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Interaction of poloxamer F-98 with free standing phospholipid films♣

G. AS. GEORGIEV[a*], A. G. JORDANOVA[b], G. R. IVANOV[c], Z. I. LALCHEV[a]
[a] *Faculty of Biology, Sofia University "St. Kliment Ohridski", 8 Dragan Tsankov Blvd., 1164 Sofia Bulgaria;*
[b]*Institute of Biophysics, Bulgarian Academy of Sciences, 20 Georgi Bonchev Str.t, 1784 Sofia, Bulgaria;*
[c]*Advanced Technologies Ltd., compl. Yavorov, bl. 38/B, 1111 Sofia, Bulgaria.*

The interaction of poloxamer F-98 with dimiristoylphosphatidylcholine (DMPC) is investigated experimentally at one (monolayer) and two interacting air/water interfaces (foam film). Monolayer tensiometry reveals the degree of co-polymer penetration in DMPC monolayers and the alterations in monolayer compression/decompression behaviour. The changes in the film morphology are registered by Brewster angle microscopy (BAM). Foam film experiments show that the steric disjoining pressure arising in presence of Poloxamer F-98 results in increase of film equivalent water thickness and stability. It is found that the interaction between the co-polymer and DMPC molecules, both in monolayers and foam films, strongly alters the interfacial properties of the mixed systems, and bears promise for the development of new drug carrier formulations.

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1. Introduction

Poloxamers are three-block polyoxyethylene (POE)-polyoxypropylene (PPO)-POE co-polymers (Fig.1) commonly used by the modern pharmacy [1].

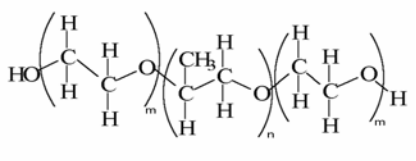

Fig. 1. Simplified scheme of the poloxamer structure.

The hydrophobic PPO part acts as an anchor, embedding the polymer molecule in the acyl chain region of phospholipid (PL) membranes. Thus, membrane structures (liposomes, micelles etc.) enriched with poloxamer molecules might be obtained. The membrane surface exposed to the water solution will be grafted with POE chains in mushroom or brush conformations (see Fig.4 below) depending on the polyoxyethylene moieties' lateral packing density. POE coating creates a steric barrier at the membrane surface, ensuring both: (i) defence against opsonisation (i.e. preventing the recognition of the membrane aggregate as a foreign body by the immune defence of the human organism) and (ii) increased colloid stability (i.e. decreased flocculation) of the resulting dispersions [2]. These properties make mixed PL-poloxamer liposomes and micelles promising "stealth" (invisible to the immune system) drug carriers.

Thus, the interfacial properties of mixed films composed by poloxamers and liposome-forming PLs deserve further study. In the current work, we characterize the interactions between the barely studied poloxamer F-98 and Dimiristoylphosphatidylcholine (DMPC). The interactions in 2-D are examined with monolayer tensiometry and Brewster Angle Microscopy (BAM). The effects of poloxamer presence on the interfacial properties of the contact occurring between two opposing head-to-head membrane planes are characterised by the foam film (FF) setup of Scheludko and Exerowa [3].

2. Experimental

DMPC was obtained from Sigma, and F-98 from BASF. All experiments were performed at room temperature.

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Monolayer tensiometry was performed with a Microtrough X tensiometer (Kibron, Finland) with the standard trough provided by the manufacturer. DMPC molecules were spread (from chloroform) at 100 Å^2 per molecule, and 30 mins were provided for $CHCl_3$ evaporation. Then, compression/decompression was performed at a low quasi-equilibrium speed (0.5 Å^2/molecule/min), in order to avoid hysteresis. After the DMPC film was compressed to 40 Å^2 per molecule, 10^{-5} M of F-98 was injected in the subphase, and during film decompression the change of the shape of surface pressure (π)-area isotherm was used to detect co-polymer insertion in the film.

BAM studies were performed with a MicroBAM 2 (NIMA, UK).

Foam Films (FFs) were formed by the method of Scheludko and Exerowa [3]. A biconcave drop (50 µl volume) of the dispersion was placed into the cylinder of the measuring cell for 30 min. After sucking the solution from the drop, a thick FF formed. Then, the film spontaneously thinned until a critical thickness was reached (ca. 300 Å). Then a black spot (BS), due to local thinning in the film, appeared and expanded to fill up the whole area of the film, and finally a Black Film (BF) was formed. The film formation was observed with an inverted light microscope. The equivalent water thickness (h_w) of the foam films was calculated (with a resolution of 0.5 nm) from the data for the intensity of the reflected light, measured by the interferometric technique. The mean value of the thickness was determined from the data for 5–10 films. The stability of the black films was characterized in terms of the dependence of the BF formation probability (W) on the surfactant (DMPC and F-98) concentration. The formation probability (*W)* depends strongly on the surfactant concentration, *C* [4] and is 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 (i.e. W varies between 0 and 1). The dependence $W(C)$ is extremely steep, which allowed us to define a threshold concentration C_t - the minimum surfactant concentration at which $W = 1$ (stable films are always formed).

3. Results

Monolayers

The results from tensiometry experiments are shown in Fig. 2. It can be seen that F-98 penetrates the monolayer at π=20 mN/m as since that point the trend of the resulting isotherm (dashed line) strongly deflects from the isotherm (solid line) of the pure DMPC (i.e. in absence of F-98 in the subphase).

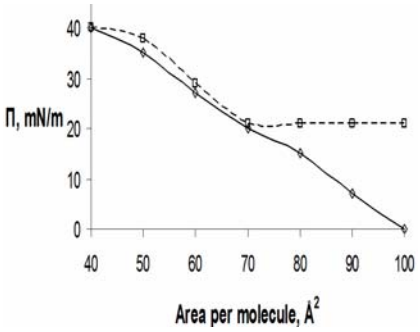

Fig. 2. Surface pressure-area isotherm of a DMPC monolayer: pure (solid line) and in the presence of 10-5 M F-98 in the subphase. Experiments were performed at 0,15 M NaCl, pH 6,8-7,0, T=25ºC.

The effect of the poloxamer on the phase heterogeneity of monolayers is visualized by BAM (Fig.3).

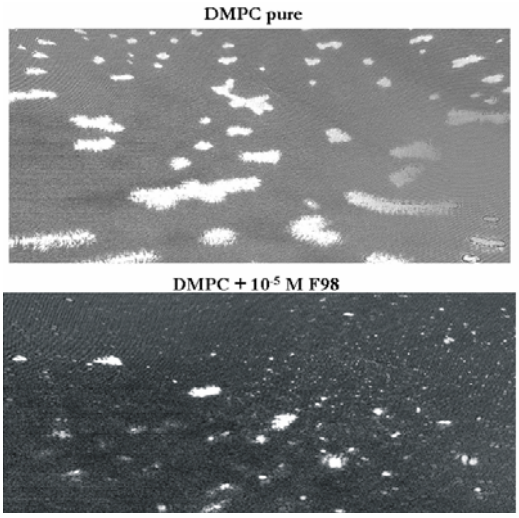

Fig. 3. BAM pictures of DMPC/three block co-polymer monolayers obtained at a surface excess (20Å^2 per DMPC molecule).

It can be seen that F-98 penetration induces disintegration of the condensed domains, and much more fluid monolayer is observed.

Foam Films (FFs)

The effect of F-98 on the equilibrium water thickness (h_w) of mixed DMPC/F-98 foam films is presented in Fig. 4.

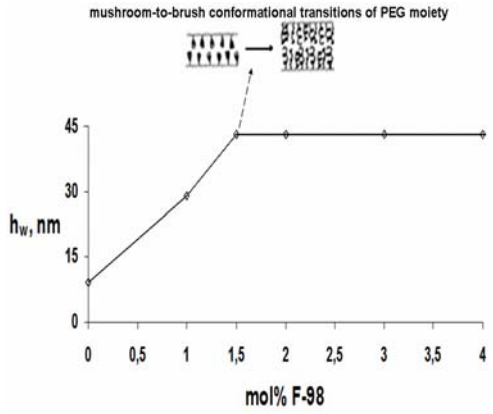

Fig. 4. Dependence of h_w of mixed DMPC/F-98 films on the concentration of F-98. Experiments were performed at 0,15 M NaCl, pH 6,8-7,0, T=25ºC.

It can be seen that h_w steeply increases from 9 nm (pure DMPC films) to 43 nm at the very low concentration of 1.5 mol% F-98.

This effect is accompanied by a striking increase in the film stability (Fig. 5) manifested by a 20x decrease of the threshold concentration value C_t.

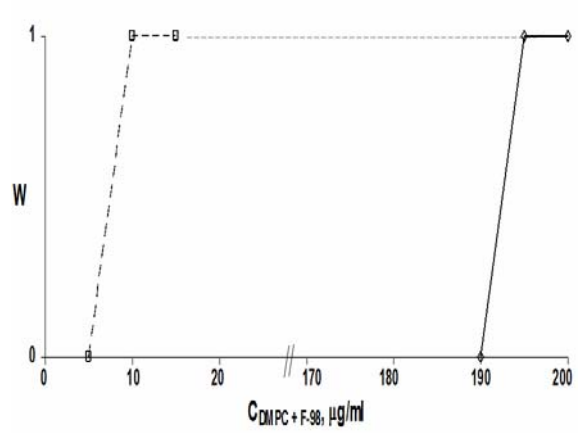

Fig. 5. Dependence of the Black Film formation probability (W) on the surfactant concentration (C) of foam films by DMPC pure (solid line) and in the presence of 1.5 mol% F-98 (dashed line). Experiments were performed at 0,15 M NaCl, pH 6,8-7,0, T=25ºC.

4. Discussion

Monolayer tensiometry experiments prove that F-98 penetrates DMPC monolayers, and strongly modifies the shape of the compression decompression isotherm. It can be seen (Fig. 2) that during film decompression, F-98 adsorbs from the subphase and inserts itself in the interfacial film at quite a high surface pressure (20 mN/m) and packing density (70 $Å^2$ per DMPC molecule). After that (upon further decompression) the isotherm of the mixed film strongly (dashed line, Fig. 2) differs from the one of the pure DMPC monolayer (solid line, Fig. 2). The mixed DMPC/F-98 film maintained a surface pressure of 20 mN/m, while for pure DMPC film π dropped to 0 mN/m. This result can be attributed to the insertion and spreading of F-98 molecules at the air/water interface during monolayer decompression. That suggestion is supported by Brewster Angle Microscopy observations, showing that poloxamer insertion strongly influences the 2-D phase coexistence and results in complete fluidization of the monolayer. BAM studies were performed at a surface excess, for two reasons: (i) condensed domains in DMPC monolayers at standard temperatures are registered only after the film collapse [5], and (ii) as FFs are obtained well above the DMPC critical micellar concentration, insoluble monolayers at a surface excess might better mimic the adsorption film at FF interfaces.

The displacement of DMPC monomers by F-98 molecules from the air/water interface (in addition to the increase in the FF thickness) may explain the strong increase of the FF stability. The increase of h_w demonstrates the steric repulsion disjoining pressure, induced by the grafting of film surfaces with POE chains. At the very low F-98 concentration of 1.5 mol% plateau in Fig. 4 is observed, corresponding to the mushroom-to-brush conformational transition of polyoxyethylene moieties (see the insert in Fig.4).

5. Conclusions

The poloxamer F-98 enters the phospholipid monolayer at pressures below 20 mN/m, causing a liquification of the solid domains. It also strongly reduces the required DMPC concentration necessary for Black Film formation. Thus, the interaction between co-polymer and DMPC molecules, both in monolayers and foam films, strongly alters the interfacial properties of the mixed model membrane systems. Thus, further study of DMPC/F-98 dispersions bears promise for the development of advanced pharmaceutically applicable materials, to be employed in the design of "stealth" drug delivery systems.

Acknowledgements

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*Corresponding author: ggeorg@biofac.uni-sofia.bg