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Phospholipid free thin liquid films with grafted poly(ethylene glycol)-2000: formation, interaction forces and phase states

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Abstract

Free thin liquid films (foam films) formed from aqueous dispersions of dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylethanolamine with covalently bound poly-(ethylene glycol) of molecular weight 2000 (DPPE-PEG-2000) were studied by the thin liquid film microinterferometric technique of Scheludko and Exerowa in the temperature range 14–36°C. The surface tension kinetics of the dispersions were studied in order to ensure equilibration of the foam films. These measurements showed that the rate of surface coverage depends slightly on the temperature and does not reach equilibrium values within reasonable time intervals for the dispersions containing only one amphiphile (DPPE-PEG-2000). The destruction of the vesicles at the air/(aqueous dispersion) interface was much faster for the dispersions containing DMPC/DPPE-PEG-2000 mixtures above 23°C, the temperature of the chain-melting phase transition of the main lipid component (DMPC). The dependence of the equilibrium thickness of the foam films on the electrolyte concentration was measured for 1 and 9 mol% DPPE-PEG-2000 at 28°C in the range 10^{-3} to 0.5 M NaCl. These results indicate that at the low electrolyte concentrations the electrostatic and van der Waals interactions are dominant similar to the foam films stabilized with DMPC alone. At the high electrolyte concentrations the steric repulsion of the PEG layers becomes dominant. The temperature–composition dependence of the bilayer thickness was measured for the foam bilayers at 0.14 M NaCl. The data for the foam bilayer thickness and the comparison with the phase diagrams of PC/PE-PEG dispersions, show that the DMPC/DPPE-PEG-2000 foam bilayers are able to exist in two phase states characterised by different conformations (mushroom and brush) of the grafted polymer.

Keywords: Foam film; Polymer; Phospholipid; Phase transition; Interaction

1. Introduction

It is now well established that the inclusion of phospholipids with grafted polyoxyethylene (polyethylene glycol, PEG) chains into the surface of phospholipid liposomes to form the so-called 'Stealth' liposomes prolongs liposome circulation in the blood stream and greatly increases the potential use of liposomes for drug delivery [1–5]. The stabilization of Stealth liposomes is attributed to the action of steric forces arising from the pendent PEG chains. These forces have been studied between lipid bilayers with grafted polymer adsorbed on mica surfaces [6] and between lipid bilayers with grafted

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polymer in multilamellar dispersions [7]. Following the work of deGennes [8], several regimes of interaction between adsorbed polymers on surfaces can be identified. These depend on the distance between the polymer chains (D) relative to the dimensions of the polymer as characterised by the Flory dimension, R_F, which is related to the monomer size (persistence length) 'a' and the number of monomer units in the polymer 'N', such that $R_F = a\ N^{3/5}$. Three regimes are detected in [7] for the polymers grafted to the lipid lamella: (1) when $D > 2R_F$ (interdigitated mushrooms); (2) $D < 2R_F$ (mushrooms); and (3) $D < R_F$ (brushes) in the case of bilayers incorporating PEG-grafted lipids. The conditions for the transitions between these regimes depend on the PEG molecular mass and on the grafting density of the polymer on the bilayer. For the mixed dispersion of distearoylphosphatidylcholine (DSPC) and distearoylethanolamine with grafted PEG-2000 (DSPE-PEG-2000) the transition between the mushroom and brush regimes occurs when the bilayer contains approximately 4 mol% DSPE-PEG-2000 [7]. We have shown that this transition affects the permeability of phospholipid liposomal bilayers to glucose causing a maximum in the permeability/grafting density dependence at approximately 4.5 mol% DPPE-PEG-2000 [9,10].

The free thin liquid films (foam films) consist of two amphiphile monolayers contacting a gas phase and an aqueous core between them of thickness dependent on the interaction forces which prevail under the experimental conditions. These films are an appropriate model for studying membrane fusion [11], interlamellar interactions [12,13], membrane permeability [14], lung surfactant [15], amniotic fluid [16,17], liposome destruction at the air/solution interface [18] and phospholipid phase transitions [19,20].

The aim of the present work was to study the formation, interaction forces and phase states of foam films formed from dispersions of dimyristolphosphatidylcholine (DMPC) and DPPE-PEG-2000. The thickness of the foam films was measured at different temperatures, DPPE-PEG-2000 grafting density and salt concentrations using the thin liquid film microinterferometric technique of Scheludko and Exerowa [21,22].

2. Materials and methods

2.1. Materials

1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) was obtained from Fluka Chemie, Buchs, Switzerland and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[poly(ethylene glycol)-2000] (DPPE-PEG-2000) from Avanti Polar Lipids, Birmingham, AL, USA, both materials were used as supplied. Quartz-bidistilled deionized water (specific conductivity $10^{-6}\ \Omega^{-1}\ \mathrm{cm}^{-1}$) and sodium chloride (Merck), roasted at 500°C for 2 h prior to use in order to remove surface active impurities, were used in the dispersion preparation. Analytical grade methanol, ethanol and chloroform were used.

2.2. Dispersion preparation

Dispersions of multilamellar vesicles (MLV) were prepared by the following procedure: 12 mg of lipid mixture was dissolved in 4 ml chloroform and 1 ml methanol. The solvent was rotary evaporated at 30°C to yield a thin lipid film. The lipid film was hydrated at 35°C in water or aqueous solution of NaCl. The mean size of the vesicles was within the 50–200 nm range [9].

2.3. Surface tension measurements

The surface tension kinetics of lipid adsorption from DPPE-PEG-2000 and DMPC/DPPE-PEG-2000 dispersions at the air/(aqueous dispersion) interface was followed by a digital tensiometer K10T (Kruss) with an accuracy of 0.1 mN m^{-1}.

2.4. Thickness determination

The foam films were studied by the thin liquid film microinterferometric technique of Scheludko and Exerowa (e.g. [21,22]). A microscopic horizontal foam film of radius 0.1 mm was formed after equilibration at 36°C in the middle of a biconcave drop of dispersion in a special glass measuring cell [23] by removal of liquid from the drop. The thinning of the foam film was followed through the optically flat glass bottom of the cell covered with a thin layer of dispersion. The equivalent water thickness h_w was calculated [24,25] from the data for the intensity of the light reflected from the film. The mean value of the thickness was determined from the data for 5–10 films. The equivalent water thickness is higher than the true thickness due to the layer structure of the film and the associated refractive index variations [26–28]. This difference is essential for the thinnest foam films (the foam bilayers). In this case, the real thickness is estimated with the aid of optical models which regard the foam film as constituted of several layers of different refractive indices. In the case of DMPC/DPPE-PEG-2000, foam bilayers the film can be regarded as consisting of two lipid layers of thickness h_1, two PEG-2000 layers of thickness h_2 and an aqueous layer of thickness h_3. According to this 5-layer model the relation between h_w and the film thickness $h = h_3 + 2h_2 + 2h_1$ is given by [26,29]:

$$h = h_w - 2h_1(n_1^2 - n_3^2)/(n_3^2 - 1) - 2h_2(n_2^2 - n_3^2)/(n_3^2 - 1)$$

where the refractive index of the lipid layer n_1 was taken as 1.464 [28], the refractive index of the polymer layer n_2 was estimated from the data for the refractive indices of PEGs of various molecular masses and water contents [30,31] and the refractive index of the aqueous layer n_3 was taken as 1.333.

3. Results and discussion

3.1. Adsorption at the air/water interface

The surface tension kinetics was studied in order to find experimental conditions for fast adsorption and equilibration of the film surfaces ensuring high reproducibility of the results. Fig. 1a shows the change in surface tension of aqueous dispersions containing 225 μg cm^{-3} DPPE-PEG-2000 at 17°C (curve 1) and 27°C (curve 3) as a function of time. For this single amphiphile the decrease in surface tension was small and slow and reached almost a constant value after approximately 2 h. Similar behaviour was observed for mixed dispersion containing the same amount DPPE-PEG-2000 (9 mol%) and 575 μg cm^{-3} DMPC (91 mol%) at 17°C as seen from curve 2. The decrease in surface tension was much greater and faster for the same dispersions at 27°C, i.e. above the chain melting temperature of DMPC (23°C [32]). The variation of the DPPE-PEG-2000 content within 1–9 mol% did not change dramatically the adsorption kinetics. Fig. 1b presents the

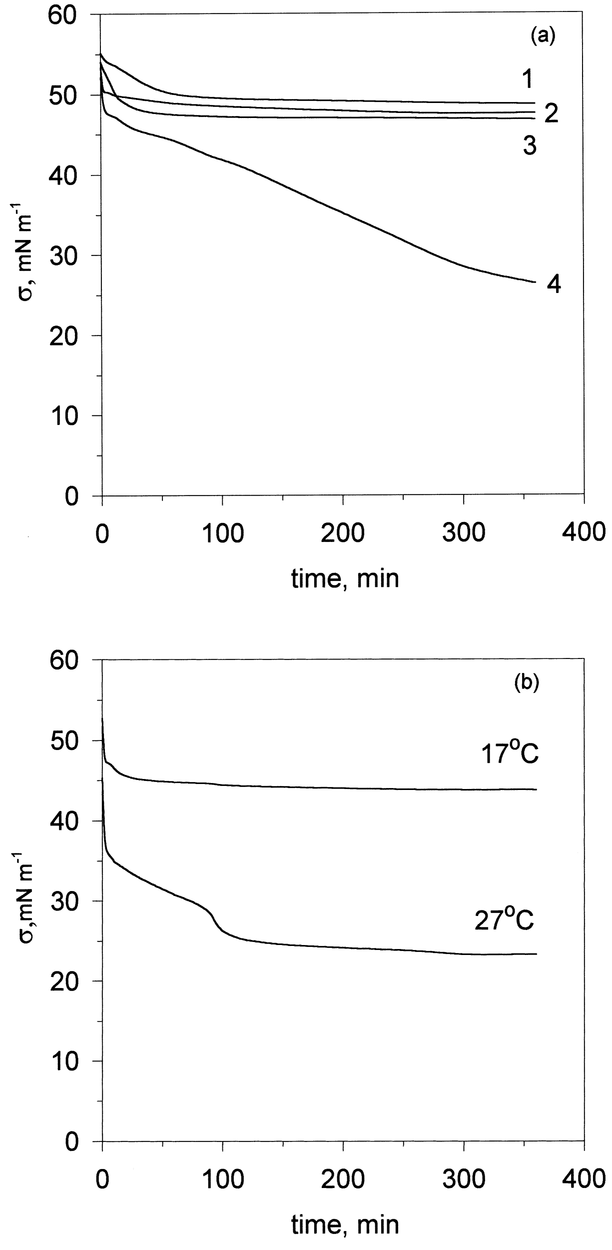

Fig. 1. Surface tension kinetics of aqueous dispersions containing DPPE-PEG-2000 and 0.14 M NaCl with and without DMPC: (a) 225 μg ml^{-1} DPPE-PEG-2000 at 17°C (curve 1) and 27°C (curve 3); 225 μg ml^{-1} DPPE-PEG-2000 (9 mol%) plus 575 μg ml^{-1} DMPC (91 mol%) at 17°C (curve 2) and 27°C (curve 4); and (b) 31 μg ml^{-1} DPPE-PEG-2000 (1 mol%) plus 769 μg ml^{-1} DMPC (99 mol%) at 17 and 27°C.

surface tension kinetic data for a dispersion containing 1 mol% DPPE-PEG-2000 (31 μg cm^{-3}) and 99 mol% DMPC (769 μg cm^{-3}). Similar to the dispersion with higher DPPE-PEG-2000 content, the surface tension lowering was large only above the chain melting temperature of DMPC and equilibrium was approached between 300 and 400 s. The equilibrium value of surface tension at 27°C (24 mN m^{-1}) was used for the calculation of the capillary pressure in the foam film measuring cell. The surface tension measurements demonstrate that the phase state of the phospholipid is crucial for the rate of coverage of the surface by the amphiphiles. A similar result was found for pure phosphatidylcholines [18,20]. Thus, a substantial adsorption was found for the DMPC/DPPE-PEG-2000 dispersions above the chain melting temperature of DMPC which is essential for the formation and the stability of the foam films.

3.2. Interaction forces

The interaction forces in the foam films formed from the DMPC/DPPE-PEG-2000 dispersions were studied by measuring the equilibrium equivalent water thickness (h_w) of the films at different electrolyte contents. The films were studied at 28°C after equilibration at the same temperature and the h_w values for 1 and 9 mol% DPPE-PEG-2000 are shown in Fig. 2a and b, respectively. These results are compared with previous measurements of the thickness of pure DMPC foam films [13] shown in the same figures. The curves are characterized by a low value of the critical electrolyte concentration for formation of foam bilayer (about 0.05 M NaCl) which is typical for foam films stabilized by non-ionic surfactants. At low electrolyte concentration the capillary pressure in the cell is balanced with the electrostatic repulsion and van der Waals attraction. With the increase of the electrolyte concentration the electrostatic repulsion is reduced and the steric repulsion of the adsorption layers becomes manifested.

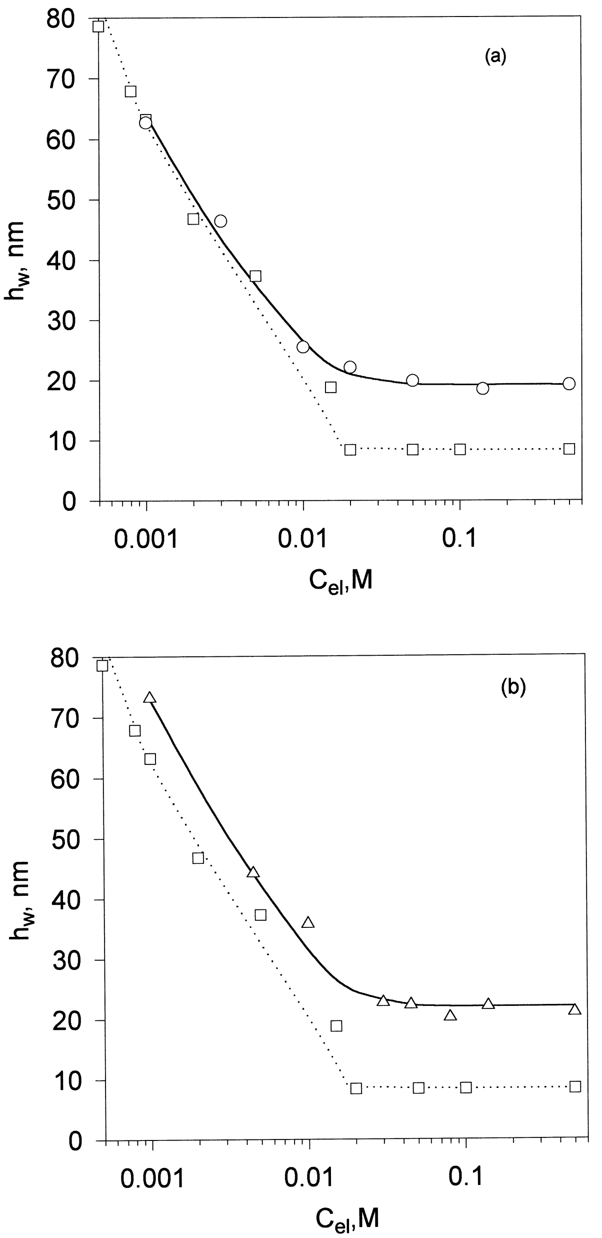

Fig. 2. Dependence of the equivalent water thickness h_w of foam films on NaCl concentration C_{el} at 28°C. The foam films were formed from dispersions containing: (a) ○, 31 μg ml^{-1} DPPE-PEG-2000 (1 mol%) plus 769 μg ml^{-1} DMPC (9 mol%), □, pure DMPC (data from [13]); and (b) △, 225 μg ml^{-1} DPPE-PEG-2000 (9 mol%) plus 575 μg ml^{-1} DMPC (91 mol%), □, pure DMPC (data from [13]).

Table 1
Thickness characteristics of DMPC/DPPE-PEG-2000 foam films

DPPE-PEG-2000 (mol%)	h_1 (nm)	h_2 (nm)	h_3 (nm)	h (nm)	h_w (nm)
1 (0.14 M NaCl)	1.8	3.5	6.9	17.5	19.2
9 (0.14 M NaCl)	1.8	6.5	2.9	19.5	21.7
1 (1 mM NaCl)	1.8	3.5	50.4	61.0	62.7
9 (1 mM NaCl)	1.8	6.5	60.3	70.9	73.1

At an electrolyte concentration of 1 mM NaCl the foam film thickness can be used to estimate the surface potential and surface charge density in the films. The thickness of the films was calculated by using the five-layer model. It was supposed that similarly to the lipid bilayers with grafted polymer adsorbed on mica surfaces [6] and in multilamellar dispersions [7] the polymer can exist in two conformations – mushroom (at the lower surface densities) and brush (at the higher surface densities). The thickness difference of the foam bilayers at 1 and 9 mol% DPPE-PEG-2000 was attributed to this phenomena. The thickness h_2 of the polymer layer for the two regimes was taken from [6]. The refractive index n_2 of the polymer layer was estimated from reference data [30,31] on the basis of evaluation of the polymer concentration in the polymer layers of thickness h_2. In this evaluation, we used the data for the area of a DMPC molecule in the liquid crystalline state [33] and of a PE molecule [6] in the gel state. The estimated values of n_2 are 1.335 for 1 mol% DPPE-PEG-2000 and 1.345 for 9 mol% DPPE-PEG-2000. The values of h_w, h_1, h_2, h_3 and h are shown in Table 1 for 10^{-3} and 0.14 M NaCl.

The linear Poisson–Boltzmann equation was used for determination of the electrostatic term and the van der Waals component was calculated according to Donners et al. [34,35]. In these calculations, the border between the hydrophilic and hydrophobic part of the foam film was chosen between the polar head group and hydrocarbon chain of the phospholipid. The thickness of the films was taken from Table 1. For the films containing 1 and 9 mol% DPPE-PEG-2000 the surface potential was found to be 27 and 48 mV and the surface charge 2 and 4 mC m^{-2}, respectively. The surface potential of the foam films containing 1 mol% DPPE-PEG-2000 is close to the estimations of 36 mV for pure DMPC foam films [13] and 29 mV for ABA triblock copolymer of polyethylene oxide and polypropylene oxide foam films [36]. The lower value of the surface potential of the foam films containing 1 mol% DPPE-PEG-2000 with respect to the DMPC foam films is due to the scattering of experimental results and to the different assumptions employed in the model calculations.

The thickness of the DPPE-PEG-2000 foam bilayers was determined in the presence of 0.14 M NaCl and 47.5 vol% ethanol in order to check the contribution of solvation forces at short distances. The equivalent thickness h_w of the foam bilayers was determined to be 20.5 nm for the mushroom regime and 15.0 nm for the brush regime. In this case, similarly to the DPPC foam bilayers [20] the solvent core between the surfactant molecules is thinner in the presence of ethanol which indicates a lower hydration of the polar parts of the lipid and lipid–polymer molecules.

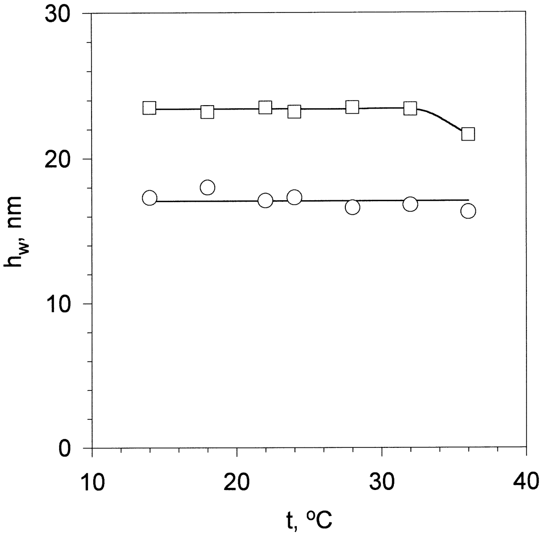

Fig. 3. Dependence of equivalent water thickness h_w of foam bilayers (C_{el} = 0.14 M NaCl) on temperature. The foam films were formed from dispersions containing: ○, 87 μg ml^{-1} DPPE-PEG-2000 (3 mol%) plus 713 μg ml^{-1} DMPC (97 mol%); □, 225 μg ml^{-1} DPPE-PEG-2000 (9 mol%) plus 575 μg ml^{-1} DMPC (91 mol%).

3.3. Phase states of the foam bilayers

The data for the thickness of the foam bilayers at 1 and 9 mol% DPPE-PEG-2000 showed clearly that these bilayers can exist in two different phase states characterized by the different conformation of the polymer molecules (mushroom and brush). Experiments were performed at various temperatures and polymer grafting densities in order to obtain the regions of existence of the two phase states of the foam bilayers.

The effect of temperature on the equivalent water thickness at 3 and 9 mol% DMPC/DPPE-PEG-2000-DMPC foam films is shown in Fig. 3. There is a

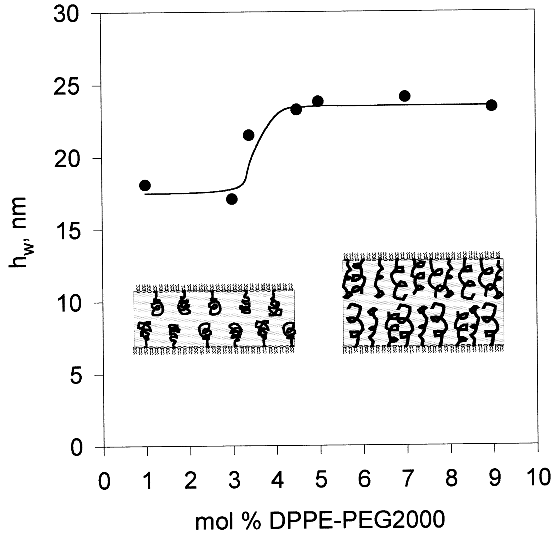

Fig. 4. Dependence of the equivalent water thickness h_w of DMPC foam bilayers ($C_{el}=0.14$ M) on the DPPE-PEG-2000 content at 18°C.

marked difference in the thickness between these films although apart from the data at the highest temperature for the 9 mol% DPPE-PEG-2000 film the thicknesses are independent of temperature. A similar thinning above 30°C was observed for all films containing 3–4 mol% and greater DPPE-PEG-2000.

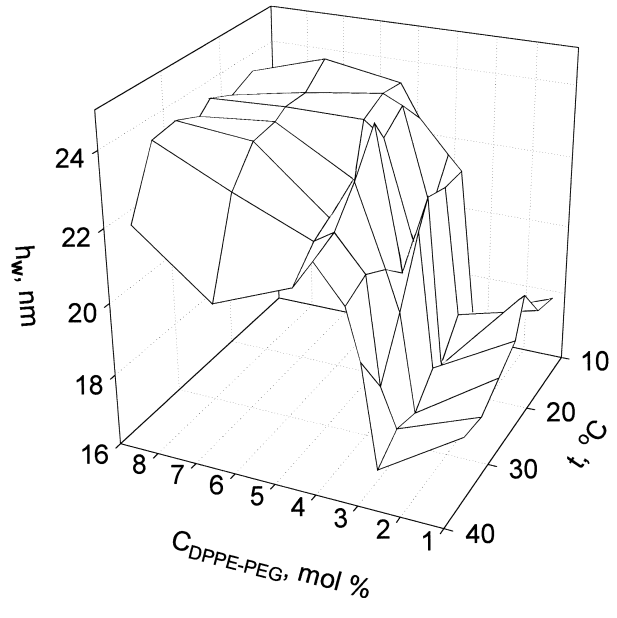

Fig. 5. Dependence of the equivalent water thickness h_w of DMPC/DPPE-PEG-2000 foam bilayers ($C_{el}=0.14$ M NaCl) on temperature and DPPE-PEG-2000 content.

The effect of the polymer grafting density on the equivalent water thickness h_w at 18°C is seen by the sharp change in the region of 3–4 mol% DPPE-PEG-2000 (Fig. 4). This value is lower than the one obtained in [7] as a result of the higher surface area of the main lipid component (DMPC). More comprehensive thickness data as a function of both mol% DPPE-PEG-2000 and temperature at a constant salt concentration of 0.14 M is shown in Fig. 5. The transition in the range 3–4 mol% DPPE-PEG-2000 between the two phase states of the foam bilayers is seen at all temperatures studied. A change of h_w was not detected in the region of the chain-melting phase transition temperature of DMPC (23°C) within the limits of the experimental accuracy (data not shown).

4. Conclusion

The measurement of the surface tension of DMPC/DPPE-PEG-2000 and DPPE-PEG-2000 liposomal dispersions show that the phase state of the phospholipid is the predominant factor determining the kinetics of vesicle disruption and lipid adsorption at the air/(aqueous dispersion) interface.

DMPC foam films modified by DPPE with chemically bound PEG-2000 were studied at different compositions and temperatures. The foam films were formed after equilibration at temperatures above the chain-melting phase transition of the main lipid component DMPC ensuring fast coverage of the air/(aqueous dispersion) interface. The electrostatic repulsion is the main interaction in the foam films formed at low electrolyte contents while the steric repulsion is dominant above 0.02 M NaCl. Estimates of the double layer potential show an increase in potential with mol% DPPE-PEG-2000. The addition of 47.5 vol% ethanol causes a lower thickness of the foam films due a reduction in solvation of the polar parts of the lipid and lipid–polymer molecules. The existence of two phase states of the foam bilayers is demonstrated. The transition between them occurs at 3–4 mol% DPPE-PEG-2000. These states are characterized by different conformation of the polymer molecules (mushroom or brush) as conceived by deGenne [8].

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