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INTERACTION OF DMPE-PEGS WITH PHOSPHATIDYLCHOLINE AND SUGAR ESTER SURFACTANT IN MONO- AND BILAYER FILMS AT THE AIR/WATER INTERFACE

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ABSTRACT. In this work Thin Liquid Films (TLFs) and monolayers at the air/water interface formed by Dimyristoyl phosphatidylcholine (DMPC) or sugar ester surfactant L-1695 pure and mixed with Dimyristoyl phosphatidylethanolamine (DMPE) Linked Poly Ethylene Glycols were compared with DMPE-PEG pure films.

DMPE-PEG concentration (C) in the film forming dispersions was- <10 mol% (dispersions mainly of liposomes), 20 mol% (liposomes/micelles mixture) and 80 mol% (completely micellar dispersions).

All DMPE-PEGs (DMPE-PEG_{550}, DMPE-PEG_{2000} and DMPE-PEG_{5000}) pure formed stable Black TLFs. DMPE-PEG_{550} formed Common Black Films (CBFs) by trivial fluctuation Black Spot (BS) mechanism (characterized by film thinning time t_{0-1} and BS expansion time t_{1-2}), while DMPE-$PEG_{2000/5000}$ formed CBF-like films by continuous thinning (characterized by film total formation time t_{0-2}) due to steric repulsion increase. Mixed with DMPC Newton Black Films (NBFs), DMPE-PEG_{550} increased t_{0-1} and t_{1-2} and transformed NBFs to thicker CBFs, while DMPE-$PEG_{2000/5000}$ changed TLF formation mechanism and CBF-like films were formed. $C_{DMPE-PEG}$ at which these effects occur decreased with increasing PEG Mw. The threshold concentration (C_t) of DMPE-PEG containing films decreased with increasing PEG mol% and Mw. Lowest $C_t=4.10^{-6}$ M was reached for DMPE-PEG_{5000} films. Thus TLFs permit to define new quantitative parameters for comparing DMPE-PEGs membrane forming properties.

Compared with DMPC monolayers, DMPE-PEG containing films showed (proportionally to DMPE-PEG mol% and Mw) improved formation kinetics and lower surface tension and compression/decompression cycle hysteresis area values.

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Similar results were obtained with L-1695 films.

INTRODUCTION

PEG moieties of Phospholipid Linked PEGs, extended in „mushroom“ (at low PL-PEG surface concentration) or „brush“ (at high PL-PEG surface concentration) chain conformation from the membrane plane towards the water solution, form at the membrane surface thick hydrophilic layer (Chapman and Jones, 1995; Kuhl et al. 2004; Johnson and Edwards, 2001). It was responsible for the increased steric disjoining pressure among PL Linked PEG coated surfaces measured with Surface Force Apparatus Technique (Kuhl et al., 1994). Thus the hydrophilic layer creates a steric barrier preventing the adsorption of lipoproteins and opsonins to the liposome surface, making the liposomes invisible for the Reticulo Endotelial System responsible for the uptake of foreign particles out of the blood flow. Thus so called „stealth“ liposomes are obtained, with increased stability and prolonged life time in the blood flow circulation (from hours to days), making them promising drug vehicles (Chapman and Jones, 1994; Johnson and Edwards, 2001). Stealth liposomes were used as vehicles of previously known anticancer and antifungal drugs (doxorubicin, alphameticin, etc.) for which increased pharmacological effect and decreased toxicity were obtained (Chapman and Jones, 1994). It was found also that apart from „stealth“ bilayer liposomes, PL Linked PEGs, pure or mixed (at high mol content) with phospholipids, form „stealth“ normal monolayer micelles, which also could be used as drug vehicles (Szeifer et al., 1998; Marsh , 2001; Johnson and Edwards, 2001; Montessano et al., 2001; Belsito et al., 2003). Recently PL Linked PEGs were used for obtaining of „stealth“ erythrocytes as universal blood substituent (Szeifer et al., 1998).

Two model membrane systems widely used for studying the effect of membrane active compounds are Phospholipid Thin Liquid Films and Monolayers at the air/water interface. Thin Liquid Films, being of several types, are composed of two mutually adsorbed, plane-parallel, oriented „head-to-head“, PL monolayers (Fig. 1) thus being structurally analogous to the *cis*-monolayers apposition occurring at the onset of membrane fusion and at close intermembrane adhesion (Naydenova et al., 1990; Exerowa and Krugliakov,1998). Despite of the successfull use of phospholipid TLFs as model system (Lalchev, 1997; Exerowa and Krugliakov,1998) in biology and medicine (regarding membrane interactions, fusion and adhesion processes, bio-surfactant action at interfaces, etc.) up to date there are only two studies (Nikolova and Jones, 1996; Nikolova and Jones, 1998) of TLFs formed by Dimyristoyl Phosphatidylethanolamine linked PEGs concerning film thickness and gas permeability. In these works the hydrodynamic behavior and the stability of the films was not discussed. PL monolayers (Fig. 1D) can be regarded as a half of TLFs or Black Lipid Membranes (Lalchev, 1997; Lalchev, 2004) and appears to be a preferred model for studying the interactions among phospholipids in the membrane plane.

Fig.1

The aim of the current work was to study Thin Liquid Films (TLFs) and monolayers at the air/water interface formed by Dimyristoyl phosphatidylcholine (DMPC)/sugar ester surfactant L-1695 pure and mixed with Dimyristoyl phosphatidylethanolamine (DMPE) Linked Poly Ethylene Glycols. The stability and hydrodynamic behavior of pure DMPC films were compared with those of PEG-containing TLFs. Surface tension (equilibrium and at compression/decompression) of pure DMPC/L-1695 monolayers and PEG-containing monolayers was also measured. TLFs and monolayers formed by pure DMPE Linked PEGs were also obtained and their properties were compared with those of DMPC pure and DMPC/DMPE-PEG mixed films. The film forming dispersions were composed of two types of particles, liposomes and micelles, which ratio depends of DMPE-PEG mol content (Montesano et al., 2001; Belsito et al., 2003). Both PL Linked PEGs (DMPE-PEG_{550}, DMPE-PEG_{2000} and DMPE-PEG_{5000}) and Free PEGs (PEG-400, PEG-1500, PEG-2000, PEG-5000) used were with PEG moiety Mw < 8000. DMPC was chosen because it is commonly used PL for preparation of pharmacologically active liposomes and one of the main constituents of the biological membranes (Chapman and Jones, 1995). L-1695 was used due to the fact that it is commonly used in pharmacological and food preparations.

MATERIAL AND METHODS

Material

Dimyristoyl phosphatidylcholine (DMPC), Dimyristoyl phosphatidylethanolamine (DMPE)-PEG_{550}, DMPE-PEG_{2000} and DMPE-PEG_{5000} were purchased from „Avanty Polar Lipids“ and L-1695 were purchased from „Sigma“. NaCl was purchased from „Merck“. Solutions were made with bidestilled water with conductivity less than 1 μS.

Determination of the type of the particles composing film forming dispersions

Three types of the film forming dispersions were used- mixture of DMPC with < 10 mol%, 20 mol % and 80 mol % DMPE-PEG. Experiments were done according to the protocol of Montesano et al. (Montesano et al., 2001) and Belsito and al. (Belsito et al., 2003) by measuring with SPEKOL -11 the optical density at 400 nm (OD_{400}) of dispersions of DMPE-PEGs pure or mixed with DMPC.

Thin Liquid Films (TLFs)

TLFs were formed by the method of Scheludko and Exerowa (Exerowa and Krugliakov,1998) using the modified measuring cell of Lalchev et al. as previously

described (Lalchev, 1997). A biconcave drop (50 μl volume) of the phospholipid dispersion (pH=6.8-7.0; C_{el}=0,5 M NaCl) was incubated into the cylinder of the measuring cell at T=37°C for 30 minutes. After sucking the solution from the drop thick TLF is formed (Fig. 1). Further the film spontaneously gets thinner and after some characteristic film thinning time, t_{0-1} (sec), critical film thickness (300 Å) is reached. Then a Black Spot (BS), local thinning in the film, appears (as schematically shown in Fig.1 A), expands with characteristics rate to fill up the whole area of the film. The kinetic of this process was measured by BS expansion time t_{1-2} (sec) detecting the time from the formation of the first black spot to the moment of its expansion to the whole film area, i.e. to black TLF formation. At different experimental conditions two types of black films is possible to be formed- common black films, CBFs, (Fig. 1B) and Newton black films, NBFs, (Fig. 1C).

The probability (W) for formation of stable black films depends strongly on the phospholipid concentration, C, (Lalchev, 1984; Exerowa and Krugliakov,1998) and can be calculated by the equation $W=\Delta N / N$, where N is the total number of trials (at least 50 for each concentration) and ΔN is the number of trials in which stable black films are formed. Thus, W varies between 0 and 1 indicating that the films always rupture (W=0) and that the films always are formed stable (W=1). The dependence W(C) is extremely steep which allowed to define a threshold concentration (C_t) as the minimum phospholipid concentration at which W=1 and stable films are always formed (Lalchev, 1984). It is proven that W(C) dependence is sensitive to the composition of the film forming dispersion, molecular shape and phase state of the film forming PLs, pH, electrolyte concentration, applied pressure, etc. (Lalchev, 1997; Exerowa and Krugliakov,1998).

Monolayers

Monolayers (spread and adsorbed) of DMPC pure or mixed with DMPE Linked or Free PEGs were formed in the Langmuir through and the surface tension γ (mN/m) was measured by the method of Wilhelmy with accuracy ±0.5 mN/m, as previously described (Christova et al., 1998). The surface tension- equilibrium, maximum and minimum (after 100 to 20 % compression/decompression of the film surface area), and the histeresis area (A_H) during compression/decompression cycling of the monolayer were measured. Experiments were made at T=37°C, pH 6.8-7.0 and electrolyte concentration C_{el}=0,5 M NaCl.

RESULTS AND DISCUSSION

Thin Liquid Films

Optical density measurements for evaluation the ratio of liposomes and micelles, composing pure and mixed with DMPC dispersions of DMPE-PEGs

Experiments were done with film forming dispersions of DMPE-PEGs pure or mixed with DMPC, composed of two types of particles- liposomes or micelles. The type and the ratio of the dispersion forming particles was regulated by varying of DMPE-PEG mol content and was determined experimentally by measuring the optical densities (OD_{400}) of the dispersions at 400 nm (Fig.2).

Fig.2

The technique was applied by Montesano et al. (Montesano et al., 2001) and Belsito and al. (Belsito and al., 2003) for determining the type and the ratio among the particles composing dispersions of DPPE linked PEGs mixed with DPPC. OD_{400} (DMPE-PEG mol%) dependence is shown at Fig.2. The upper region of the plot curved part corresponds to dispersions consisting predominantly by liposomes (Montesano et al., 2001; Belsito and al., 2003). With increasing of DMPE-PEG content OD_{400} decreases due to the decreased size of the dispersion forming particles transforming from bilayer liposomes to monolayer normal micelles. When the whole dispersion is composed by micelles the plateau region of minimum OD_{400} is reached. It can be seen that the plateau region was reached at lower concentration for DMPE-PEG_{2000} (35 mol %) in comparison with DMPE-PEG_{550} and DMPE-PEG_{5000} (73 mol %). Similar nonlinear dependence of the particle transformation from liposomes to micelles on PEG moiety molecular weight was also observed for dispersions of DPPE-PEGs with DPPC (Montesano et al., 2001; Belsito and al., 2003). In the current work for most of the experiments with DMPE-PEG containing dispersions, three concentrations of DMPE linked PEGs were used- < 10 mol% (where dispersions are formed predominantly by liposomes), 20 mol % (where probably mixture of liposomes and micelles exist in the dispersions) and 80 mol % (where DMPC/DMPE-PEG dispersions consist of micelles only).

Thin Liquid Films

TLFs of DMPE-PEGs pure and mixed with DMPC

W(C) curves of Thin Liquid Films of pure DMPC and pure DMPE-PEG dispersions are shown in Fig.3 A and B.

Fig.3

The threshold concentrations of DMPC/DMPE-PEGs mixed dispersions are listed in Table 1. It can be seen that all DMPE linked PEGs used formed stable Black Films at threshold concentration values lower than the threshold concentration for DMPC films ($2{,}9.10^{-4}$ M DMPC). For DMPE-PEGs pure films the value of C_t decreased with increasing of PEG moiety length and molecular weight and the lowest value of C_t (4.10^{-6} M) was reached for DMPE-PEG_{5000}. It is important to note that at

constant electrolyte concentration (C_{EL}=0,5 M NaCl) the type of the Black Films formed by DMPE-PEGs and DMPC were different (see Table 1). DMPC films were Newton Black Films. Only the films of the shortest DMPE linked PEG-550 were Common Black Films. Both CBFs and NBFs were formed by the common mechanism of fluctuation BS formation (Exerowa and Krugliakov, 1998). However the films of longer chain DMPE-PEG_{2000} and DMPE-PEG_{5000} were formed not by the common fluctuation BS formation mechanism (Exerowa and Krugliakov, 1998), but by continuous thinning without BS formation until black, we called, CBF-like films were obtained. That behaviour could be explained in terms of very strong steric repulsion disjoining pressure arising due to the overlapping of hydrophilic polymer „brushes". Increased steric repulsion disjoining pressure was measured with Surface Force Apparatus between PL linked PEG_{2000} coated surfaces by Kuhl et al. (Kuhl et al., 1994) and in TLFs stabilised by DPPE-PEG_{2000}, and by DMPC/ DPPE-PEG_{2000} mixture (Nikolova and Jones, 1998) or by three-block co-polymeric surfactants (Exerowa and Krugliakov, 1998). However these studies (Nikolova and Jones, 1996; Nikolova and Jones, 1998) did not discuss the formation mechanism of the Black Films. Black Film formation by continuous thinning, due to steric repulsion between the apposed surfaces was previously observed in Films by long chain polyalcohols (Exerowa and Krugliakov, 1998).

Table 1.

The threshold concentration values and the formation mechanisms (Table 1) of mixed films of DMPC and DMPE-PEGs depend both on DMPE-PEG content and on the PEG moiety length and molecular weight. The Ct of mixed films was lower than that of DMPC films and higher in comparison with pure DMPE-PEG films. Analogously to DMPE-PEG Black Films, the lowest C_t value ($0{,}5.10^{-5}$ M) was obtained in presence of the longest chain PL linked PEG- for 80 mol% DMPE-PEG_{5000}. When DMPE-PEG concentration was increased at some minimal DMPE-PEG concentration ($C_{DMPE\text{-}PEG}^{min}$) Thin Liquid Films became to thin not to NBFs (characteristic for DMPC), but to thicker CBFs and CBF-like films, characteristic for DMPE linked PEGs (Table 1). CBFs were formed at $C_{DMPE\text{-}PEG550}^{min} \geq 9$ mol %, and CBF-like films formed by continuous thinning were obtained at ≥ 7 mol % DMPE-PEG_{2000} and at ≥ 3 mol % DMPE-PEG_{5000}. Both DMPE-PEG_{2000} and DMPE-PEG_{5000} induce TLF thinning to CBFs (without thinning to truly bilayer NBFs) but at extremely low concentrations (less than 0,1 mol %) so the minimal DMPE-$PEG_{2000/5000}$ concentration for this film thinning „pre-transition" was not determined. For the three DMPE-PEGs the minimal $C_{DMPE\text{-}PEG}^{min}$ was lower than 10 mol %, i.e. the film forming dispersions were composed mainly by liposomes (Montesano et al., 2001; Belsito et al., 2003). It can be seen that $C_{DMPE\text{-}PEG}^{min}$ decreased with increasing of PEG moiety molecular weight. The values of C_t of the mixed films for the minimal DMPE linked PEG concentrations are shown at Table 1.

Fig.4

The hydrodynamic behavior of mixed DMPC/DMPE-PEG films also strongly depend on the mol % of DMPE linked PEG. The results concerning film thinning time (t_{0-1}) and Black Spot expansion time (t_{1-2}) of DMPC/ DMPE-PEG_{550} films are shown in Fig. 4 A and B. It can be seen (Fig. 4A) that t_{0-1} increased linearly from 30 sec (for DMPC pure films at 0 mol % DMPE-PEG_{550}) to 40 sec (at 42 mol % DMPE-PEG_{550}). At 9 mol% DMPE-PEG_{550} when films became to thin to CBFs (but not to NBFs, as DMPC films) t_{0-1} =35 sec. For DMPE-PEG_{550}>42 mol% plateau value of t_{0-1} was observed. The value of BS expansion time, t_{1-2} (Fig. 4B), steeply increased from 1 sec (for DMPC pure NBFs) to plateau value of 6 sec (at ≥30 mol % DMPE-PEG_{550}). At DMPE-PEG_{550} concentration (9 mol% DMPE-PEG_{550}) when films became to thin to CBFs, t_{1-2} value was 4 sec.

Fig.5

Fig.5 A, B and C represents the dependence of film thinning time and BS expansion time of films containing DMPE-$PEG_{2000/5000}$ (pure and mixed with DMPC) on DMPE-$PEG_{2000/5000}$ mol %. For concentrations up to 7 mol % DMPE-PEG_{2000} and up to 3 mol % DMPE-PEG_{5000}, CBFs were observed with t_{0-1} (Fig.5 A) linearly increasing to 35 sec (for both DMPE-linked PEGs) and t_{1-2} (Fig. 5 B) reaching 8 and 10 sec, respectively. At ≥ 7 mol % DMPE-PEG_{2000} and ≥ 3 mol % DMPE-PEG_{5000}, TLFs became to thin to CBF-like films. In this case, when the film is formed by continuous thinning we define a new parameter of CBF formation called total film formation time t_{0-2} (Fig. 5 C). The value of t_{0-2} increases from 45 sec to a plateau values of 75 sec (at 80 mol % DMPE-PEG_{2000}) and 85 sec (at 75 mol % DMPE-PEG_{5000}) equal to the total film formation times of pure DMPE-$PEG_{2000/5000}$ films, respectively.

L-1695 TLFs pure and mixed with DMPE linked PEGs

When the molecular ratio DMPE-PEG-2000/L-1695 is more than 10 (Fig. 6), stable CBFs were formed not by the common fluctuation BS formation mechanism, but by continuous thinning without BS formation. Similar behaviour explained in terms of very strong steric repulsion disjoining pressure arising due to the overlapping of hydrophilic polymer „brushes“ is observed in FFs stabilised by DMPE-PEG-2000, and mixture DMPC/DMPE-PEG-2000 . Appearance of white spots was also observed. In this case, when the film is formed by continuous thinning we define a new parameter of CBF formation called total film formation time t_{o-2}. The latter was defined as the time from thick FF formation until equilibrium CBF, with defined diameter, was formed. The results about t_{o-2} are represented at Fig.7. At DMPE-PEG-2000/L-1695 ratio ≥90 a plateau value of t_{o-2}=900 sec was reached. The increase of t_{0-2} agrees with the results reported for the formation of highly viscous hydrogels by PL linked PEGs. In CBFs formed by continuous thinning, white spots

were also observed leaving the film until CBFs with homogenous surfaces were formed.

Fig.6

When TLFs are formed by pure DMPE-PEG-2000 at C_t=3,7.10^{-5} M CBFs are formed by continuous thinning of the film, which was similar to the mixed DMPE-PEG-2000/L-1695 films at ratio higher than 10. Thus we can conclude that DMPE-PEG-2000 inclusion in the FF monolayers occurred.

Monolayers

Monolayers of DMPC pure and mixed with DMPE-PEGs

The monolayers used, adsorbed and spread, were pure DMPC, pure DMPE-PEG and mixed DMPC/DMPE-PEG monolayers. Mixed monolayers content of DMPE-PEGs was: <10 mol % (in concentrations equal to $C_{DMPE-PEG}^{min}$ at which TLFs became to thin to CBF/CBF-like films without thinning to NBFs), 20 mol % and 80 mol % DMPE-PEG. It was revealed that the effect of 20 mol % and 80 mol % DMPE-PEG on the monolayer surface tension (both at equilibrium and during compression/decompression) was practically identical so only the results for 20 mol % DMPE-PEG are shown below. Thus no difference was observed in the monolayer forming properties of DMPC/DMPE-PEG dispersions composed by liposomes/micelles mixture or only by micelles.

The γ(t) dependences of DMPC, DMPE-PEG and DMPC/DMPE-PEG spread monolayers (200 $Å^2$ area per molecule) are represented at Fig. 7.

Fig.7

DMPC monolayers reached equilibrium surface tension γ_{eq} = 58 mN/m after equilibration time (t_{eq}) of 8 sec. The presence of DMPE-PEGs in the monolayers resulted both in improving the kinetics of film spreading and decreasing of γ_{eq} value. Both effects were proportional to DMPE-PEG content in the film and to PEG moiety molecular weight. At 20 mol% DMPE-PEG the film spreading kinetics and γ_{eq} were practically the same as for pure DMPE-PEG monolayers. It can be seen that at $\geq$20 mol% DMPE-PEG the kinetics of film spreading was the same for all DMPE-linked PEGs used (t_{eq}=5 sec), while γ_{eq} value depends on PEG moiety Mw and lowest γ_{eq} of 42 mN/m for the highest molecular weight DMPE-PEG_{5000} was reached. These results are in agreement with the data for the increase of the effective molecular area and lateral steric repulsion force between PL linked PEGs with the increase of PEG moiety length and Mw (Kuhl et al., 1994; Marsh, 2001). The latter could result in accelerated kinetics of film spreading and uniform molecular packing at the air/water interface, necessary for reaching of low equilibrium surface tension (Majewski et al., 1997).

Similar results were obtained with L-1695 (data not shown).

CONCLUSIONS

In this work Thin Liquid Films and monolayers at the air/water interface formed by Dimyristoyl phosphatidylcholine (DMPC) pure and mixed with Dimyristoyl phosphatidylethanolamine (DMPE) Linked Poly Ethylene Glycols or Free Poly Ethylene Glycols were studied. For both- DMPE Linked and Free PEGs- PEG moiety molecular weight (Mw) was < 8000. The stability and hydrodynamic behavior of pure DMPC films were compared with those of DMPE-PEG and DMC/DMPE-PEG Thin Liquid Films. Surface tension (equilibrium and at compression/decompression) of pure DMPC monolayers and DMPE-PEG and DMC/DMPE-PEG monolayers was also measured. Films formed by pure DMPE Linked PEGs were also obtained and their properties were compared with those of DMPC pure and DMPC/DMPE-PEG mixed films.

Experiments were conducted at three concentrations of DMPE-PEGs in the film forming dispersions- < 10 mol% (when dispersions are formed predominantly by liposomes), 20 mol % (when mixture of liposomes and micelles exist in the dispersions) and 80 mol % (when all the particles forming DMPE-PEG / DMPC dispersions are micelles).

It was found that all used DMPE-linked PEGs (DMPE-PEG_{550}, DMPE-PEG_{2000} and DMPE-PEG_{5000}) pure formed stable Black TLFs. While DMPE-PEG_{550} formed CBFs by the well known trivial mechanism of fluctuation BS formation (characterized by film thining time t_{0-1} and BS expansion time t_{1-2}), higher molecular weight DMPE-$PEG_{2000/5000}$ formed CBF-like films by continuous thinning (characterized by film total formation time t_{0-2}) due to the steric repulsion between the overlapping in the film subphase long PEG chains. When included in DMPC/DMPE-PEG films, DMPE-PEG_{550} increased t_{0-1} and t_{1-2} and changed the film thinning, from thinning to NBFs to thinning to thicker CBFs, while DMPE-$PEG_{2000/5000}$ changed the film formation mechanism and CBF-like films formed by continuous thinning were obtained. The minimal DMPE-PEG concentration ($C_{DMPE-PEG}^{min}$) at which the above effects were observed decreased with increasing PEG moiety Mw. The value of $C_{DMPE-PEG}^{min}$ was lower than 10 mol% at which no significant change in the type of the particles composing DMPC/DMPE-PEG dispersions was realized, and dispersions were formed mainly by liposomes. The threshold concentration of DMPE-PEG pure films decreased with increasing of PEG moiety Mw and the lowest $C_t = 4.10^{-6}$ M was reached for DMPE-PEG_{5000} films, while the threshold concentrations of DMPC/DMPE-PEG mixed films decreased both with increasing DMPE-linked PEG mol% and with increasing of PEG moiety Mw. Thus TLFs provide the opportunity to define new quantitative parameters for comparison between the membrane forming properties of DMPE-linked PEGs.

For monolayers improved formation kinetics of adsorbed and spread films, decrease of surface tension values (equilibrium, minimal and maximal) and of the film compression/decompression cycle histeresis area were observed in DMPE-PEG

containing monolayers in comparison with DMPC pure films. These effects also were proportional to both - the mol % DMPE-linked PEG and PEG moiety Mw. However it was revealed that the effect of 20 mol % (when mixture of liposomes and micelles exist in the film forming dispersions) and 80 mol % DMPE-PEG (when all the particles composing DMPE-PEG/DMPC dispersions are micelles) on the monolayer surface tension (both at equilibrium and during compression/decompression) was practically identical. Thus no difference in the monolayer forming properties of DMPC/DMPE-PEG dispersions composed by liposomes/micelles mixture or only by micelles was observed.

Similar results were obtained with films of L-1695.

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FIGURE AND TABLE

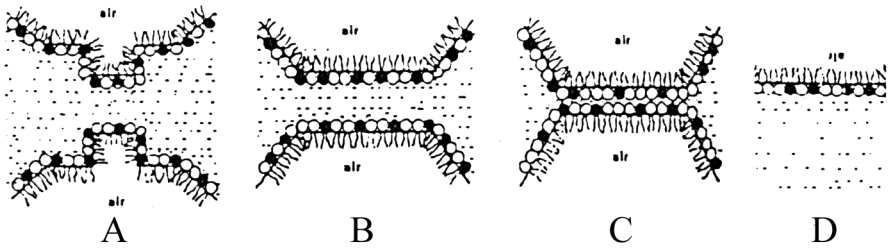


Fig. 1. *Schematic representation of TLFs and monolayers at the air/water interface used in our study. Two types of TLFs are shown: Common black Film (B) and Newton Black Film (C). Thick TLF with black spot (A) is also presented. On D is shown phospholipid monolayer which can be regarded as a half of bilayer film.*

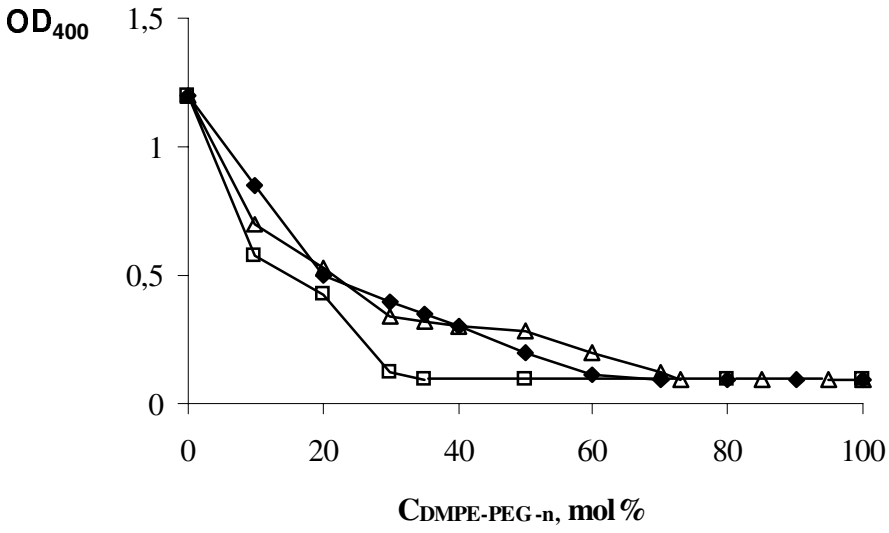


Fig. 2. *OD_{400} ($C_{DMPE-PEG-n}$) dependence of mixed dispersions DMPC/DMPE-PEG.. Data labels- DMPE-PEG_{550} (♦), DMPE-PEG_{2000} (□), DMPE-PEG_{5000} (Δ). Experiments were done at C=1000 μg lipid/ml, T=37°C, Cel=0,5 M NaCl, pH 6,8-7,0.*

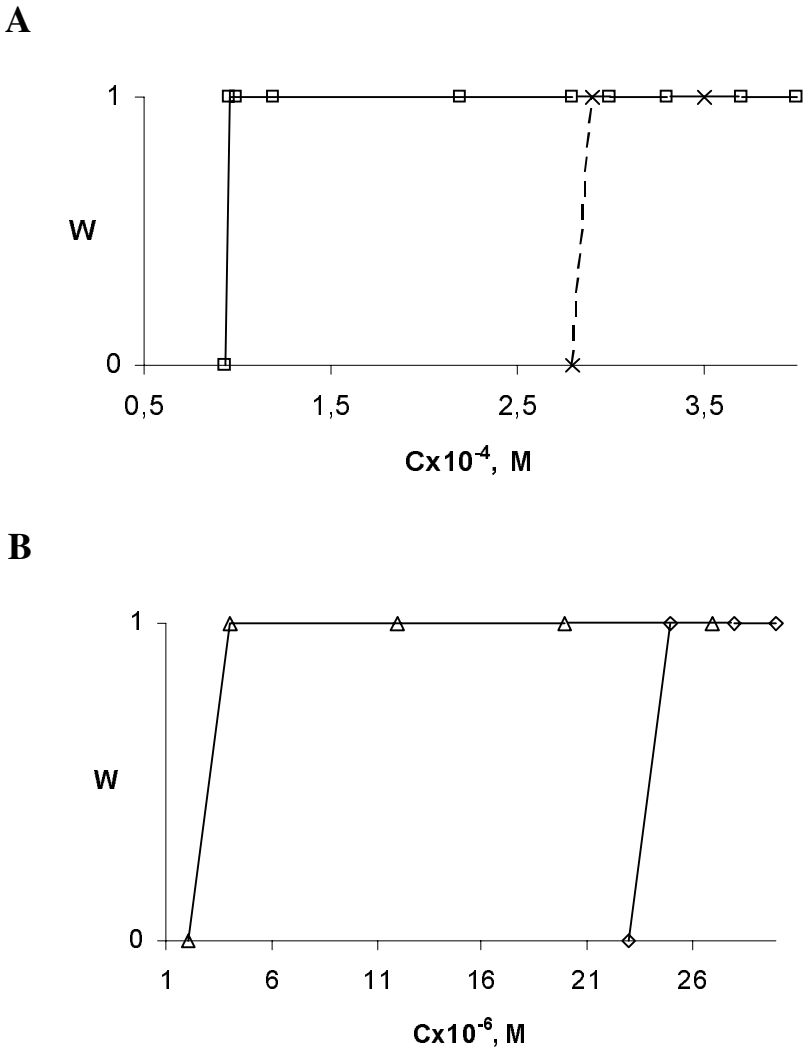


Fig. 3. *Comparison between W(C) dependences of DMPC and DMPE linked PEGs films at Cel=0,5 M NaCl . Experiments were done at T=37°C, Cel=0,5 M NaCl, pH 6.8-7.0 with film diameter d_f = 200 μm. Data labels: panel A- (x and rough dashed line)- DMPC (the film is NBFs, Fig. 1C); (□) DMPE-PEG$_{550}$ (the film is CBF, Fig. 1B); panel B- (◊)- DMPE-PEG$_{2000}$ (the film is CBF-like) and (Δ)- DMPE-PEG$_{5000}$ (the film is CBF-like).*

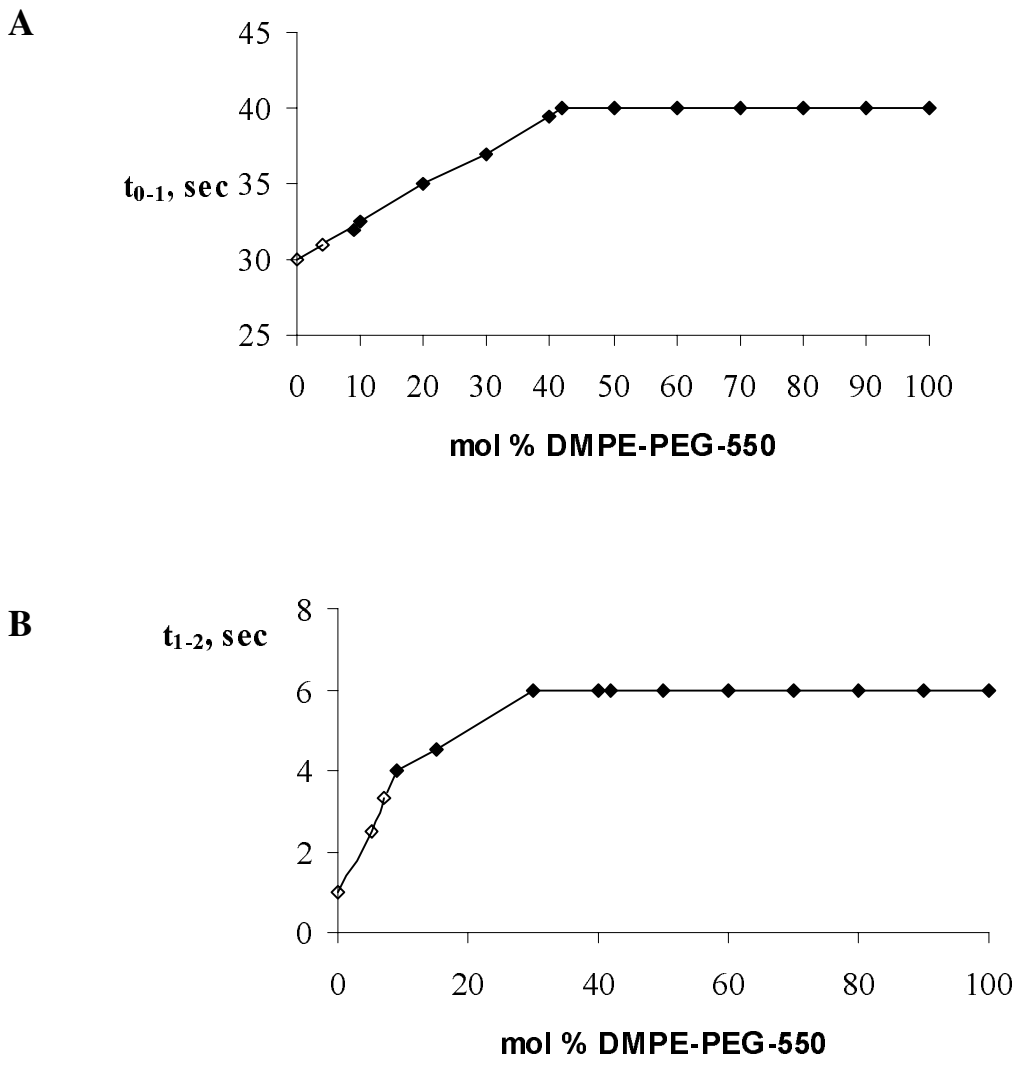
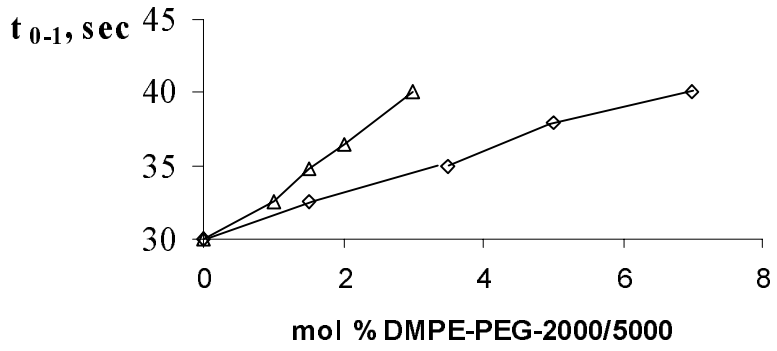
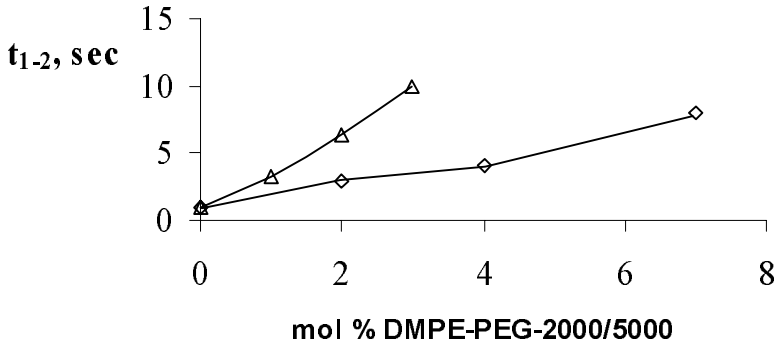


Fig. 4. *Film thinning time (t_{0-1}) and BS expansion time (t_{1-2}) dependences on mol % DMPE-PEG$_{550}$ in DMPE-PEG$_{550}$ + DMPC mixed TLFs (panel A and B respectively) with film diameter d_f = 200 μm. Data labels designate film thinning to NBFs (◊) or to CBFs (♦) at 9 mol % DMPE-PEG$_{550}$. Experiments were done at T=37°C, Cel=0,5 M NaCl, pH 6.8-7.0.*

A



B



C

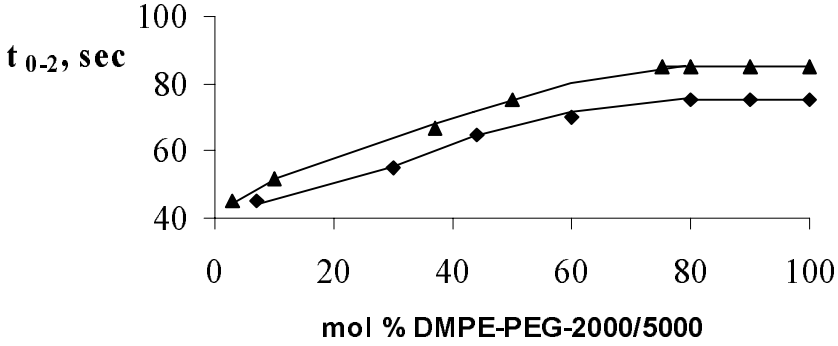


Fig. 5. *Film thinning time (t_{0-1}; panel A), BS expansion time (t_{1-2}; panel B), film formation time (t_{0-2}; panel C) dependence on mol % of DMPE-PEG$_{2000}$ (◊ and ♦) or DMPE-PEG$_{5000}$ (Δ and ▲) with film diameter d_f = 200 μm. Open symbols are used for CBFs, while closed symbols are used for CBF-like films formed by continuous thinning. DMPE-PEG concentration at which TLFs became to thin to CBFs (and not to NBFs) was not measured (see main text for comment). Experiments were done at T=37°C, Cel=0,5 M NaCl, pH 6.8-7.0.*

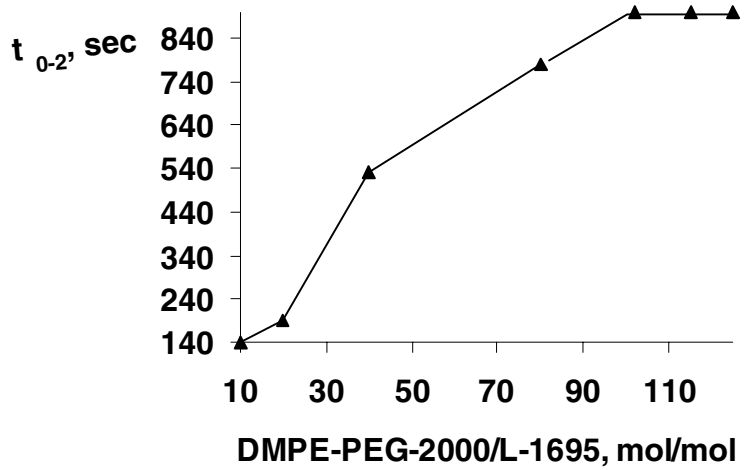


Fig.6. *Dependence of the total film formation time (t_{0-2}) of CBFs formed by sugar ester surfactant L-1695 on DMPE-PEG-2000/L-1695 ratio. CBFs were formed without BS formation. Experiments were done at T= 24ºC, C_{el}=0.5 M NaCl, C_{L-1695}= 70 μg/ml, pH 6.8-7.0, d_f=200 μm.*

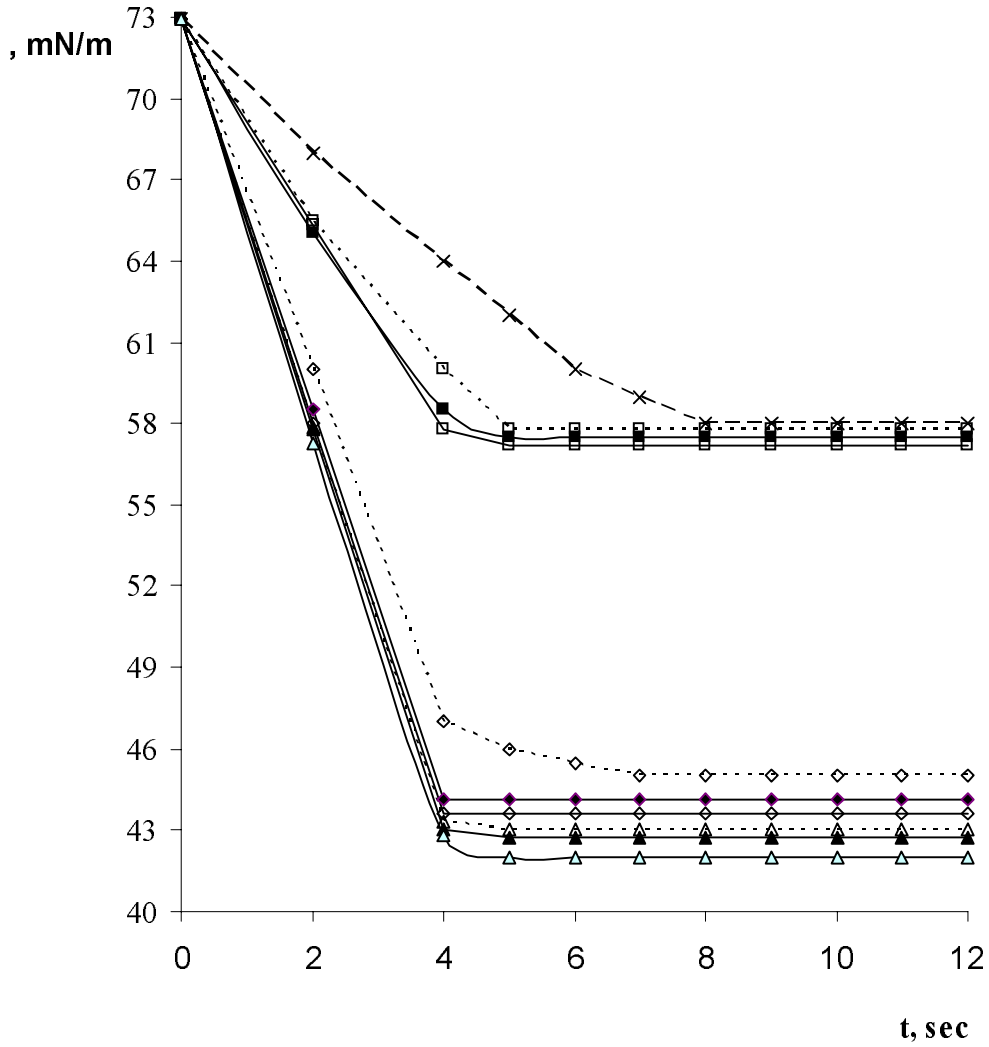


Fig. 7. *Comparison of γ(t) dependence of spreading kinetics of DMPC pure (x and rough dashed line) spread monolayers γ(t) dependence of DMPE-PEG containing films: DMPE-PEG_{550} pure (□),DMPE-PEG_{550} (20 mol%) + DMPC (■), DMPE-PEG_{550} (9 mol%) + DMPC (□ and fine dashed line); DMPE-PEG_{2000} pure (◊), DMPE-PEG_{2000} (20 mol %) + DMPC (♦), DMPE-PEG_{2000} (7 mol %) + DMPC (◊ and fine dashed line); DMPE-PEG_{5000} (Δ),DMPE-PEG_{5000} (20 mol%) + DMPC (▲),DMPE-PEG_{5000} (3 mol%) + DMPC (Δ and fine dashed line). Experiments were done at 200 $Å^2$ per molecule, T=37°C, Cel=0,5 M NaCl, pH 6.8-7.0.*

Table 1. *t_{0-1}, t_{1-2}, C_t, and type of TLFs stabilized by DMPC and free- and lipid-linked PEGs with different molecular weight. Experiments were done at T=37°C, Cel=0,5 M NaCl, pH 6,8-7,0, d_f=100 μm.*

Composition	TLF type	C_t, mol/l	t_{0-1}, sec	t_{1-2}, sec	Formation mechanism
DMPC	NBF	$2{,}9.10^{-4}$	30	1	Fluctuation BS(1) formation
DMPE-PEG_{550}	CBF	$9{,}6.10^{-5}$	40	6	Fluctuation BS(2-5) formation
* DMPE-PEG_{550} (9 mol%) + DMPC	CBF	2.10^{-4}	31	4	Fluctuation BS (2-5) formation
DMPE-PEG_{550} (20 mol%) + DMPC	CBF	$1{,}2.10^{-4}$	38	5	Fluctuation BS (2-5) formation
DMPE-PEG_{550} (80 mol%) + DMPC	CBF	$9{,}8.10^{-5}$	40	6	Fluctuation BS (2-5) formation
DMPE-PEG_{2000}	CBF-like	$2{,}5.10^{-5}$	*75* **		Continuous thinning without BS formation
* DMPE-PEG_{2000} (7 mol%) + DMPC	CBF-like	7.10^{-5}	*45*		Continuous thinning without BS formation
DMPE-PEG_{2000} (20 mol%) + DMPC	CBF-like	$3{,}2.10^{-5}$	*60*		Continuous thinning without BS formation
DMPE-PEG_{2000} (80 mol%) + DMPC	CBF-like	$2{,}7.10^{-5}$	*70*		Continuous thinning without BS formation
DMPE-PEG_{5000}	CBF-like	4.10^{-6}	*85*		Continuous thinning without BS formation
* DMPE-PEG_{5000} (3 mol%) + DMPC	CBF-like	1.10^{-5}	45		Continuous thinning without BS formation
DMPE-PEG_{5000} (20 mol%) + DMPC	CBF-like	7.10^{-6}	*70*		Continuous thinning without BS formation
DMPE-PEG_{5000} (80 mol%) + DMPC	CBF-like	5.10^{-6}	*70*		Continuous thinning without BS formation
Free PEG-6000	CBF	5.10^{-3}	720	14	Fluctuation BS(2-5) formation

*-denotes $C_{DMPE\text{-}PEG}^{min}$ above which TLFs became to thin to CBFs (but not to NBFs)
**- with Italic are shown t_{0-2} values