Foam films stabilized with lysophosphatidylglycerol in the presence of Na$^+$ and Ca^{2+}

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Abstract: This work presents investigation of foam films stabilized with soluble phospholipid palmitoyl lysophosphatidylglycerol (LPG). The films have been studied by measuring the thickness/electrolyte concentration and disjoining pressure/thickness isotherms in the presence of Na$^+$ and Ca^{2+} in the solution. At low NaCl we have obtained formation of thick silver-colored films with equilibrium thickness. With the increase of the concentration of Na$^+$ the films became thinner and at 0.5 mol dm^{-3} their equilibrium thickness was ~9 nm (common black films). Foam films in the presence of Ca^{2+} followed the same dependence, but at concentration of electrolyte 5×10^{-2} mol dm^{-3} and higher, Newton black films were formed. The formation of equilibrium Newton black films happened with "jump" from silver films during the thinning process. The isotherms of disjoining pressure for both electrolytes had shown lightly decrease for the thickness of the films, without any transitions.

Key words: lysophospholipids, black foam films, disjoining pressure isotherms of LPG

INTRODUCTION

Many studies, using different techniques, have been carried out on model bilayer systems, as is well known the cell membrane is mainly constituted of zwitterionic phospholipids [1]. Lysophospholipids attract the interest of the investigators, because of the role they play in the functioning of biological membranes. Also their presence in alveoli in small amounts is important for the dysfunction of pulmonary surfactant [2].

Phosphatidylglycerol is a second major phospholipid in pulmonary surfactant which constitutes ~10% of the surfactant phospholipid pool. Phospholipase-mediated surfactant hydrolysis may disrupt surfactant function by generation of lysophospholipids and free fatty acids and/or depletion of native phospholipids. Small amounts of lysophosphatidylcholine, lysophosphatidylethanol-amine, lysophosphatidylglycerol (LPG) are generated by this mechanism [2].

Although not nearly as well studied as protein inhibition of pulmonary surfactant, an inhibition mechanism involving unsaturated membrane phospholipids, lysophospholipids, and other amphipathic molecules has been identified [3]. Lysophospholipids are phospholipids containing a single fatty acid chain per molecule and are generated by phospholipase A2 secreted by white blood cells and likely type II cells, particularly during respiratory distress syndrome (RDS), an important cause of neonatal mortality of premature newborns [4]. Such lipid substances can be considered diluents of the specific surfactant lipid assembly. Thus, insertion and mixing of these unsaturated amphipathic lipid and fatty acid molecules with the surfactant phospholipid molecules would significantly fluidize the phospholipid monolayers and could promote early collapse, thus preventing low surface tension from being reached. The inactivation due to lipid penetration cannot be effectively overcome by raising surfactant concentration [3].

A long time ago thin liquid films have been studied as a good model on stability of colloidal systems in liquid dispersion media. Thin liquid films form when two compartments of disperse phase (droplets, or bubbles, or particles) come close to each other. Foam films between two gas phases are an example of symmetrical thin liquid films. The most important factor which determines the properties of foam films is the interaction between the two adjacent phases across the liquid film. The thermodynamic quantity disjoining pressure Π is a result of these attractive or repulsive interactions due to different types of surface forces acting in the films [5]. The thicker common black film appears at lower electrolyte concentrations. The electrostatic double

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layer repulsion is suppressed at higher electrolyte concentrations, and the equilibrium state in this case is a very thin Newton black film. Once this state is reached, the film thickness is independent on the electrolyte concentration and is only determined by the direct interaction of the surfactant adsorption layers by short-range surface forces. Although the mechanism of Ca^{2+} binding by charged phospholipids is not completely clarified, it seems that it depends on the structure and properties of the polar head groups.

During the last three decades have been published several data about foam films stabilized by biosurfactants, such as pure phospholipids. After that, lipid and lipid–protein thin liquid films gained a significant interest and many papers and books proved the foam films as an adequate model system for studying of pulmonary surfactant [6].

The aim of this work was to study foam films stabilized with LPG with microscopic foam films in the presence of monovalent and divalent ions. For that purpose the equilibrium thicknesses of the obtained foam films have been measured at different conditions (electrolyte concentration, external pressure).

EXPERIMENTAL

Materials. Palmitoyl lysophosphatidylglycerol (LPG), purchased from Avanti Polar Lipids, was used in the experiments.
NaCl (Suprapur) and $CaCl_2 \cdot 2H_2O$ (p.a.) were obtained from Merck. NaCl was roasted at 500 °C for 4 h to remove surface active contaminations. $CaCl_2 \cdot 2H_2O$ (p.a.) was used as obtained.
All solutions were prepared with triple distilled water. All experiments were carried out at a temperature of 22 °C.
Methods. The measurements were performed using the well-known microinterferometric experimental technique [7]. All films in our experiments were formed with the same radius, 0.01 cm. The capillary pressure, Pσ, in these films was determined as Pσ = 2σ/R, where σ is the surface tension and R is the radius of the glass capillary, where the microscopic horizontal film forms in the center of a double-concave drop.
The measuring cell of Scheludko and Exerowa [7] has proven to be a suitable and reliable tool for formation of microscopic horizontal foam films. It is presented in Figure 1. The foam film **c** is formed in the middle of a biconcave drop **b**, situated in a glass

tube **a** of radius R, by withdrawing liquid from it (variant A) and in the hole of porous plate **g** (variant B). The periphery of the foam film is in contact with the bulk phase, i.e. with the solution from which it is obtained.
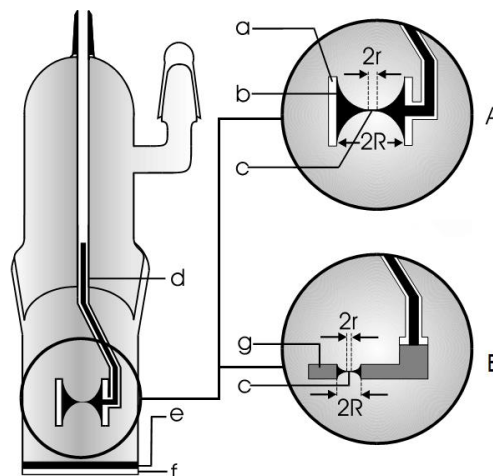

Fig. 1. Scheme of the measuring cell of Scheludko and Exerowa for the study of microscopic foam films; **A**: in a glass tube; **B**: in a porous plate; **a** - glass tube film holder; **b** - biconcave drop; **c** - microscopic foam film; **d** - glass capillary; **e** - surfactant solution; **f** - optically flat glass; **g** - porous plate. (taken from D. Exerowa, P. Kruglyakov, *Foam and foam films*, Elsevier, Amsterdamm, 1998.)

Classical optics provides relations that can link the thickness of the film with its optical characteristics. If foam films are observed in white light, it can be seen that during thinning their coloration changes periodically. Initially the process runs rather rapidly and gradually slows down. Such a course of the interference can be registered as a curve photo-current/time in which the extrema correspond to the interference maxima and minima, i.e. film thicknesses are divisible to λ/4n (where λ is the wavelength and n is the refractive index). Film thickness (between a maximum and a minimum) is calculated from the ratio between the intensities measured of the reflected monochromatic light I, corresponding to a certain thickness, and I_{max}, corresponding to the interference maximum [7].
The direct measurements of the disjoining pressure isotherms were performed by means of the specially designed "porous-plate technique" which has repeatedly been used in microscopic foam film studies. The external pressure that balances the pressure in the film was applied utilizing the special membrane pump and measured with an accuracy of ±5 Pa [8].

The processing of the interferometrically obtained photometric data yields the so-called equivalent film thickness, which is found considering the film as homogeneous with an index of refraction equal to the refraction coefficient of the solution from which the film is obtained, in our case 1.33. The accuracy of the so determined microscopic thin liquid film thicknesses is ±0.2 nm.

According to the classical DLVO theory, $\Pi = \Pi_{el} + \Pi_{vw}$, where Π_{el} is the electrostatic and Π_{vw} is the van der Waals component of the disjoining pressure Π. The following expression for the van der Waals component of disjoining pressure in a symmetric film bordering gas or condensed phases is obtained $\Pi_{vw} = -K_{vw}/h^3$, where K_{vw} is the van der Waals-Hamaker constant [7]. Calculations for Π_{el} were based on asymptotic equations for 1:1 electrolyte [10] and 2:1 electrolyte [11]. For electrolyte solution the thickness of diffusive electric double layer (κ^{-1}) for aqueous solution can be calculated with the following equation: $\kappa^{-1} = \left(\dfrac{\varepsilon_0 \varepsilon k T}{2\rho_{\infty i} z_i^2 e^2} \right)^{1/2}$ [9].

RESULTS AND DISCUSSION

The results of measurements of equilibrium thickness of foam films from LPG as a function of NaCl concentration are shown in Figure 2. The investigations were carried out at two different phospholipid concentrations (C_s) - 2.4×10^{-6} mol dm⁻³ and 1.5×10^{-5} mol dm⁻³. For both surfactant concentrations at low electrolyte concentration (C_{el}) thick equilibrium films were formed and gradually decreased in thickness with the increase in C_{el}. At $C_{el} = 5\times10^{-2}$ mol dm⁻³ a transition to common black film formation occurred. Further increase of C_{el} did not affect the type of black films. The thickness is slightly decreased to ~9 nm at 0.5 dm mol⁻³ NaCl. The dependence of the foam film thickness by reducing the concentration of the electrolyte follows a similar tendency, but at the higher concentration of lipid lies entirely up of the curve for the lowest C_s. These findings are in accordance with previous studies with mixtures between uncharged and charged lipids in which have shown [13] that the thickness of the investigated films increases with the concentration of the charged surfactant.

An experimental scatter of about ±3 nm was observed only in the region of formation of the thicker silver-colored films at the low C_s. In all other experiments the experimental film thickness values were obtained within the accuracy of the

microinterferometric technique. According to the frame of experimental error there is no significant difference in the thickness of the films.
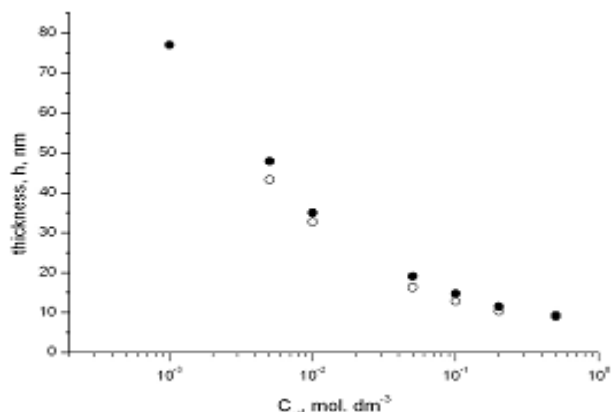

Fig. 2. Equilibrium thickness (h, nm) of microscopic foam films stabilized with LPG (C_s) as a function of NaCl concentration (C_{el}): ○ $C_s = 2.4\times10^{-6}$ mol dm⁻³; ● $C_s = 1.5\times10^{-5}$ mol dm⁻³.
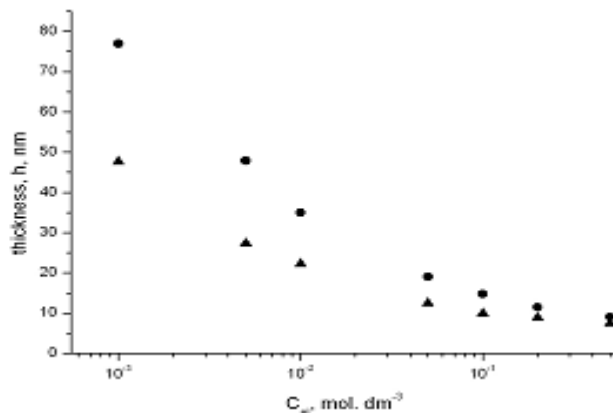

Fig. 3. Equilibrium thickness (h, nm) of microscopic foam films, stabilized with LPG ($C_s = 1.5\times10^{-5}$ mol dm⁻³) as a function of $CaCl_2$ concentration (▲). For comparison are given data at the same C_s from Figure 2 in the presence of NaCl concentration (●).

The effect of $CaCl_2$ on equilibrium thickness of the foam films studied is shown in Figure 3. The results are compared at the same figure with the results, obtained with NaCl at the same C_s for LPG. The experiments were carried out only with $C_s = 1.5\times10^{-5}$ mol dm⁻³.

At $C_{el} = 5\times10^{-3}$ mol dm⁻³ the thinning of foam films leads to formation of common black films without observation of black spots. Curiously, the process of thinning changed dramatically at $C_{el} = 5\times10^{-2}$ mol dm⁻³ where Newton black films were observed. The transition from thick silver films to Newton black films happens without observation of black spots. At $C_{el} = 0.5$ mol dm⁻³ $CaCl_2$ the

thickness ~7.5 nm. Probably, in the case when the positive Ca^{2+} bind to the phospholipids head group, they reduce the initially negative surface charge and lead to weaker repulsive electrostatic interactions than in the case with NaCl added. That's why the comparison of the results for Na^+ and Ca^{2+} shows that the presence of divalent ions lead to significant decrease of the thickness of foam films.

Important information on foam film interactions have been obtained from directly measured disjoining pressure isotherm. All experiments were carried out at $C_s = 1.5 \times 10^{-5}$ mol dm^{-3} LPG. The results obtained with 1×10^{-4} mol dm^{-3} NaCl added and 2×10^{-4} mol dm^{-3} $CaCl_2$ added are shown in Figures 4 and 5, respectively.
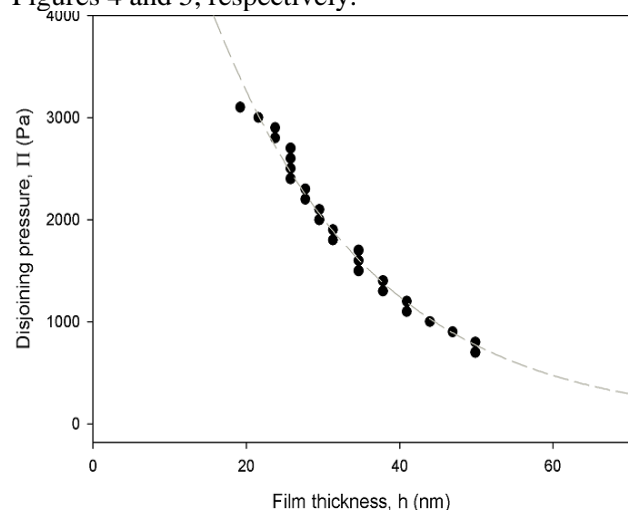

Fig. 4. $\Pi(h)$ isotherm (with guideline), measured at $C_s = 1.5 \times 10^{-5}$ mol dm^{-3} LPG with $C_{el} = 1 \times 10^{-4}$ mol dm^{-3} NaCl added.

As seen in Figure 4, the increase in the applied pressure leads to a gradual decrease of the equilibrium thicknesses. At pressure 3.1 kPa we have measured ~19 nm film thickness. No "jump" transitions have been detected. The course of the obtained isotherm with $CaCl_2$ added, shown in Figure 5, is similar to the obtained with NaCl although the C_{el} is different (in Figure 4). The film thickness at pressure 3.1 kPa we have measured is lower ~14.5 nm.

According to classical DLVO theory we are able to determine the diffuse electric layer φ_o at the film interfaces. Obtained values for φ_o in the measurements with NaCl is 2,45 mV and for solution with $CaCl_2$ is 1,7 mV. For low potentials, below 25 mV [9], the potential becomes proportional to the surface charge density. The magnitude of the Debye length depends solely on the properties of the

solution and not on any property of the surface such as its charge or potential. For solutions with Ca^{2+} the obtained value for κ according to estimated predictions is 0.08035 nm^{-1} and as for the experimental data estimated value is twice lower - 0.04373 nm^{-1}. When recalculating the concentration of Ca^{2+}, based on the theory for Debye length, we obtain estimated very low value ~ 6.10^{-5} mol dm^{-3}. These data compared with low potential for solution with Ca^{2+} can be explained with further interaction between divalent ions and phosphate residues from the polar head of the lipid molecules [10].

As in previous reports for negative-charged lipids [12] our observations also indicate that Ca^{2+} has a marked effect on the nature of the bilayer surface.
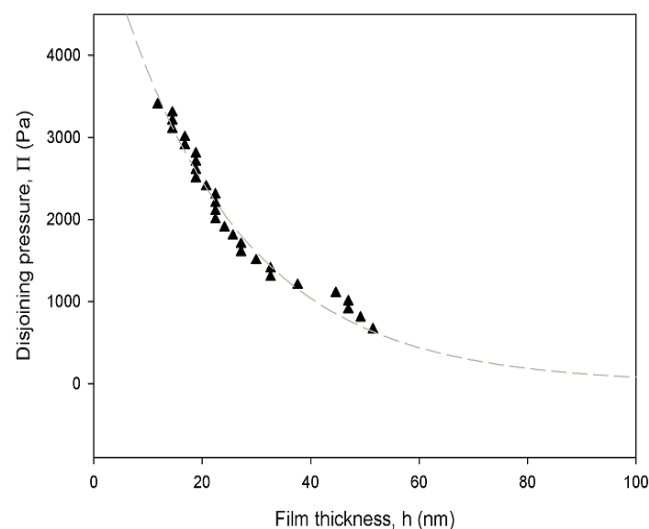

Fig. 5. $\Pi(h)$ isotherm (with guideline), measured at $C_s = 1.5 \times 10^{-5}$ mol dm^{-3} LPG with $C_{el} = 2 \times 10^{-4}$ mol dm^{-3} $CaCl_2$ added.

Probably, apart from double layer interactions, other more specific factors like bridging, conformational changes might play an important role.

CONCLUSION

This work shows properties of thin liquid films stabilized with charged phospholipids in the presence of monovalent and the influence of divalent ions on the properties of the foam films. The process of thinning of the equilibrium thickness of the films of LPG in the presence of Na^+ with the increase of the electrolyte concentration results in the formation of the common black films. At higher concentration of the lipid, were measured thicker films and the effect is significant at middle electrolyte concentrations. The possible explanation may be

founded in the repulsion forces between the layers in the film structure.

The study of the foam films of LPG in the presence of Ca^{2+} shows a very strong interaction of these ions with the polar group of the lipid molecules resulted in formation of Newton black films at higher concentration of electrolyte. The equilibrium thickness in these very thin foam films probably depends on the short-range interactions between the monolayers of LPG. This statement is supported by recalculated and much lower concentration of electrolyte from obtained experimental results, obtained low value for φ_o and low value for k. Probably, the reason is in decisive influence of phosphoric polar head group of lipid structure in connection with Ca^{2+}. In the case when the positive Ca^{2+} bind to the phospholipid head group, they reduce the initially negative surface charge and lead to weaker repulsive electrostatic interactions than in the case with NaCl added.

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ПЕННИ ФИЛМИ ОТ ЛИЗОФОСФАТИДИЛГЛИЦЕРОЛ В ПРИСЪСТВИЕ НА Na⁺ И Ca²⁺

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(Резюме)

Лизолипидите привличат интереса на изследователите заради участието и значението им при функционирането на биологичните мембрани. Тази работа представя изследване на пенни филми, стабилизирани с разтворимия фосфолипид палмитинов лизофосфатидилглицерол. Проучени бяха зависимостта на дебелината на филма от концентрацията на електролита в присъствие на натриеви и калциеви йони в разтвора, както и изотерми на разклиняющо налягане. При ниска концентрация на NaCl се образуват дебели сиви филми с равновесна дебелина. С увеличаване на концентрацията на Na⁺ филмите изтъняват и при 0.5 mol dm⁻³ достигат до равновесна дебелина около 9 nm (обикновени черни филми). Пенните филми в присъствие на Ca²⁺ следват същата зависимост, но при концентрация на електролита по-висока от 5×10⁻² mol dm⁻³ се образуват нютонови черни филми (НЧФ). Вероятно, равновесната дебелина на тънките НЧФ зависи от близкодействащи сили между взимодействащите монослоеве от лизолипида във филма. В подкрепа на това твърдение е преизчислената много по-ниска стойност на концентрацията на електролита, стойността на потенциала, както и Дебаевата дебелина. Изотермите на разклиняющо налягане и при двата електролита показват плавно изтъняване на филмите без преходи.