Phase Transitions in Phosphatidylcholine Foam Bilayers

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Foam bilayers from individual and mixed phosphatidylcholines are experimentally studied at different temperatures. Occurrence of a chain-melting phase transition in the foam bilayers is detected by two independent parameters—the critical concentration C_c for formation of foam bilayer and the foam bilayer thickness. The data for C_c are discussed on the basis of the hole-nucleation theory, which applies the Ising model to foam bilayers and uses the mean-field approximation for interpretation of their stability. This allows the determination of the binding energy of a phospholipid molecule in gel and liquid-crystalline foam bilayers. New possibilities to relate the microscopic and macroscopic characteristics of foam bilayers are demonstrated.

KEY WORDS: Foam bilayer; phase transition; Ising model; mean-field; binding energy.

1. INTRODUCTION

Amphiphile bilayers are formed at the border of two identical or different phases. These bilayers are constituted of two monolayers of amphiphile molecules (synthetic surfactants, lipids, etc.). Familiar examples of amphiphile bilayers are foam bilayers (which are in contact with gas phase) and emulsion bilayers (which are in contact with oil or water phase, depending on the type of emulsion). Special attention has been paid to lipid bilayers, which are of major importance for biology and medicine.

Some of the main studies of amphiphile bilayers are focused on understanding the mechanism of their rupture. A number of theories for the stability of amphiphile bilayers regard their rupture as a result of spontaneous[1,2] or deliberate[3,4] formation of holes in them. Kashchiev and

Exerowa[1,2,5,6] interpreted the hole formation in amphiphile bilayers as fluctuation process occurring in a two-dimensional system in which the short-range interactions are dominant. Generally, the bilayer rupture is considered as a result of a two-dimensional phase transition of condensation of the "gas" of vacancies of amphiphile molecules. Later it was shown experimentally[7] that a necessary condition for the existence of an amphiphile bilayer was the condensed state (fluid or solid) of monolayers constituting the bilayer.

The stability of foam and emulsion bilayers was investigated theoretically by Chowdhury, Stauffer, and coworkers[8,9] by Monte Carlo simulations. Their approach has contributed to a better understanding of the mechanism of rupture of these bilayers, clarifying, for example, the role of the exchange of amphiphile molecules between the bulk and the surface for the kinetics of hole nucleation.

It is well known that water dispersions of amphiphile molecules may undergo different phase transitions when temperature or composition is varied.[10,11] These phase transitions were studied systematically for phospholipid dispersions and phase diagrams were obtained for some of the systems.[12,13] Occurrence of phase transitions in monolayers of amphiphile molecules at the air/water interface[14] and in bilayer lipid membranes[15] was also reported. Of special interest for biology and medicine is the chain-melting phase transition[10,11,14,16] found both for water dispersions and insoluble monolayers of amphiphile molecules.

The aim of the present work is to check the possibility for the occurrence of phase transitions between condensed phases of foam bilayers and particularly for the occurrence of the chain-melting phase transition.

The object of our study is foam bilayers (Newton black films) from individual and mixed phosphatidylcholines. We used the microinterferometric method for investigation of foam bilayers,[17,18] which permits a precise determination of bilayer thickness under strictly controlled equilibrium experimental conditions.

In this paper we present a brief review of some important results of the hole-nucleation theory for the stability of bilayers (Section 2). The occurrence of the chain-melting phase transition in phosphatidylcholine foam bilayers is demonstrated by experimental investigation of the temperature dependence of the bilayer thickness (Section 3.2). Section 3.3 gives experimental results on the temperature dependence of the critical concentration for the formation of foam bilayers. These data are analyzed on the basis of the hole-nucleation theory, and the binding energy of a phospholipid molecule in the foam bilayer, the interaction energy of two neighboring phospholipid molecules, and the specific edge free energy of the nucleus hole are estimated.

2. THEORETICAL BACKGROUND

In the theory of Kashchiev and Exerowa[1,2,5,6] the stability and permeability of bilayers are analyzed on the basis of short-range interactions in a two-dimensional ordered system. This theory considers the foam bilayer as constituted of two mutually adsorbed monolayers of amphiphile molecules (Fig. 1). The foam bilayer periphery is in contact with a bulk solution (meniscus) with concentration C of amphiphile molecules and its surfaces are in contact with gas phase (air). The rupture of the foam bilayer is regarded as the result of a two-dimensional phase transition of the "gas" of amphiphile vacancies into a "condensed phase" of vacancies, the latter being equivalent to a ruptured bilayer.

The adsorption isotherm of amphiphile vacancies in the foam bilayer is obtained[1] from the analysis of the thermodynamic equilibrium of the amphiphile molecules in the foam bilayer with those in the bulk solution. At thermodynamic equilibrium the chemical potential of amphiphile molecules in the foam bilayer μ_f is equal to the chemical potential of amphiphile molecules in the bulk solution μ_b:

$$\mu_f = \mu_b \tag{1}$$

The chemical potential of an amphiphile molecule in the bulk solution is given by

$$\mu_b = kT \ln C + \mu_b^* \tag{2}$$

where T is the absolute temperature, k is the Boltzmann constant, and μ_b^* is a reference chemical potential.

The chemical potential of an amphiphile molecule in the foam bilayer μ_f is obtained[1] by employing the Ising model[19] for localized adsorption
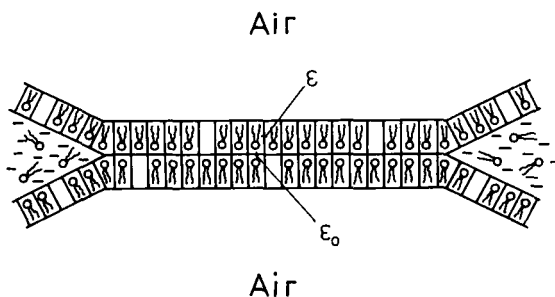
Air

Air

Fig. 1.   Schematic cross section of a phospholipid foam bilayer according to the lattice model.

of lattice gas to foam bilayer, accounting only for the nearest-neighbor interactions (the mean-field approximation):

$$\mu_f = kT \ln[\theta/(1-\theta)] - z\varepsilon\theta - z_0\varepsilon_0\theta + \mu_f^* \qquad (3)$$

where θ is the degree of filling by amphiphile molecules of each monolayer constituting the foam bilayer, ε and ε_0 are, respectively, the energy of lateral and normal interaction of two neighboring amphiphile molecules in the bilayer, z and z_0 are, respectively, the number of nearest neighbors in the lateral and normal directions and μ_f^* is a reference chemical potential. The first term on the right side of Eq. (3) is the entropy contribution to the chemical potential. The second and the third terms account for the lateral and normal interaction components of the free energy of the foam bilayer. The latter terms include the degree of filling θ, as a given amphiphile molecule from one of the monolayers is in contact with its nearest neighbors from the other monolayer with probability θ.

The adsorption isotherm of amphiphile vacancies in the foam bilayer is obtained[1] from Eqs. (1)–(3), bearing in mind the filling by vacancies of the foam bilayer $\theta_v = 1 - \theta$:

$$C/C_0 = [(1-\theta_v)/\theta_v] \exp[-Q(1-\theta_v)/kT] \qquad (4)$$

where $Q = z\varepsilon + z_0\varepsilon_0$ is the binding energy of an amphiphile molecule in the foam bilayer and C_0 is a reference concentration of amphiphile molecules.

An example for an adsorption isotherm of amphiphile vacancies in the foam bilayer, representing the case of strong attraction between the amphiphile molecules ($Q \geqslant 8kT$), is shown in Fig. 2. The minimum bulk
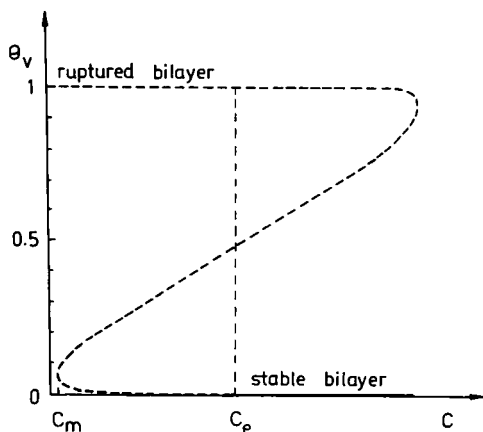

Fig. 2.   Adsorption isotherm of amphiphile vacancies in a foam bilayer.

amphiphile concentration which is necessary for the formation of a thermo-dynamically stable foam bilayer is marked by C_e. This concentration is called[1] the "equilibrium bulk concentration of amphiphile molecules" insofar as it represents the hypothetical equilibrium between the diluted and the condensed phase of vacancies. According to Maxwell's rule, C_e is determined[1] from Eq. (4) at $\theta_v = 1/2$:

$$C_e = C_0 \exp(-Q/2kT) \tag{5}$$

Practically Eq. (5) can be regarded as some kind of Clausius–Clapeyron equation, because it defines the conditions for the coexistence of the condensed and the diluted phases of vacancies. That is why the enthalpy λ of two-dimensional evaporation of amphiphiles from the foam bilayer can be estimated by the relation $\lambda = QN_a/2$, where N_a is the Avogadro constant.

Theoretically, the formation of metastable foam bilayers can also be expected. This is possible when the bulk amphiphile concentration C is in the range $C_m < C < C_e$ (see Fig. 2). The hole-nucleation theory of bilayer stability defines the critical concentration of amphiphile molecules C_c at which metastable foam bilayers are formed[2]:

$$C_c = C_e \exp[-B/\ln(\tau_c/A)] \tag{6}$$

where τ_c is the mean lifetime of the metastable foam bilayer, the parameter B is a function of the specific edge free energy of the nucleus hole and A is a parameter accounting for the kinetics of incorporation of single vacancies into the nucleus hole. Obviously, at very short τ_c, for example, equal to the resolution time of the equipment (about 0.5 sec), C_c appears to be the minimum bulk concentration of amphiphile molecules at which the existence of a foam bilayer can be detected. For that reason C_c is called the "critical concentration for formation of foam bilayer." It is important to note that C_c is experimental parameter, which is practically higher than C_m.[20]

The concentrations C_c and C_e are specific constants for each particular system and they determine the ability of a foam bilayer to exist in metastable state in the range $C_c < C < C_e$. When $C \geqslant C_e$, the foam bilayers are thermodynamically stable and do not rupture spontaneously. When there is no metastable region and only stable bilayers are formed ($\tau \to \infty$, $C_c \to C_e$) the critical surfactant concentration is equal to the equilibrium one ($C_c = C_e$). In this case it is possible to calculate the value of the binding energy Q of an amphiphile molecule in the foam bilayer from an experimentally determined temperature dependence of C_c.

3. EXPERIMENTAL RESULTS AND DISCUSSION

3.1. Materials

The following individual and mixed phosphatidylcholines were used in experiments with foam films: 1, 2-dimyristoylphosphatidylcholine (DMPC) from Fluka, 1, 2-dipalmitoylphosphatidylcholine (DPPC) from Avanti Polar Lipids, and egg phosphatidylcholine (Egg PC) from Sigma. The foam bilayers were formed from water–ethanol solutions. The addition of ethanol (47.5 vol%) increases the critical micelle concentration[21] and ensures sufficiently high concentration of amphiphile monomer. The water–ethanol solutions contained also 0.14 or 0.07 mol dm^{-3} NaCl (Merck), which was necessary for formation of the foam bilayer.[22]

As is well known,[10,23] the fully hydrated dispersions of DMPC, DPPC, and Egg PC undergo a high-energy phase transition due to the melting of hydrocarbon chains of phospholipid molecules. The temperature of this transition for the above-mentioned phospholipids is respectively 23.7° C,[23] 41.8° C,[23] and $-15°$ C.[10] In order to check for possible occurrence of phase transitions in phosphatidylcholine foam bilayers we investigated DMPC, DPPC, and Egg PC foam bilayers in the temperature ranges 16–31° C, 35–45° C, and 15–35° C, respectively.

3.2. Thickness Transition in Phosphatidylcholine Foam Bilayers

The foam bilayers were studied by the microinterferometric method of Sheludko and Exerowa.[17,18] The thickness of horizontal microscopic foam bilayers was determined by measuring the intensity of the light reflected from the foam film. The equivalent thickness h_w was calculated from the data for the intensity of the reflected light,[18] regarding the foam bilayer as an optically homogeneous structure, with refractive index equal to that of the solution from which the foam bilayer was formed.

More accurate analysis of the optical data was done by using the so-called triple-layer model.[24] According to this model, the foam bilayer is conventionally regarded as constituted of three layers. It is accepted that the outer layers of thickness h_1 and refractive index n_1 include the hydrocarbon tails of amphiphile molecules, while the inner layer of thickness h_2 and refractive index n_2 includes the polar head groups of amphiphile molecules and the water core between them. The estimation of the thickness h ($h = 2h_1 + h_2$) according to the triple-layer model was carried out with the help of the following equation:

$$h_2 = h_w - 2h_1[(n_1^2 - 1)/(n_2^2 - 1)] \tag{7}$$

Table I presents the obtained results for foam bilayer thickness. The accuracy of the equivalent thickness determination was $\pm 3\%$.[18] The equivalent thickness of DMPC and DPPC foam bilayers changes significantly at 23° C and 39° C, respectively. These temperatures are very close to those found for the chain-melting phase transition of fully hydrated dispersions of DMPC and DPPC.[23] The slightly lower values for the foam bilayers are probably due to the presence of ethanol in the solutions from which these bilayers were formed. Such an effect was observed for water–ethanol dispersions of DPPC.[25,26] The analysis of the data by the triple-layer model (see Table I) supports the assumption that the temperature-driven change in the foam bilayer thickness is due to the melting of hydrocarbon tails of phospholipid molecules (i.e., to the sharp change in the number of *gauche* conformations of the carbon–carbon bonds). The experimentally obtained difference in the thickness of the hydrocarbon regions for the liquid-crystalline and gel foam bilayers (0.3 nm) seems to be small in comparison with the theoretical estimations for the change in the hydrocarbon chainlength due to their melting. This result can be explained by the assumption that in the gel foam bilayers the hydrocarbon tails of amphiphile molecules are tilted by 30° with respect to the bilayer normal similarly to the tails of the molecules in the lamellar gel phase of fully hydrated dispersions of DMPC and DPPC[28] and in the liquid-condensed state of DPPC monolayers at the air/water interface.[29]

In the case of Egg PC foam bilayers the equivalent thickness h_w was constant within the temperature range investigated (15–35° C), which is in agreement with the lack of phase transition in the fully hydrated dispersions of Egg PC within this temperature interval.[10]

Table I.   Experimental Values of Equivalent Thickness h_W of Foam Bilayers and of Thickness h_1 of Hydrocarbon Regions, h_2 of Inner Polar Region, and h of Foam Bilayer According to the Triple-Layer Model.[a]

	Liquid-crystalline			Gel	
	DMPC	DPPC	Egg PC	DMPC	DPPC
h_w, nm	6.2	6.4	6.7	7.0	7.1
h_1, nm	1.1	1.3	1.4	1.4	1.6
h_2, nm	3.5	3.1	3.2	3.5	3.2
h, nm	5.7	5.7	6.0	6.3	6.4

[a] The data of Yellin and Levin[27] for the number of *gauche* conformations of carbon–carbon bonds are used in the calculation of h_1.

3.3. Binding Energy of a Phospholipid Molecule in the Foam Bilayer

The binding energy of a phospholipid molecule in the foam bilayer was calculated from the experimental data for the temperature dependence of the critical concentration C_c for formation of a foam bilayer.

The determination of C_c was carried out on the basis of the final state which the foam film reaches during its drainage, in particular, rupture at critical thickness without formation of black spots or formation of foam bilayer via black spots. In fact the determination of the critical concentration for formation of foam bilayer was carried out by studying the dependence of the probability W for observation of black spots in the foam film on the bulk phospholipid concentration C at a certain temperature. Such a $W(C)$ dependence, obtained at $10°$ C for foam films stabilized by DMPC, is presented in Fig. 3. It is seen from the figure that at $C < C_c$, $W = 0$ and at $C \geqslant C_c$, $W = 1$.

A special peculiarity of phospholipid foam bilayers which distinguishes them from foam bilayers stabilized by common synthetic surfactants[30] is that the former are infinitely stable at all investigated temperatures and concentrations allowing their formation. In the present study we accepted as infinitely stable a foam bilayer which did not rupture for 5–6 hrs. The high stability of the phospholipid foam bilayers is the cause[20] for the sharp change of the $W(C)$ dependence at C_c (see Fig. 3).

The high stability of phospholipid foam bilayers may be demonstrated also by an attempt to destroy them by α-particle irradiation, since it is well known[31] that the irradiation shortens significantly the mean lifetime of metastable foam bilayers. Such experiments were carried out with DMPC, DPPC, and Egg PC foam bilayers in a special measuring cell,[6] the
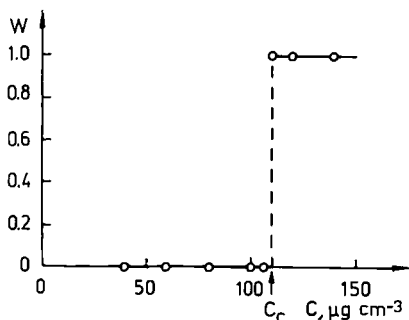

Fig. 3. Dependence of the probability W for observation of a foam bilayer in a foam film at $10°$ C on the concentration C of DMPC in the solution, containing also 47.5 vol% ethanol and 0.07 mol dm^{-3} NaCl.

maximum intensity of irradiation being 700 μC. At all investigated temperatures and concentrations (equal to or slightly higher than C_c) the phospholipid foam bilayers did not rupture.

The critical concentration C_c for formation of DMPC foam bilayer was determined from the experimental $W(C)$ curves at different temperatures; the obtained experimental data are represented by circles in Fig. 4. The high stability of DMPC foam bilayers at C_c allows us to suppose that $C_c = C_e$ and to calculate the binding energy Q of a DMPC molecule in the foam bilayer from the slope of the straight lines in Fig. 4, which represent the best fit of the linearized equation (5) with experimental data. The two values of the binding energy Q of a DMPC molecule in the gel and liquid-crystalline foam bilayers obtained from the slopes of the straight lines are, respectively, $(1.9 \pm 0.1) \times 10^{-19}$ J for temperatures lower than 23° C and $(8.0 \pm 0.2) \times 10^{-20}$ J for temperatures higher than 23° C.

The clearly pronounced change in the slope of the Arrhenius dependence at 23° C corresponds to a sharp change in the value of Q. Obviously this change is due to the chain-melting phase transition of DMPC foam bilayers, detected also by the temperature dependence of the foam bilayer thickness. It is clear that the parameter C_c is very sensitive to the occurrence of first-order phase transitions in foam bilayers and may be used for their registration.

The experimental value of the critical concentration C_c for the formation of DPPC foam bilayers was also determined from the experimental $W(C)$ curves at different temperatures. These data are shown in Fig. 5 by circles and their best fit with the linearized Eq. (5) is shown by the straight
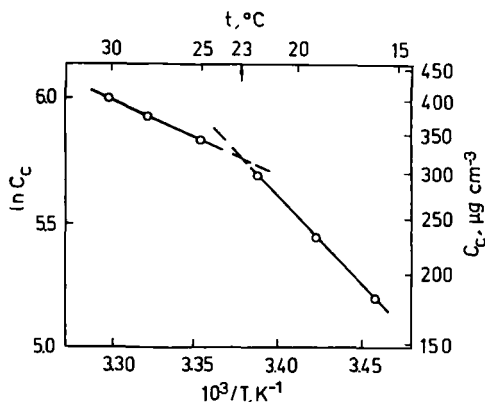

Fig. 4. Arrhenius dependence of the critical concentration C_c for the formation of a DMPC foam bilayer: circles, experimental data; straight line, theoretical dependence, according to Eq. (5) and assuming $C_c = C_e$.

lines. It is seen from the figure that exactly as in the case of DMPC foam
bilayers, the slope of the Arrhenius dependence changes sharply in the
range of the chain-melting phase transition (about 39° C), which corre-
sponds to a change in the binding energy of a DPPC molecule in the foam
bilayer. The values of Q of $(7.1 \pm 0.2) \times 10^{-20}$ J and $(4.9 \pm 0.2) \times 10^{-19}$ J
were calculated under the assumption $C_c = C_e$ for the liquid-crystalline and
gel state of the DPPC foam bilayers, respectively. The value of the binding
energy of a DPPC molecule in the liquid-crystalline foam bilayer should be
regarded as identical with the corresponding value of Q for a DMPC foam
bilayer insofar as one accounts for the narrow experimental temperature
range and for the accuracy of determination of C_c (about 5 %).

In the case of Egg PC foam bilayers the temperature dependence of C_c
(Fig. 6) did not detect the occurrence of a phase transition in the tem-
perature interval investigated, which is in agreement with the data for the
temperature dependence of the foam bilayer thickness. The value of the
binding energy of a phospholipid molecule in Egg PC foam bilayer,
$Q = (1.38 \pm 0.02) \times 10^{-19}$ J, was obtained from the best fit of Eq. (5) with
the experimental data for C_c under the assumption $C_c = C_e$. It is important
to note that this value must be regarded as an effective one, as in the case
of Egg PC foam bilayers it applies for a mixture of unsaturated and
saturated phosphatidylcholines of different chain lengths. The high value of
Q is probably due to the lateral segregation of liquid-crystalline and gel
domains of phosphatidylcholines in the foam bilayer similar to that
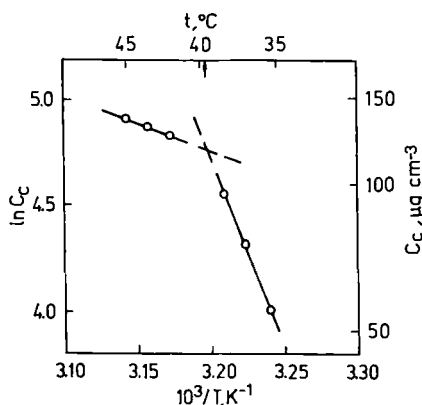observed in Egg PC bilayer lipid membranes.[32]

Fig. 5.   Arrhenius dependence of the critical concentration C_c for the formation of a DPPC
foam bilayer: circles, experimental data; straight line, theoretical dependence, according to
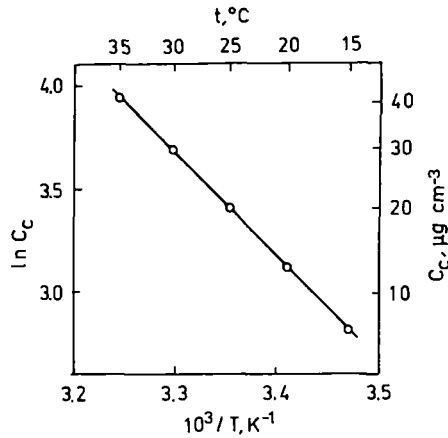Eq. (5) and assuming $C_c = C_e$.

Fig. 6. Arrhenius dependence of the critical concentration C_c for the formation of an Egg PC foam bilayer: circles, experimental data; straight line, theoretical dependence, according to Eq. (5) and assuming $C_c = C_c$.

The energy of lateral interaction ε of two neighboring molecules can be calculated from Q under the assumption that the normal short-range molecular interactions may be neglected in comparison to the lateral ones ($\varepsilon \gg \varepsilon_0$). This assumption is reasonable, bearing in mind the strong van der Waals attraction between the tails of phosphatidylcholine molecules and is in agreement with the analysis of Nagel[16] for the interactions in bilayers. It is reasonable to assume also that the phosphatidylcholine molecules are arranged in a two-dimensional triangular lattice in the foam bilayer, which arrangement is similar to that in the fully hydrated lamellar dispersions[28] and in the monolayers at the air/water interface.[14] As a result of the above assumptions, combined with the theoretical model described in Section 2, we can write $\varepsilon = Q/6$. The values of ε obtained by using this equation are shown in Table II together with the values of Q. Also included in this

Table II. Values of the Binding Energy Q of Phosphatidylcholine Molecule, Lateral Interaction Energy ϵ of Two Neighboring Molecules, and Specific Edge Free Energy γ of Holes for Foam Bilayers of DMPC, DPPC, and Egg PC

	Liquid-crystalline			Gel	
	DMPC	DPPC	Egg PC	DMPC	DPPC
$Q \times 10^{20}$, J	8.0	7.1	13.8	19.0	49.0
$\varepsilon \times 10^{20}$, J	1.3	1.2	2.3	3.2	8.2
$\gamma \times 10^{11}$, Jm^{-1}	1.6	1.4	2.9	4.5	9.1

table are the values of the specific edge free energy γ of holes in the phosphatidylcholine foam bilayers, calculated from the relation $\varepsilon = \gamma d$.[1,33] Here d is the mean distance between the centers of two phospholipid molecules neighboring in the lateral direction.

The analysis of the data for the liquid-crystalline state of foam bilayers, shown in Table II, together with their comparison with the theoretical[16,34-36] and experimental[4,37] values of the corresponding quantities for fully hydrated dispersions of phosphatidylcholines, shows that our experimental results (for example, γ) are reasonable and are in agreement with the data for bulk systems obtained by other authors. At the same time, the values of Q, ε, and γ for the gel state of foam bilayers seem to be higher than expected, given the similarity of interactions in fully hydrated dispersions and foam bilayers. This difference may be attributed to several reasons. First, there are mostly theoretical estimations of interactions in fully hydrated gel dispersions of phospholipids and they cannot be regarded as conclusive. On the other hand, the study of foam bilayers involves some peculiarities related to the experimental procedure (first of all the high ethanol content in the solutions from which the foam bilayers were formed) and to the simple theoretical model described in Section 2.

4. CONCLUDING REMARKS

The experiments with phosphatidylcholine foam bilayers demonstrate that these bilayers may exist in different phase states, corresponding to different condensed states of amphiphile monolayers constituting the foam bilayer.

The temperature dependence of the thickness of foam bilayers shows the occurrence of a first-order phase transition of melting of hydrocarbon tails of the phospholipid molecules. This melting is realized at a temperature very close to the temperature of the corresponding phase transition in fully hydrated water dispersions of phosphatidylcholines. This result is in agreement with the theoretical considerations of Nagel[16] for the decisive role of van der Waals attractions between hydrocarbon chains of phospholipid molecules for the chain-melting phase transition in bilayer systems.

The binding energy Q of a phosphatidylcholine molecule in liquid-crystalline and gel foam bilayers, the lateral interaction energy ε of two neighboring phospholipid molecules, and the specific edge free energy γ of holes in the foam bilayers have been calculated.

These results are a reliable basis for further improvement of the theoretical model here presented and for new experimental research in order to relate better the macroscopic and microscopic characteristics of foam bilayers.

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