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SHORT COMMUNICATION

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# Phospholipid foam films studied by contact angle measurements and fluorescence microscopy

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**Abstract** Foam films drawn from suspensions of the phospholipid 1,2-dimyristoyl-*sn*-glycero-3-phosphorylcholine (DMPC) in water/ethanol mixtures were used for the investigation of the relation between the properties of the monolayers and the interaction between the film surfaces. The film thickness and the contact angle between the film and the meniscus were measured as a function of the temperature in a range around the temperature of the main phase transition for the lipid. Additionally, fluorescence microscopy was applied to investigate the distribution of a fluorescent lipidlike dye in the surface of the film and the meniscus. From the contact angle the free energy of film formation was calculated. At the temperature of the chain-melting phase transition the film thickness decreases by 0.7 nm. This can be related to a decrease in the thickness of the hydrocarbon layers of the lipid monolayers at this temperature. The decrease in the film thickness leads to a reduction in the free energy by increasing the van der Waals attraction between the film surfaces. No structures were observed in the monolayers of the film in the fluorescence investigation. However, on formation of the very thin equilibrium film the dye was expelled from the film area, indicating an increase in the packing density of the lipid, if the monolayers are in adhesive contact in the film.

**Key words** Black foam films · Contact angle · Film thickness · DMPC · Fluorescence microscopy

## Introduction

Phospholipid bilayers are one of the major structural elements of biological membranes. Vesicles [1–3] and monolayers [4–7] have been used as models for the investigation of the structure of bilayers and of the interactions between them. A foam film stabilized by phospholipid is another suitable model for studying the forces between two approaching bilayers [8–15]. The properties of foam films are summarized in several reviews and monographs [16–18]. Foam films consist of an aqueous layer covered with two monolayers of adsorbed amphiphile molecules. The alkyl chains of the amphiphile are directed to the air. The headgroup layers face each other in the film. If electrical double layer interaction is suppressed by the addition of sufficient electrolyte, very thin Newton black films are formed. The aqueous core in such films is very thin and the two amphiphilic monolayers are in an adhesive energetic minimum.

In the case of adhesion of the two monolayers in the film the free energy, $\Delta g^f$, of film formation per unit area is negative and can be determined from measurements of the contact angle between the film and the surrounding meniscus of liquid [16, 19, 20]. It was found that $\Delta g^f$ of Newton black foam films stabilized by the soluble surfactant sodium dodecyl sulphate (SDS) increases with the temperature [19, 21]. In the case of a foam film of

phospholipids the situation is more complicated as it is known that the monolayers experience the chain-melting phase transition. Since the thickness of phospholipid Newton black foam films decreases jumpwise on increasing the temperature above the chain-melting temperature [10], some consequences for $\Delta g^f$ should be expected.

Recently, a concept of "enhanced colloidal interaction" (ECI) has been developed [22], taking into account changes in the composition of the matter in the film and especially in the stabilizing monolayers on approach of the two interacting interfaces. In the case of strongly surface active amphiphiles the ECI theory allows the calculation of the change of the packing density in the monolayers from the free energy of film formation. According to the theory, the packing density of the amphiphile in the stabilizing monolayers of a film should increase when the film thins under the action of attractive forces. Some evidence for this effect in the case of Newton films stabilized with SDS was found. The expected effect of an increase in the packing density is small (of the order of 1%) and is therefore hard to measure with direct methods [23]; however, some properties of the film, such as permeability for gas, lifetime, or solubility of dye probes, may react sensitively to this change.

In a series of articles we have reported investigations of the properties of foam films made from phospholipids [13–15]. The aim of the present work is to determine the dependence of the free energy of formation of black foam films stabilized with 1,2-dimyristoyl-*sn*-glycero-3-phosphorylcholine (DMPC) on temperature and to investigate the influence of $\Delta g^f$ on the packing density of the lipid in the monolayers of the film; therefore, the contact angles of DMPC foam films were measured in the range from 20 to 30 °C. This range includes the main phase transition of DMPC at 23 °C. The packing density of the lipid was investigated by observation of the distribution of a fluorescent dye in the film and the surrounding surface of the meniscus.

## Experimental

### Materials and preparation

DMPC from Fluka was used without further purification. The lipid dispersions were prepared with ethanol (extra pure) from Merck and bidistilled water purified using a Millipore-Q desktop apparatus (pH 5.5, specific resistance 18 MΩ cm). Sodium chloride from Riedel de Haen was roasted at 600 °C for 5 h to remove surface-active contamination. L-α-Phosphatidylcholin-β-(nitrobenzoxadiazole-aminohexanoyl)-γ-palmitoyl (NBD-DPPC) from Sigma was used as a fluorescent dye without further treatment. The structure of the dye molecule is similar to that of DMPC and it is assumed to be less soluble in a densly packed lipid phase [24]. In the experiments a small amount of the dye was used in order to exclude the process of self-quenching [24].

Since the lipids are not soluble in water, lipid monomers in the bulk phase cannot be a source for the formation of a stabilising monolayer; therefore, the films were prepared from suspensions of lipid vesicles in water/ethanol (52.5:47.5% v/v) mixture. The degradation of vesicles delivers the material for densly packed monolayers of DMPC molecules at the film surfaces. The concentration of DMPC in the suspensions was 0.4 g/l in all experiments. The suspensions were prepared by adding the lipid to a water/ethanol mixture with a constant concentration of NaCl (0.07 M). The prepared suspension was kept in a refrigerator overnight. It was sonicated (Sonorex RK52, Bandelin Electronic, Berlin, Germany) for 30 min at 45 °C before the measurements. This procedure leads to the formation of small unilamellar vesicles with diameters below 100 nm [25]. For fluorescence experiments the fluorescent dye was added with concentration of 1 mol% of the total lipid.

### Fluorescence investigation

Fluorescence microscopy investigations were carried out using an Olympus AX 70 universal microscope. The phospholipid films were prepared and investigated in a homemade experimental cell (Fig. 1). The cell was mounted on the table of the microscope. It consists of a metal body and a cover. The body of the cell allows the circulation of water from a thermostat using the connection pipes, thus, the temperature in the cell is set to be constant with an accuracy of ±0.1 °C. The room temperature was adjusted to be equal to the temperature of the measurement, and it was kept constant with an accuracy of ±0.5 °C.

Horizontal microscopic foam films with a diameter of around 0.4 mm were formed in the middle of a biconcave drop in a film-holding glass ring of 4-mm diameter. With a piston made of Teflon the liquid can be sucked off the ring via a capillary. The film holder can be removed easily from the cell, which allows good cleaning. After filling the ring with the suspension, a biconcave drop is formed. Using the piston the liquid is sucked from the drop and the two surfaces approach and a foam film is formed in the middle of the ring. The groove D in the bottom of the cell was filled with the suspension to saturate the atomosphere in the cell with its vapour.

The foam film was illuminated from the top through the objective of the microscope. The probe was irradiated with an excitation wavelength of about 480 nm and it emitted visible light of longer wavelength (of about 530 nm). The illuminating light passes through a quartz window. The bottom of the cell below the film is tilted to avoid superposition of the signal from the film with light reflected from the bottom.

The change in the fluorescence during the process of film drainage was captured by a highly sensitive charge-coupled-device (CCD) camera PROXICAM HR5 (Proxitronic, Bensheim, Germany) and recorded on a video tape.

### Film thickness and contact-angle measurements

The measurements of the thickness and contact angle were performed with a Scheludko-type experimental cell as described elsewhere [13, 18, 26]. The cell was placed in a jacket with circulating water. The temperature in the cell was constant with an accuracy of ±0.1 °C. Additionally, the room temperature was adjusted to be equal to the temperature of the measurement and was kept constant with an accuracy of ±0.5 °C. The temperature in the cell was measured close to the foam film holder using a KELVIMAT 4322-V000 device (Burster Präzisionsmesstechnik, Germany) with an accuracy of ±0.01 °C.

The equivalent foam film thickness ($h_w$) was determined using the microinterferometric method [18, 26] by applying the model of an optical homogeneous film. The contact angle between the film and the surrounding meniscus ($\theta$) was measured using the expansion method [18, 20, 27, 28].

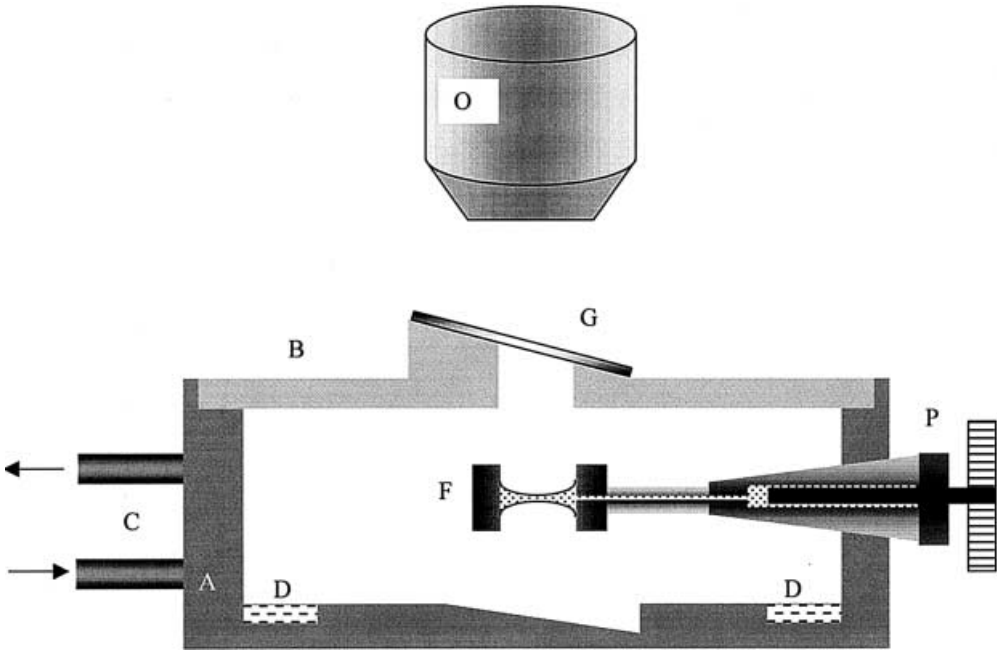


**Fig. 1** Experimental cell for fluorescence investigation of microscopic foam films: *A* – metal body; *B* – cover; *C* – connection pipes for water circulation; *F* – film-holding glass ring of 4-mm diameter connected with a capillary side arm; *P* – piston which consists of a glass jacket and a Teflon rod; *D* – a groove in the bottom of the cell was filled with the suspension to saturate the atmosphere in the cell with its vapour; *O* – objective of the microscope; *G* – quartz window. The bottom of the cell is tilted with respect to the normal direction of the incident light. This avoids observation of the reflection of the incident excitation light

Surface tension measurements

For the determination of the surface tension of the suspensions a tensiometer (KM5, Lauda, Germany) equipped with a du Nouy ring made from platinum–irridium of 6-cm circumference was used. The surface tension was measured at every temperature investigated in the range from 20 to 30 °C. A constant equilibrium value of the surface tension of about 27 mN/m was reached after a few minutes for every temperature.

## Results and discussion

The contact angles between foam films stabilized by the lipid DMPC and the surrounding meniscus were measured in the range of temperature from 20 to 30 °C. The dependence of the contact angle and the equivalent thickness of the foam films on the temperature is shown in Fig. 2. Both dependencies clearly show the influence of the chain-melting phase transition of the DMPC monolayer on the film properties. The film thickness decreases and the contact angle increases abruptly at around 23 °C, where the chain-melting transition of DMPC occurs [1, 29]. Our values of the film thickness are slightly (0.5 nm) larger than that given in Ref. [10]; however, the general behaviour is in good agreement.

The free energy of film formation, $\Delta g^{\mathrm{f}}$, is defined as

$$\Delta g^{\mathrm{f}} = \int_{h_2}^{\infty} \Pi(h)\mathrm{d}h + \Pi h_2 \ . \tag{2}$$

Here, $\Pi$ is the disjoining pressure in the foam film.

The free energy is connected with the contact angle between the film and surrounding meniscus by

$$\Delta g^{\mathrm{f}} = 2\sigma(\cos\theta - 1) \ . \tag{3}$$

The foam film thickness $h = 2h_1 + h_2$ is composed [30, 31] of the solvent core thickness, $h_2$, and the thickness of the hydrophobic chain on the DMPC molecule, $h_1$. The solvent core thickness can be obtained from the experimentally measured equivalent solution film thickness, $h_{\mathrm{w}}$ using the equation [30, 32]

$$h_2 = h_{\mathrm{w}} - 2h_1 \frac{n_1^2 - 1}{n_2^2 - 1} \ , \tag{4}$$

Here $n_1$ and $n_2$ are the refractive indices of the adsorbed monolayer and the solution core, respectively. The monolayer thickness $h_1$ could be obtained from ellipsometry [14, 33] or from X-ray diffraction [34] measurements on monolayers of the lipid. The value of $h_1$ obtained with pure water as the subphase at temperatures higher than the temperature of the main phase transition is 1.15 nm and the tilt angle is around 30° [35]. The lipid layers become more condensed in the presence of ethanol [14]. This leads to a decrease in the tilt angle to 19° [36]. From this we find for the thickness of the hydrocarbon layer $h_1$ = 1.3 nm in our system for temperatures above the temperature of the main phase transition of DMPC. At temperatures below that of the main phase transition it is accepted [29] that the thickness of the hydrocarbon layer increases by 30%, which leads to, $h_1$ = 1.6 nm. These values of $h_1$ were used to calculate the total thickness of the film, $h$, and the thickness of the solution core, $h_2$. The results obtained for $h_2$, $h$ and $\Delta g^{\mathrm{f}}$ are presented in Table 1.

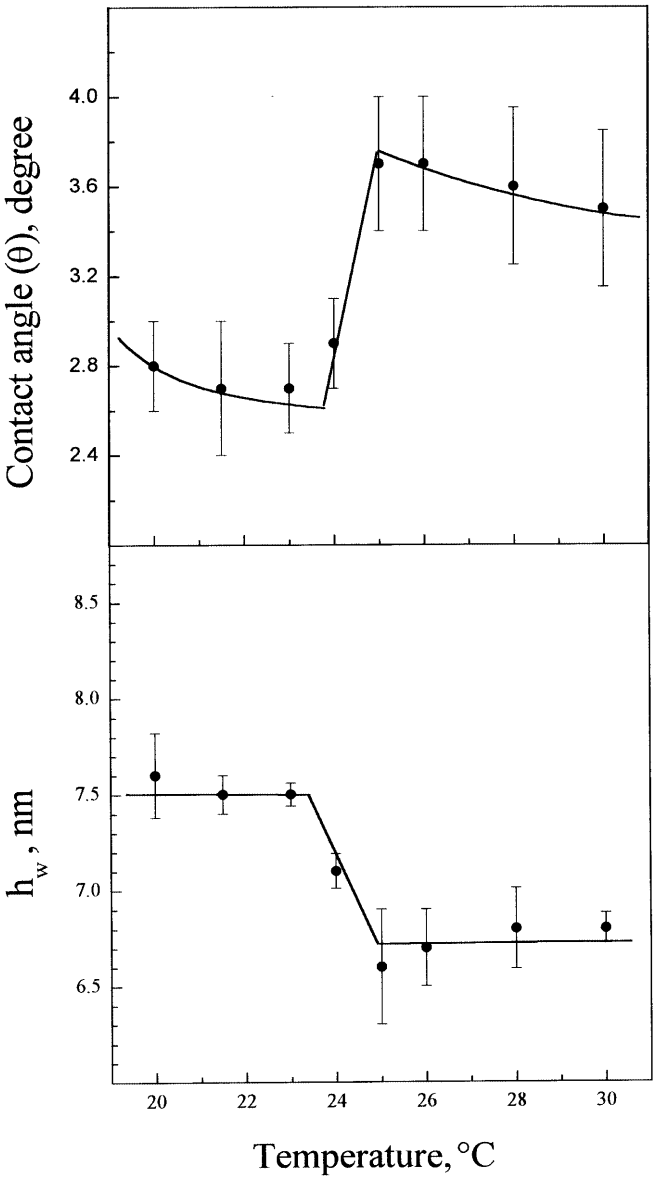


**Fig. 2** Equivalent foam film thickness $h_w$ and contact angle $\theta$ as a function of the temperature for foam films prepared from DMPC (0.4 g/l) and NaCl (0.07 M) in water/ethanol (52.5:47.5% v/v) mixture

The variation of $\Delta g^f$ shows that the attractive interaction between both interfaces of the foam film stabilised by DMPC increases above the temperature of the main phase transition. This is expected in the case of film interaction composed from van der Waals attraction between the film surfaces and a steep hydration or steric repulsion between the layers of the head groups of the lipid molecules as discussed in our earlier work [14]. The dependence of the film formation free energy of Newton black foam films from solutions of SDS on the temperature is reported in Ref. [21]. The thickness of the

**Table 1** Values of the foam film thickness, $h$, the water–core thickness, $h_2$, and the free energy of film formation, $\Delta g^f$, as a function of the temperature

| Temperature (°C) | $h_2$ (nm) | $h$ (nm) | $\Delta g^f$ (mJ/m$^2$) |
|---|---|---|---|
| 20 | 3.3 | 6.5 | −0.063 |
| 21.5 | 3.2 | 6.4 | −0.061 |
| 23 | 3.2 | 6.4 | −0.060 |
| 24 | 2.8 | 6.0 | −0.068 |
| 25 | 3.1 | 5.7 | −0.112 |
| 26 | 3.2 | 5.8 | −0.110 |
| 28 | 3.3 | 5.9 | −0.111 |
| 30 | 3.3 | 5.9 | −0.104 |

Newton black foam films does not change with the temperature, but a decrease in the absolute value of $\Delta g^f$ with increasing temperature was found. The dependence of $\Delta g^f$ on temperature is dominated in our case by the phase transition; however, the results may be described by a superposition of a weak decrease in the absolute value of $\Delta g^f$ with the temperature and an abrupt increase at the phase transition temperature (Fig. 2).

The ECI theory [22] enables, in principle, the calculation of the change in the packing density of the amphiphile molecules in the stabilising monolayers of the film from the measured contact angles. The chemical potential of the amphiphile molecule has to be known for this purpose. In the case of lipid monolayers obtained from suspensions this quantity cannot be estimated with sufficient accuracy; therefore, were are not able to calculate the possible difference in the packing density between monolayers of the film and that at the surface of the bulk phase. However, from the pure existence of a contact angle we expect an increasing packing density in the film surfaces in comparison to a monolayer on the bulk phase of the same composition, in agreement with the ECI theory.

To prove the above-mentioned idea we investigated the distribution of the fluorescent dye probe NBD-DPPC at the surface of the film and at the surface of the meniscus around the film. It is known that the dye is located in the more expanded phase of the lipid monolayer and that is not soluble in the more condensed phase [24]. The changes in the distribution of the fluorescent dye are shown in the process of film drainage to the thin equilibrium state in Fig. 3. The contrast is caused by the difference in the concentration of the dye, as its solubility decreases with an increasing packing density of the lipid monolayer. A lower intensity of the fluorescent light indicates areas with a higher packing density than in areas with higher fluorescence intensity.

The intensity of the fluorescence is homogeneously distributed in the thick film just after film formation. As the film becomes thinner dark areas appear in the film. When the final state of the thin equilibrium film is reached (Fig. 3d) the film is homogeneously dark. This

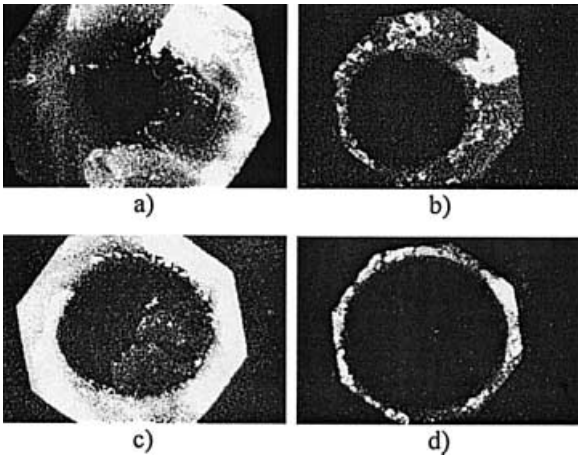


**Fig. 3a–d** Fluorescence microscope images taken at different stages of the film drainage

indicates the expulsion of the dye out of the film monolayers. We conclude that this expulsion is caused by a higher packing density of the lipid in the film surfaces similar to the case of formation of domains of the amphiphile molecules in the two-phase coexistence range of monolayers. Such a conclusion is in agreement with the results presented in Refs. [37–39] for lateral diffusion of a fluorescent dye in the plane of lipid monolayers which form the film. These results also confirm that the monolayers are more condensed in the case of Newton black foam films than in thick and in common black foam films.

## Conclusions

A decrease in the film thickness above the temperature of the main phase transition of DMPC was found in agreement with earlier results [10]. The decrease in the film thickness is a result of the decrease in the thickness of the layer of hydrocarbon chains of the lipid. The dependence of the contact angle of the film on the temperature shows that a decrease in the film thickness is connected with a decrease in the free energy of film formation.

Fluorescent images from the process of thinning of foam films qualitatively demonstrate that bringing the two interfaces of the film in contact leads to condensation of the lipid molecules not only in the direction normal to the interface but in a lateral direction as well. These results confirm the prediction of the ECI theory [22] for the increasing packing density of the film monolayers at an adhesive contact.

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