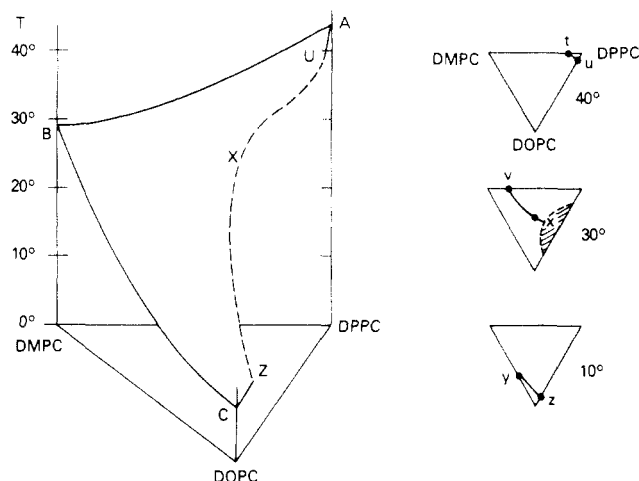
Figure 8. (a, left) Ternary lipid-phase diagram for mixtures of DMPC–DPPC–DOPC dispersed in water; vertical axis is T. The diagram was constructed from the binary T^*-composition relations given in Figures 2, 5, and 7; the bulk-phase relations in these figures have been omitted in the ternary diagram. All points that represent the possible combinations of the three lipids that form the unilamellar state will fall on the T^*-composition surface AUXZCB. See text for complete description of this diagram. (b, right) Triangular diagrams representing the loci from the intersection of isothermal planes with the T^*-composition surface in (a). Lines tu, vx, and yz are the loci of all points representing the compositions that form the unilamellar state at 40, 30, and 10 °C, respectively. The shaded area shown for 30 °C indicates the heterogeneous bulk-phase domain in which both gel and liquid crystal are present in the dispersion. Point x was obtained with an equimolar mixture of DMPC–DPPC–DOPC; data are shown in Figure 9.

physical state of the system and is independent of the number of components. For a homogeneous liquid crystal dispersion there will always be one independent variable that must be fixed to completely define the critical bilayer state. Typically, either temperature or composition is chosen. For dispersions containing more than two lipid components choosing temperature as the independent variable will always yield T^* from the surface pressure maximum in the Π–T phase diagram. However, when composition is the independent variable, one or more states may form, where $(\partial \Pi / \partial \bar{X}_i^b)_T = 0$.

To illustrate the type of behavior observed in multicomponent dispersions when composition is chosen as the independent variable, consider mixtures of the three lipids DMPC, DOPC, and DPPC. A ternary phase diagram may be constructed from the binary diagrams in Figures 2, 5, and 7, and it is given in Figure 8a. The vertical axis is T, and the vertices of the triangle represent the compositions for each of the three pure lipids dispersed in water. Lines AB and BC represent the T^*-composition relations for binary mixtures of DPPC–DMPC and DMPC–DOPC, respectively. For approximately the same temperature range mixtures of DPPC and DOPC are heterogeneous (Figure 7). The dotted line UXZ represents the limits of the domain where both gel and liquid crystal phases coexist; the drawn boundaries give only an approximation of this region and are included for illustration purposes. The points representing T^* for all possible combinations of the three lipids are contained on the surface formed within these lines.

Precise details of the surface contours require measuring T^* for many ternary lipid mixtures. For example, T^* for the mixture containing equimolar amounts of all three lipids is obtained by measuring the surface pressure–temperature relation for this composition; it is given in Figure 9. Included in Figure 9 is the differential scanning calorimeter thermogram for this mixture. The surface pressure maximum occurs at 30 °C; the thermogram indicates that at this temperature only the liquid crystal state is present.

Although details of the T^*-composition surface in the ternary phase diagram are omitted, a number of general properties of this system may be illustrated. The bulk-phase relations have not been included in this diagram because the unilamellar state for these
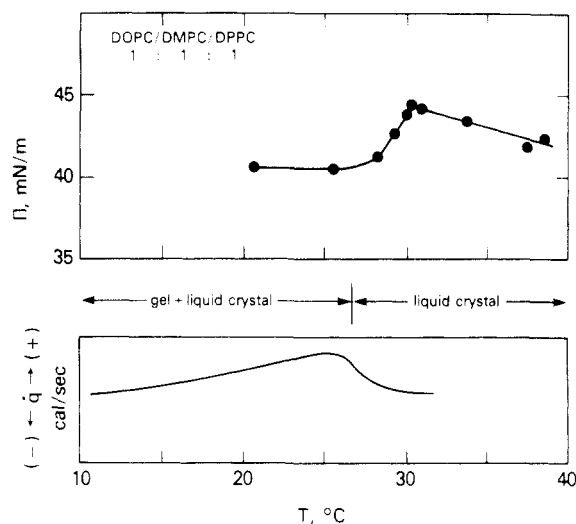

Figure 9. (A, top) Surface pressure–temperature relation for an equimolar mixture of DMPC–DPPC–DOPC dispersed in water. From the surface pressure maximum, $T^* = 30$ °C. (B, bottom) Differential scanning calorimeter tracing of heat flow for the equimolar mixture as a function of temperature. The gel–liquid crystal transition temperature is approximately 27 °C. Thus, at 30 °C the unilamellar vesicles are liquid crystalline.

lipids generally forms as a liquid crystal. In the ternary diagram an isotherm is represented by a plane intersecting the surface containing all possible values for T^*; the resulting locus is a line in the triangular diagram for each isotherm. The line will contain all the points representing the ternary compositions which form the unilamellar state at the specified temperature. Examples for temperatures of 10, 30, and 40 °C are given in Figure 8b. The points shown are taken from the data of the binary diagrams at these temperatures. Also included is one point taken from Figure 9A for the ternary mixture containing equimolar amounts of the three lipids.

The range of compositions which form the unilamellar critical state lies along the line tu at $T^* = 40$ °C, line vx at 30 °C, and line yz at 10 °C. In accordance with the phase rule (eq 8), choosing either the temperature or the composition of one of the lipid components as the independent variable is sufficient to determine a critical unilamellar state. This deduction is generally applicable to all homogeneous multicomponent systems.

For heterogeneous systems containing both gel and liquid crystal, the conditions that describe the critical unilamellar state are fixed. In this case the relative amounts of gel and liquid crystal and the overall composition of the dispersion may vary, but T^* and the composition of each phase are constant.

Bilayer Assembly in Cell Membranes as a Critical Phenomenon. The thermodynamic conditions for the formation of the critical bilayer at T^* have been described by considering the equilibrium between the surface film and the dispersion. How these properties relate to bilayer assembly in cells, where no air–water interface exists, is demonstrated by examining the equilibrium between the lipid dispersion and the aqueous solution. Although the solubilities of phospholipids in water may be extremely low, in principle, a rigorous analysis of the phase relations at the critical point may be based on the solution properties of the lipids in water.[8,23] Thus, relationships analogous to eq 5 must also exist between the composition of the solution and the bulk lipid phase. These relations may be deduced for a liquid crystal dispersion with an air–water surface of constant area by imagining a process at $T = T^*$ in which some water is evaporated, causing precipitation of more liquid crystal. The composition of the fresh precipitate must be unchanged if the equilibrium relationship between the bulk lipid and the surface film expressed by eq 5 is

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to be maintained. The concentration of each component in solution must therefore be proportional to its concentration in the liquid crystal, i.e.

$$c_j(\text{bilayer}) = kc_j(\text{solution}) \qquad j = 2, 3, ..., C \qquad (9)$$

where k is a constant. In contrast to the corresponding relations between the surface film and the bilayer where the mole fractions are equal (eq 5), the respective mole fractions are only proportional to each other. This arises because the water composition in the solution is obviously greater than that in the equilibrium liquid crystal. The total number of relations among the composition variables is still $C - 1$; the phase rule remains as $F^* = 3 - P$.

Equation 9 represents an additional set of conditions for assembly of the unilamellar state at T^*. Thus, whenever the concentration of a phospholipid solution ($C > 3$) reaches its saturation point, and the concentration ratios of the lipids given by eq 9 are satisfied, the unilamellar state will form spontaneously. In growing cells where phospholipids are synthesized by enzymes located on membranes,[24] by analogy, assembly of the unilamellar state may be considered to occur when the conditions of eq 9 are satisfied. It is noteworthy, therefore, that for aqueous dispersions of the total lipid extracts from red blood cells and from two strains of bacteria grown at several temperatures a unique T^* was found for each natural mixture of membrane lipids that was identical with the growth temperature of the cell from which the lipids were obtained.[16] According to the phase rule ($F^* = 1$), the unilamellar state which forms at T^* is completely defined by the lipid composition of the dispersion. Hence, wherever the same equilibrium conditions of temperature and composition exist this state will form. These conditions are obviously present in the cell when the growth temperature equals T^*. Since it is generally believed that bilayers in cell membranes are unilamellar,[1] conditions for cellular

assembly are likely to include those examined in this study.

The properties of the unilamellar state may be summarized as follows: (a) It forms only at a critical temperature T^*. (b) Critical conditions for the formation of the unilamellar state occur when the concentration of each lipid component in solution is proportional to its concentration in the equilibrium bilayer (eq 9). (c) When the bilayer is a homogeneous liquid crystal, T^* varies with composition. For isothermal mixtures of three or more lipid components the unilamellar state may form from a number of compositions utilizing the available lipid. The concentration of only one of the components may be arbitrarily varied, and the isothermal points that represent the unilamellar state will fall on a line. This signifies that at T^* only one concentration variable may be arbitrarily chosen; all other lipid compositions will then adjust to conform to the conditions of assembly. It also implies that this composition variable represents another critical condition for assembly; if the concentration of the selected component does not fall on this line, assembly of the unilamellar state will not occur. (d) When both gel and liquid crystal coexist, only the liquid crystal is unilamellar. T^* and the composition are fixed, but the relative amounts of gel and liquid crystal phases may vary.

The unilamellar state will form whenever the required conditions of T^* and composition are satisfied. These conditions appear to be met in cell membranes. T^* for the total membrane lipid extracts of various cells equals the growth temperature.[16] Thus, at the time of membrane bilayer assembly, the temperature and composition fulfill the requirements for the formation of the unilamellar state. How the critical conditions of assembly and the phase relations presented in this study are manifested in cell membrane properties will be presented elsewhere.[25]

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pH Dependence of the Internal Quantum Efficiency and of the Peltier Heat for Photoanodic Water Oxidation at Flame-Oxidized Titanium. An in Situ Photoacoustic and Photoelectrochemical Study

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The photocurrent and the photoacoustic signal have been measured simultaneously as a function of electrode potential at an n-type TiO_2 (rutile) thin-film electrode (flame-oxidized Ti) in aqueous Na_2SO_4 solution at different pH values (0.3–13.7). The internal quantum efficiency of the photocurrent as referred to the number of photons absorbed by the electrode and the Peltier heat for photoanodic oxidation of water have been determined as a function of pH from photoacoustic measurements at 360 nm. The internal quantum efficiency at a band bending of 0.8 V increases with pH in alkaline solution, in accord with a similar finding reported previously by Salvador for the photocurrent efficiency at a rutile sintered electrode. For the Peltier heat two ranges of linear dependence on pH (–58 meV/pH) have been found which have been assigned to the net reactions $2H_2O + 4h^+ \rightarrow O_2 + 4H^+$ and $4OH^- + 4h^+ \rightarrow O_2 + 2H_2O$.

Introduction

Photoelectrochemical reactions at semiconductor electrodes involve heat effects. Usually, only a fraction of the light energy absorbed by the semiconductor is utilized in the photoassisted reaction, while the remaining portion is converted into heat by various processes, e.g., radiationless electron–hole recombination.[1,2] The heat can be monitored in situ as a function of electrode potential either by measuring the temperature change at the electrode during continuous illumination[1] or by measuring the photoacoustic signal at a thermally transparent electrode during

illumination with intermittent monochromatic light.[2] Such measurements have been used mainly for the determination of the internal quantum efficiency of the photocurrent and of the energy conversion efficiency of photoelectrochemical reactions.[1–6]

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