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Thermal transitions in dimyristoylphosphatidylcholine foam bilayers

A. Nikolova[1], **D. Exerowa**[1], **Z. Lalchev**[2], **L. Tsonev**[3]

[1] Institute of Physical Chemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., BG-1113 Sofia, Bulgaria
[2] Department of Biochemistry, Faculty of Biology, University of Sofia, BG-1421 Sofia, Bulgaria
[3] Agricultural Academy, Institute of Cryobiology and Lyophilization, Cherny Vruh Blvd., BG-1407 Sofia, Bulgaria

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Abstract. Thermal transitions in the system dimyristoylphosphatidylcholine/water/ethanol/sodium chloride were studied in the temperature range 10–31 °C. The water – ethanol dispersions were investigated by differential scanning calorimetry and the foam bilayers by the microinterferometric method for investigation of thin liquid films. Calorimetry showed that an increase in ethanol content (up to 47.5 vol.% – the concentration used in the experiments with foam bilayers) did not significantly influence the temperature of the main phase transition and led to the disappearance of the pretransition. The microinterferometric study of the foam bilayer thickness showed that there were two thermal transitions – at 13 and 23 °C. An Arrhenius type dependence was obtained for the critical concentration of dimyristoylphosphatidylcholine (DMPC) in the solution, which was necessary for the formation of the foam bilayer. A steep change in the slope of the linearized Arrhenius dependence was found at 23 °C. Values of the binding energy of a DMPC molecule in the foam bilayers were calculated using the hole-nucleation theory of stability and permeability of bilayers. It was proved that the phase transition at 23 °C was due to melting of the hydrocarbon tails of phospholipid molecules. The low-temperature phase transition was assumed to be due to a change in the tilt of the hydrocarbon tails. These experiments demonstrate for the first time the occurrence of phase transitions in foam bilayers.

Key words: Phase transition – Bilayer – Phosphatidylcholine – Chain-melting – Foam

Introduction

It is well known that water dispersions of lipids may undergo different phase transitions due to variation of temperature or composition (Lee 1977; Jones 1979; Larsson 1986; Yeagle 1987). These transitions have been investigated in detail for different phospholipids and for some of the them phase diagrams have been obtained (Small 1967; Grabielle-Madelmont and Perron 1983). There are also numerous studies of phase transitions in lipid monolayers at the air/solution interface (Knobler 1990). Of special interest are surveys on phase transitions in lamellar multilayer and bilayer systems – liposomes (Lee 1977; Chapman 1980; Evans and Needham 1987) and synthetic bilayer lipid membranes (Mikayelyan and Adzhyan 1986). Some authors (Chapman 1980; Knobler 1990) have found some similarity between phase transitions in lipid monolayers at the air/solution interface and in water dispersions of lipids, underlining the case for the chain-melting phase transition.

Newton black films (foam bilayers) are composed of two mutally adsorbed monolayers of amphiphile molecules, which are in contact with a gas phase (Perrin 1918). An important difference between the foam bilayers and the bilayer lipid membranes is that in the former the two monolayers contact each other through their polar head groups (Fig. 1). This difference in configuration should not significantly influence the chain-melting phase transition since the theoretical considerations of Nagel (1980) showed that the Van der Waal's interaction between hydrocarbon chains of phospholipid molecules is of major importance for this transition. All this allows us to suppose that the foam bilayers may be used successfully as a model system for the investigation of some phase transitions. They have been used already in the investigations of membran fusion (Naydenova et al. 1990), interlamellar interactions (Yamanaka et al. 1982; Cohen et al. 1991), membrane permeability (Kashchiev and Exerowa 1983), lung surfactant (Exerowa and Lalchev 1986; Scarpelli et al. 1993), amniotic fluid (Exerowa et al. 1984) and liposome destruction at the air/solution interface (Vassilieff 1991).

Balmbra, Clunie and co-workers (Clunie et al. 1971; Balmbra et al. 1873) are pioneers in connecting the properties of a foam bilayer with the phase state of a solution of the surfactant, stabilizing the bilayer. They found a

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Correspondence to: D. Exerowa

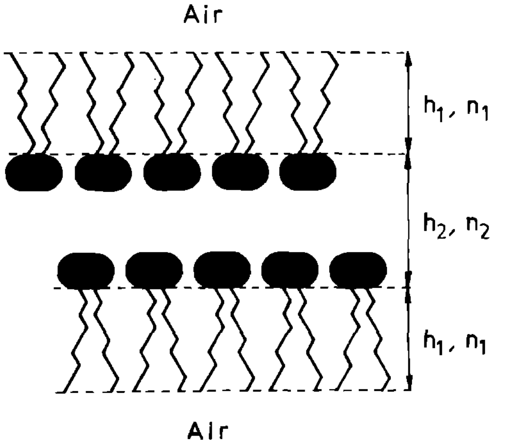

Fig. 1. Schematic representation of a lipid foam bilayer

correlation between the thickness of black foam films from decyltrimethylammonium decylsulfate and the interlamellar distance of structurally similar lamellar mesomorphic phase (neat phase) by using X-ray diffraction and polarization microscopy. More recently, Sidorova and co-workers (1976) found an analogous temperature dependence of the specific electroconductivity of extremely concentrated surfactant solutions and of the black foam films formed from them. The phase state of vertical black foam films was investigated by FT-IR spectroscopy (Umemura et al. 1985; Tian 1991). It was shown that in Newton black films from sodium dodecylsulfate (Umemura et al. 1985) and from cetyltrimethylammonium chloride (Tian 1991) the methylene chains, located outside the aqueous core of the sandwich structure, were in the liquid crystalline state.

It is of interest to investigate some properties of phospholipid foam bilayers, for example their thickness or other parameters, connected with their stability at temperature corresponding to different phase states of a bulk phase with a composition similar to that of foam bilayers. Such an investigation would prove the possibility for the occurrence of phase transitions in foam bilayers and would present an opportunity to compare these transitions with those in a bulk phase of similar composition. This is the aim of the present work.

We used the microinterferometric method for the investigation of foam films in its current variant (Exerowa et al. 1987), which allows an accurate determination of foam bilayer thickness under strictly controlled equilibrium experimental conditions and ensures formation of stable foam bilayers at very low phospholipid concentration. The experimental results of the investigation of foam bilayer stability are analyzed on the basis of the hole-nucleation theory of stability and permeability of bilayers (Kashchiev and Exerowa 1980, 1983). This approach allows the determination of important thermodynamic characteristics of foam bilayers in different phase states, principally the binding energy of a phospholipid molecule in the foam bilayer. The data for the phase state of bilayers are compared with those for the phase state of a bulk phase of similar composition studied by differential scanning calorimetry (DSC).

Materials and methods

Materials

DMPC (1,2-dimyristoyl-sn-glycero-3-phosphorylcholine) from two different sources (Fluka and Sigma) was used. Water-ethanol solutions of DMPC were prepared with bidistilled water and contained 0.07 mol dm^{-3} sodium chloride (Merck), which was heated at 500 °C prior to use in order to eliminate surface active contaminations. The electrolyte concentration was especially chosen to ensure formation of foam bilayers (Cohen et al. 1991). Foam bilayer existence is possible only at sufficiently high surfactant concentration in the solution, which is necessary for close packing of amphiphile molecules in the adsorption layers (Exerowa et al. 1981). The low solubility of DMPC in water and the low value of the critical micelle concentration (CMC) require the addition of 47.5 vol.% ethanol (p a), which ensures a significant increase in the CMC (Tanford 1972; Exerowa et al. 1984). We found a value for CMC of 400 µg ml^{-1} at 25 °C by measuring the turbidity of solutions at a wavelength of 420 nm with a SPEKOL 11 (Carl Zeiss).

In the refractive index measurements, necessary for evaluation of DMPC foam bilayer thickness, we used n-tetradecane (Merck).

Differential scanning calorimetry

Conventional differential scanning calorimetry (DSC) was carried using a Du Pont 1090 Thermal Analyzer interfaced with a Du Pont 1091 Disk memory and fitted with a subambient liquid nitrogen accessory. The water dispersions investigated contained 2 mg ml^{-1} DMPC, 0.07 mol dm^{-3} NaCl and different amounts of ethanol. Samples were prepared according to a procedure which ensured full hydration of lipid molecules (Rowe 1985). Afterwards the samples were hermetically closed in aluminium containers and put in the calorimeter. Measurements were carried out using a rate of heating of 2 °C min^{-1}. Each sample was heated and cooled sequentially between 0 and 30 °C.

Microinterferometric method for investigation of foam films

The foam bilayer investigations were carried out using the microinterferometric method (Sheludko 1967), which is regularly used in a number of studies of the kinetic and thermodynamic properties of foam films. This method was applied in its current variant, described in detail elsewhere (Exerowa et al. 1987; 1992). Microscopic horizontal foam films of radius 0.1 mm were formed in a special measuring cell (Exerowa et al. 1979) in the middle of a biconcave drop. The use of these films proved to be effective for performing investigations at very low phospholipid concentrations.

The measuring cell was fixed in a thermostating device joined to a microscope, which was equipped with a special optical and electronic system for the observation of the films and for recording the reflected light from the film (Exerowa et al. 1987). An additional thermostating jacket and thermosensor ensured constancy of temperature within ±0.1 °C.

The thickness of foam bilayers was determined by photoregistration of light reflected by the film. The equivalent water-ethanol thickness h_w was calculated from the data for the intensity of the reflected light (Exerowa et al. 1987), with the conventional assumption that the foam bilayer is an optically homogeneous structure (Duyvis 1962) with refractive index n equal to that of the solution from which it was formed.

More accurate analysis of the data for foam bilayer thickness was performed by using the triple-layer model, applied by many authors for the determination of the thickness of foam films (Duyvis 1962; Smart and Senior 1966; Frankel and Mysels 1966) and bilayer lipid membranes (Tien 1967; Cherry and Chapman 1969). According to this model, the foam bilayer is conventionally regarded as consisting of three layers (Fig. 1). It is accepted (Scherer 1989) that the outer layers with thickness h_1 and refractive index n_1 include the hydrocarbon tails of amphiphile molecules and the inner layer with thickness h_2 and refractive index n_2 includes the polar head groups of amphiphile molecules and the water core between them.

The calculation of the thickness h according to the triple-layer model ($h = 2h_1 + h_2$) is carried out with the help of the following equation (Frankel and Mysels 1966):

$$h_2 = h_w - 2h_1 [(n_1^2 - 1)/(n_2^2 - 1)]. \quad (1)$$

Refractive indices were determined using an Abbe refractometer (Carl Zeiss). The data obtained for refractive indices of tetradecane, n_1, and of water-ethanol solutions, n_2, at different temperatures are shown in Table 1. These measurements showed that DMPC and NaCl did not influence the value of the refractive index at concentrations corresponding to those used in experiments with foam bilayers.

Experimental results

Differential scanning calorimetry

DSC thermograms of water – ethanol solutions of DMPC containing NaCl, which were obtained during the first heating after a sample equilibration at 4 °C for 15 days, are presented in Fig. 2. It is well known (Hinz and Surtevant 1972; Janiak et al. 1976) that water dispersions of DMPC undergo a low-energy pretransition and a high-energy chain-melting transition. The values of temperature and enthalpy of the pretransition (T_1, ΔH_1) and of the main phase transition (T_2, ΔH_2) obtained for fully hydrated DMPC in the presence of NaCl and different ethanol contents are shown in Table 2. It is seen that in the case without ethanol (curve 1) the peak temperatures of the two transitions are slightly higher and the transition enthalpies are slightly lower than the data reported by other authors for water dispersions of DMPC (Hinz and Surtevant 1972; Janiak et al. 1976). These differences are probably due to the presence of NaCl, because similar deviations are observed for water dispersions of dipalmitoylphosphatidylcholine (DPPC), containing monovalent cations (Cunningham et al. 1988).

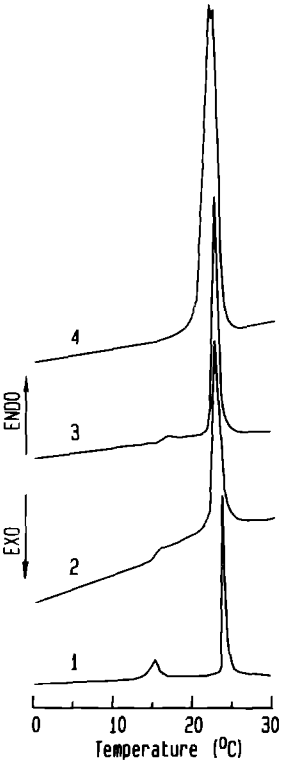

Fig. 2. DSC-thermograms of water-ethanol solution of DMPC, containing 2 mg ml^{-1} DMPC and 0.07 mol dm^{-3} NaCl and ethanol concentrations 0 vol.%-curve 1, 10 vol.%-curve 2, 20 vol.%-curve 3 and 47.5 vol.%-curve 4

Table 1. Refractive index values of tetradecane (n_1) and of water solutions containing 47.5 vol.% ethanol (n_2) for different temperatures

Temperature, [°C]	n_1	n_2
10	1.4323	1.3619
15	1.4305	1.3602
20	1.4282	1.3586
25	1.4260	1.3571
30	1.4240	1.3556
35	1.4218	1.3540

Table 2. Thermodynamic characteristics of the pretransition and of the main phase transition in the system DMPC/H_2O/NaCl/C_2H_5OH (2 mg ml^{-1} DMPC and 0.07 mol dm^{-3} NaCl)

Concentration of C_2H_5OH [vol.%]	T_1 [°C]	ΔH_1 [kcal · mol^{-1}]	T_2 [°C]	ΔH_2 [kcal · mol^{-1}]
0.0	15.0	0.90	24.2	5.50
10.0	16.1	0.09	23.4	5.96
20.0	17.4	0.06	23.5	7.65
47.5	–	–	22.6	10.60

Note: T_1 and T_2 represent the peak temperatures of the pretransition and of the main transition

Figure 2 also shows an increase in pretransition temperature with increase in ethanol concentration, leading to overlapping of the pretransition peak with that of the main phase transition at 47.5 vol.% ethanol. Such a disappearance of the pretransition at high ethanol concentration in water dispersions of DPPC has been reported by many authors (Simon and McIntosh 1984; Verio et al. 1987; Tenchov et al. 1989; Tamura-Lis et al. 1990), who regard it as a result of formation of interdigitated phases. Another result shown in Table 2 is the slight shift of the main phase transition towards lower temperatures. Similar results have been found for water-ethanol dispersions of DPPC (Rowe 1985; Tenchov et al. 1989; Tamura-Lis et al. 1990). The strong influence of ethanol on the enthalpy of the main phase transition of DMPC water-ethanol dispersions shown in Table 2 is similar to the substantial increase in this enthalpy in the case of water-ethanol disperions of DPPC (Tenchov et al. 1989; Tamura-Lis et al. 1990).

Microinterferometric determination of foam bilayer thickness

The equivalent thickness h_w of foam bilayers was determined by the microinterferometric method in the range 10–30 °C. The thickness of about 15 foam bilayers was determined at each investigated temperature in two independent experiments. The mean values of the results obtained for h_w are presented as circles in Fig. 3. Three temperature ranges are clearly distinguished by formation of foam bilayers with constant thickness – respectively 10–12 °C ($h_w = 7.5$ nm), 13–20 °C ($h_w = 6.9$ nm) and 24–30 °C ($h_w = 6.2$ nm). The values of the equivalent thickness are average ones for the whole temperature interval and are also shown in Table 3. The two thickness transitions may be regarded as reliable because the accuracy of the microinterferometric method is ± 0.2 nm (Exerowa et al. 1987) and because of the good reproducibility of experimental results. The absence of data for equivalent thickness at temperatures 21–22 °C is due to the existence of heterogeneities in the thickness of the foam films, resulting in a significant error in the thickness determination.

An important result is the coincidence of the temperature of the main phase transition determined for the water-ethanol dispersion by DSC (Table 2) with the temperature of the steep change in the foam bilayer thickness (23 °C). Within the range 22–24 °C the foam bilayer thickness variation is similar to the variation of interlamellar distance in water dispersions of DMPC (Janiak et al. 1976). These facts show that both in the bulk phase and in the foam bilayer a chain-melting phase transition occurs, which is characterized by a sharp shift in the number of gauche conformations of carbon-carbon bonds (Yellin and Levin 1977; Yeagle 1987).

The thickness transition at 13 °C is a very interesting experimental observation. In contrast to the pretransition in water dispersions of DMPC, for which the interlamellar spacing decreases with decreasing temperature (Janiak et al. 1976), the foam bilayer thickness increases in the range of this transition under cooling.

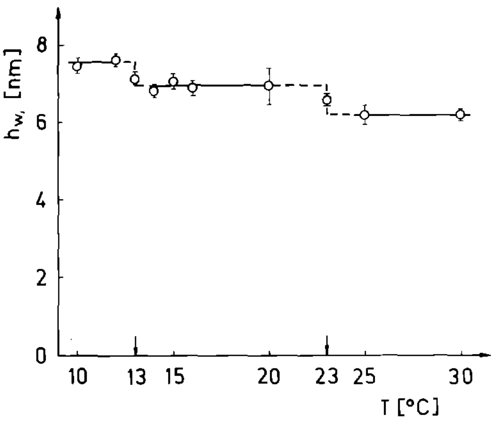

Fig. 3. Temperature dependence of the equivalent thickness h_w of foam bilayers with radius 0.1 mm, formed from solutions containing 47.5 vol.% ethanol and 0.07 mol dm^{-3} NaCl and different concentrations DMPC

Table 3. Values of the equivalent water-alcohol thickness h_w, the thickness of the hydrocarbon layers h_1, the thickness of the polar core h_2 and the thickness h (according to the triple-layer model) of DMPC foam bilayers at different temperatures

Temperature range [°C]	h_w [nm]	h_1 [nm]	h_2 [nm]	h [nm]
10–12	7.5	1.625	3.5	6.75
13–20	6.9	1.440	3.5	6.38
25–30	6.2	1.125	3.5	5.75

Additional experiments at various electrolyte contents showed that an increase in the NaCl concentration did not influence the foam bilayer thickness, which means that these transitions are not electrostatic in nature and are probably due to the occurrence of phase transitions in foam bilayers. Data in Fig. 3 suggest the existence of different types of DMPC foam bilayers, which are distinguished by their phase state.

Temperature dependence of the critical concentration for formation of the foam bilayer

The critical concentration C_c for formation of Newton black film (foam bilayer) was determined on the basis of observations of the final state which the foam film reached during its drainage (Sheludko 1967; Exerowa et al. 1992). In particular, rupture at a definite critical thickness without formation of black spots or formation of foam bilayer via black spots. Rupture at critical thickness occurred at lower concentrations C of DMPC in the solution ($C < C_c$) and black spots were formed at higher concentrations ($C > C_c$). These black spots grew up all over the foam film, turning it into a foam bilayer of constant radius. At each temperature a series of observations were carried out at various DMPC concentrations for the determination of C_c (the minimum DMPC concentration at which a foam bilayer is formed). This concentration is frequently used as a physicochemical characteristic of different surfactants (Exerowa et al. 1981, 1992). In practical

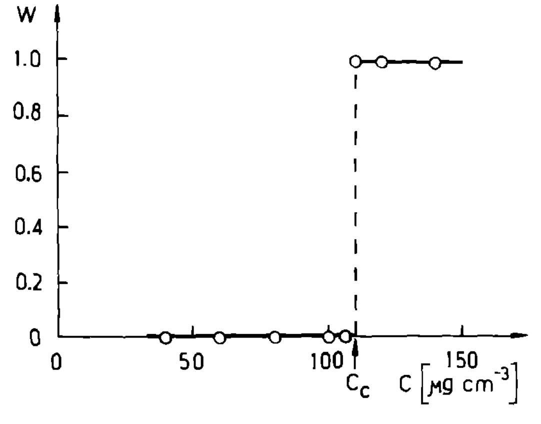

Fig. 4. Dependence of the probability for observation of a foam bilayer in foam film at 10 °C on the concentration C of DMPC in the solution, containing 47.5 vol.% ethanol and 0.07 mol dm^{-3} NaCl

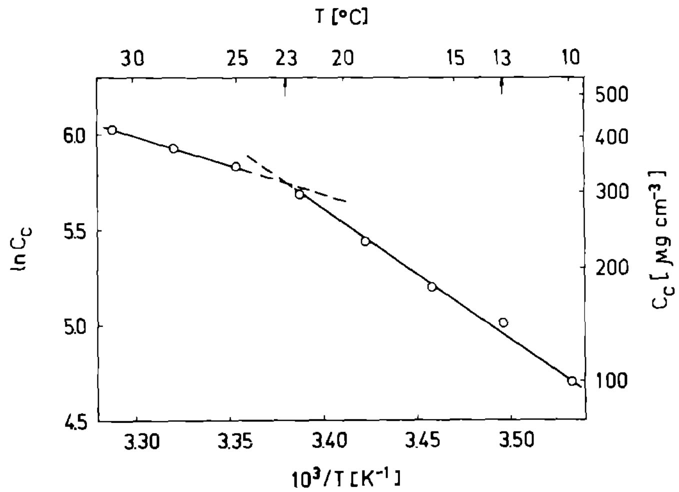

Fig. 5. Arrhenius plot of the dependence of the critical concentration C_c of DMPC in the solution for formation of foam bilayer on the temperature: *circles* – experimental results; *straight lines* – theoretical dependence according to Eq. (2)

terms, C_c was obtained from the dependence of the probability W for the observation of a foam bilayer on the DMPC bulk concentration C, at a given temperature. About 25 films were observed at each DMPC concentration and the accuracy of C_c determination was 5%. The $W(C)$ dependence obtained at 10 °C is shown in Fig. 4. In this case $C_c = 110$ μg ml^{-1}; at $C < C_c$, $W = 0$ and at $C \geq C_c$, $W = 1$.

A specific property of phospholipid foam bilayers, distinguishing them from foam bilayers stabilized by common synthetic surfactants (Exerowa et al. 1992), is their infinite stability at all concentrations allowing their formation. This stability is the reason for the steep W(C) dependence in the case of phospholipid foam bilayers (Exerowa et al. 1983). A measure of kinetic stability of foam bilayers is their mean lifetime (Kashchiev and Exerowa 1980). Conventionally we regarded the foam bilayer as "infinitely stable" if it did not rupture within 5–6 hours. The high stability of DMPC foam bilayers may be demonstrated by an attempt to rupture them by α-particle irradiation. Such experiments were carried out in a special measuring cell (Exerowa et al. 1992), the intensity of irradiation being up to 700 μC. DMPC foam bilayers dit not rupture at all investigated temperatures and concentrations, even at the highest intensity of 700 μC.

The temperature dependence of the critical concentration of DMPC for formation of a foam bilayer is shown in Fig. 5 (coordinates ln C_c vs. 1/T). Experimental results are denoted by circles and the straight lines are the least-squares fits. A clearly pronounced bend in the ln C_c vs. $1/T$ dependence corresponds to the temperature of the thickness transition at 23 °C. This should be contrasted with the invariable slope of this dependence in the range of the low-temperature thickness transition of foam bilayers. It is clear that the temperature dependence of C_c is very sensitive to the occurrence of some phase transitions in foam bilayers and may be used for their detection.

Discussion

Thermal transitions in foam bilayer thickness

The more accurate analysis of the data for foam bilayer thickness obtained by the triple-layer model (Duyvis 1962; Smart and Senior 1966; Den Engelsen and Frens 1974) enables a better understanding of the structural changes in foam bilayers due to temperature variation.

In order to calculate the thickness of a foam bilayer using the triple-layer model (Eq. (1)) it is necessary to estimate h_1 or h_2. It is well known that the main phase transition is due to the melting of hydrocarbon tails of amphiphile molecules (Nagle 1980; Yeagle 1987). The average number of gauche conformations for each DMPC molecule is about 7 in the liquid crystalline state (Yellin and Levin 1977). Having in mind this fact, we can estimate the thickness of the hydrocarbon layers of a foam bilayer as $h_1 = 1.125$ nm. Substituting the values of h_1 and h_w for the liquid crystalline state of foam bilayers in Eq. (1), we find the thickness of the polar inner layer as $h_2 = 3.50$ nm and the total thickness of the foam bilayer as $h = 5.75$ nm. These values are relevant to the liquid crystalline state of foam bilayers (24–30 °C) and are shown in Table 3. The value obtained for the thickness of the DMPC foam bilayer in the liquid crystalline state seems to be reasonable when compared with the value of the interlamellar distance in liquid crystalline DPPC water-alcohol dispersions (Tamura-Lis et al. 1990) extrapolated to high ethanol concentrations.

If we assume that the thickness of the polar core does not change significantly during the phase transitions, we can use the above value of h_2, together with the value of h_w for 13–20 °C, to calculate, using Eq. (1), $h_1 = 1.44$ nm and $h = 6.38$ nm for the first gel state of foam bilayers (Table 3). In the case of all-trans configuration of hydrocarbon tails, we can explain the above value of h_1 by the tilting of hydrocarbon tails with respect to the plane of the foam bilayer, the angle of tilt being 34 °C. This value is very close to the one obtained for the gel state of water dispersions of DMPC by X-ray diffraction (Janiak et al. 1976) and shows that the application of triple-layer model is reasonable.

The determination of foam bilayer thickness by the triple-layer model in the range 10–12 °C is more difficult, owing to the lack of data for large interlamellar distances of water dispersions of phosphatidylcholines comparable with the value of the equivalent thickness of foam bilayers. The simplest assumption for calculating the foam bilayer thickness according to the triple-layer model is that at temperatures within the range 10–12 °C the hydrocarbon chains are in the all-trans configuration without any tilting. As pointed out earlier (Nagle 1980) the tilt of hydrocarbon chains is due to repulsive forces between polar head-groups of phospholipid molecules. At high alcohol concentrations the ethanol molecules are localized among the polar head-groups of phospholipid molecules (Lohner 1991) and are screening this repulsion, which may explain the lack of tilt at low temperatures. This assumption seems to be a reasonable way of accounting for the lack of tilting of hydrocarbon chains in the gel phase of water dispersions of phosphatidylethanolamines (McIntosh 1980). If there is no tilting of hydrocarbon tails in DMPC foam bilayers the thickness of the hydrocarbon layer $h_1 = 1.625$ nm is determined for the all-trans configuration. Substituting this value, together with corresponding h_w, in Eq. (1) we obtain $h_2 = 3.5$ nm and $h = 6.75$ nm (Table 3). The value obtained for the thickness of the polar core is equal to that at higher temperatures and supports the assumption for invariance of h_2 (and probably of polar head-group configuration) during occurrence of phase transitions in phosphatidylcholine foam bilayers.

Determination of the binding energy of a DMPC molecule in the foam bilayer

The determination of the binding energy of a DMPC molecule in the foam bilayer was carried out using the experimental results for the temperature dependence of the critical concentration for formation of the foam bilayer (Fig. 5) and the theory of Kashchiev and Exerowa (Kashchiev and Exerowa 1980, 1983; Exerowa et al. 1992; Exerowa and Kashchiev 1986) of stability and permeability of bilayers. According to this theory, the short-range interactions are of decisive importance for the stability of foam bilayers, which are two-dimensional systems with a high degree of ordering. The bilayer is regarded as constituted of two mutually adsorbed monolayers of amphiphile molecules. It is assumed that within the bilayer there are vacancies for amphiphile molecules, which cluster randomly and form holes of different size. The driving force for this hole-nucleation process is the supersaturation, which depends on the concentration C of amphiphile molecules in the bulk solution which contacts the bilayer. The rupture of the bilayers is considered to result from the two-dimensional phase transition of the "gas of vacancies" into a "condensed phase of vacancies", which is equivalent to a ruptured bilayer. The equilibrium concentration C_e of amphiphile molecules in the solution at which the diluted and the condensed phase of vacancies are in thermodynamic equilibrium is a particularly important parameter of the theory, connected with the driving force of the nucleation process – the supersaturation. An Arrhenius-type dependence of the equilibrium concentration is assumed (Kashchiev and Exerowa 1980, 1983; Exerowa et al. 1992):

$$C_e = C_0 \exp(-Q/2kT) \qquad (2)$$

where Q is the binding energy of an amphiphile molecule in the bilayer, C_0 is a reference concentration, k is the Boltzmann constant and T is the absolute temperature. The concentrations C_c and C_e are specific constants of each particular system, which determine the ability of a foam bilayer to exsist in a metastable state within the concentration range $C_c < C < C_e$. When $C \geq C_e$ the foam bilayer is thermodynamically stable (there isn't a driving force for the hole-nucleation process in the foam bilayer). It follows from the theory (Exerowa and Kashchiew 1986; Krugljakov and Exerowa 1990; Exerowa and Nikolova 1992) that the critical concentration of amphiphile molecules in the solution equals the equilibrium one ($C_c = C_e$) in the case of a missing metastable region, when only thermodynamically stable foam bilayers are formed. As mentioned in the experimental results, the DMPC foam bilayers studied are extremely stable and do not rupture even under α-particle irradiation, which is well known (Penev et al. 1987) to decrease the mean lifetime of metastable foam bilayers significantly. This high stability of DMPC foam bilayers allows us to assume that $C_c = C_e$, thus permitting us to calculate the binding energy Q, with the aid of Eq. (2) from the experimental dependence of the critical concentration C_c on temperature, shown in Fig. 5.

The values of Q obtained from the best fit of Eq. (2) (the straight lines in Fig. 5) to the experimental data (the circles in Fig. 5), assuming $C_c = C_e$, are $(1.93 \pm 0.04) \times 10^{-19}$ J for temperatures lower than 23 °C and $(8.03 \pm 0.19) \times 10^{-20}$ J for temperatures higher than 23 °C. The possible error arising from the assumption that $C_c = C_e$ is analyzed elsewhere (Exerowa and Nikolova 1992) and leads to an increase in the value of Q by up to 20%. The good fit of the experimental results to the theoretical dependence and the high stability of the foam bilayers with respect to their rupture, even under α-particle irradiation, show that in the case of DMPC bilayers the assumption $C_c = C_e$ is probably accurate.

The sharp change in the binding energy of a DMPC molecule in the foam bilayer is obviously due to the occurrence of a chain-melting phase transition in the bilayer. The value of Q 1.93×10^{-19} J relates to the gel state and the value 8.03×10^{-20} J relates to the liquid crystaline state of the foam bilayer. The higher value of Q for the gel state is natural, as it refers to a state with a higher degree of order.

The above value of Q for the liquid crystalline DMPC foam bilayers is very similar to that for sodium dodecylsulfate foam bilayers 6.7×10^{-20} J (Nikolova et al. 1989).

It is worth noting the considerable difference between the Q values for the liquid crystalline and gel states of DMPC foam bilayers. Analogous to the well known case of evaporation from a crystal surface (Volmer 1939) the enthalpy ΔH_e of two-dimensional evaporation of DMPC from the foam bilayer may be estimated from the relationship $\Delta H_e = Q N_a/2$ (N_a being the Avogadro constant).

Consequently, the enthalpy change ΔH_m for the gel to liquid crystalline transition of foam bilayers may be approximately estimated from the difference between the enthalpies of two-dimensional evaporation for the gel and liquid crystalline foam bilayers. The calculations showed that $\Delta H_m = 8.1$ kcal mol^{-1}. As seen from Table 2, this value is lower than the enthalpy of the main phase transition for the water-alcohol dispersion, containing 47.5% ethanol ($\Delta H_2 = 10.6$ kcal mol^{-1}), which is probably due to the fact that the DMPC molecules in the foam bilayer cannot interdigitate. On the other hand, ΔH_m is higher than the enthalpy of the main phase transition of the water dispersion ($\Delta H_2 = 5.5$ kcal mol^{-1}, see Table 2). A possible cause for this difference might be the high ethanol content in the solution from which the foam bilayers are formed, bearing in mind the earlier mentioned fact that ethanol molecules are situated between the polar head groups of DMPC molecules, thus affecting the short-range molecular interactions.

Figure 5 also shows that in the range of the low-temperature phase transition (12–13 °C) there is no detectable change in the slope of the ln C_c vs. $1/T$ dependence, i.e. there is no change in the binding energy of a DMPC molecule in the foam bilayer. This fact is in agreement with the above proposed supposition that the low-temperature phase transition of the foam bilayer is due to a variation in the tilt of DMPC molecules, as it is considered (Nagle 1980) that the tilt change does not significantly influence the energy of short-range interactions.

Phase diagram of DMPC foam bilayers

The above analysis of experimental results for foam bilayer thickness and for the critical concentration for formation of the foam bilayer enable the construction of a phase diagram of DMPC foam bilayers within the temperature and concentration ranges investigated. This phase diagram is shown in Fig. 6. The experimental dependence of C_c on temperature determines the boundary between the stable foam bilayers in the gel or liquid crystalline states and a hypothetical "gas" phase representing a ruptured foam film. The experimental determination of the temperatures of the two phase transitions (liquid crystalline foam bilayer → gel I foam bilayer and gel I foam bilayer → gel II foam bilayer) from the foam bilayer thickness shows independence of these temperatures on the DMPC concentration in the solution, which is illustrated in the figure and is in agreement with the data for phase transitions in fully hydrated dispersions of phosphatidylcholines (Janiak et al. 1976; Grabielle-Madelmont and Perron 1983). As with the main phase transition in water dispersions of phospholipids it may be supposed that the thermal transition, liquid crystalline foam bilayer → gel I foam bilayer, is a first-order phase transition. The sharp change in Q and h values at 23 °C and the relaxation in the C_c value in the range 19–23 °C support this assumption. The lack of a distinctive change in Q and of relaxation in the value of C_c, as well as the slight change in h in the range of the low-temperature phase transition, permit us to suppose that this transition is probably of second-order. Additional investigations by other methods are obviously necessary to verify this assumption.

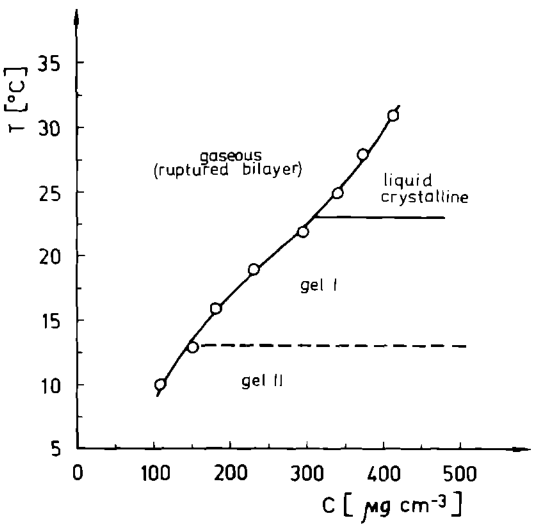

Fig. 6. Phase diagram of DMPC foam bilayers, formed from aqueous solutions, containing 47.5 vol.% ethanol and 0.07 mol dm^{-3} NaCl

Conclusions

An experimental study of the phase behavior of DMPC foam bilayers has been carried out. For the first time, the occurrence of phase transitions in foam bilayers has been detected by investigation of the temperature dependence of foam bilayer thickness and of critical concentration of DMPC in the solution needed for formation of the foam bilayer. A quantitative interpretation of these data has been carried out with the aid of the hole-nucleation theory of stability of bilayers, which considers the rupture of bilayers on the basis of short-range interactions. The binding energy of a DMPC molecule in liquid crystalline and gel foam bilayers has been calculated. A correspondence is found for the temperature of the chain-melting phase transition in the cases of foam bilayers and fully hydrated water-ethanol disperions of DMPC. A low-temperature phase transition is detected and it is explained by a change in the tilt of DMPC molecules, accounting for the high ethanol content and specific configuration of DMPC molecules in the foam bilayers.

These results may be of interest for modelling the molecular interactions in bilayer and multilayer lipid structures. In this respect, investigations of other individual lipids and of mixtures of lipids would be very useful, as well as application of various independent experimental techniques for investigation of foam bilayers (X-ray diffraction, X-ray reflectivity, FT-IR spectroscopy, etc.).

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