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DPPC monolayers as simple models of biological membranes for studies of interactions with perfluorinated compounds♣

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Langmuir monolayers of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) formed at the air-water interface were used as simple models of biological membranes to study interactions with two selected perfluorinated compounds: perfluorooctanoic acid (PFOA) and perfluorooctanesulphonic acid (PFOS). The presence of those common pollutants in the subphase led to the formation of more fluidic monolayer possessing different surface and barrier properties. The observed increase in the area per molecule in the Langmuir monolayer spread at the air-water interface was attributed to the incorporation of perfluorinated compounds into the layer. Moreover, perfluorooctanesulphonic acid was found to interact with DPPC monolayers transferred onto the electrode surface. Exposure of DPPC modified electrode to PFOS solution led to changes in electrode capacitance and in the efficiency of electron transfer rate observed for selected electroactive probes added to the solution.

1. INTRODUCTION

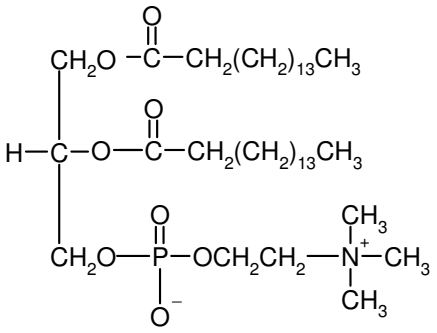
Biological membrane is a complex structure which contains a variety of chemicals including different types of proteins, lipids, glycolipids and carbohydrates. It is responsible for a number of important processes such as transport of substantial compounds, which determine proper functioning of a cell and the entire organism. According to the fluid mosaic model of a cellular membrane, its main frame is formed by a phospholipid bilayer, in which other components are located [1]. It also plays the role of a barrier for any toxic

♣ Dedicated to Professor Emil Chibowski on the occasion of his 65 birthday

substances which interact with membranes by attaching to proteins or by incorporation into the phospholipid bilayer. As a result of such interactions, changes of the properties of the membrane and of processes taking place in the membrane may be observed.

Langmuir-Blodgett technique allows to form phospholipid monolayers at the air-water interface which may serve as simple models of a biological membrane to study the interactions with solution species such as drugs, toxins or pollutants [2,3]. This approach is especially convenient since the compounds can be purposely added to the subphase in a controlled concentration [4,5]. 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) is one of the phospholipids occurring in natural membranes (Figure 1). Its monolayer at the air-water interface is well characterized [6,7] and therefore this phospholipid is often used as a model of the outer membrane cell leaflet [2,8,9].

(A)



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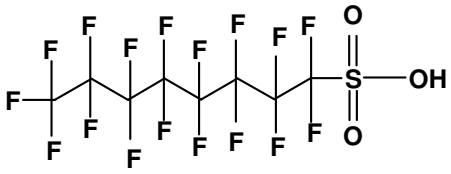

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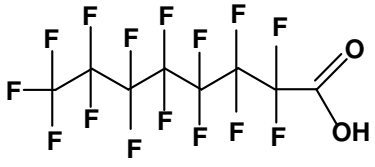

Fig. 1. The molecular structures of A) 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), B) perfluorooctanesulphonic acid (PFOS), C) pefluorooctanoic acid (PFOA).

Perfluorinated compounds (PFCs) are fully fluorinated analogues of fatty acids and are used in a variety of industrial applications including the synthesis of fluoropolymers, the production of paints, fire-fighting foams, lubricants and water repellents [10]. Due to their exceptional chemical stability caused by the high energy of C-F bond, these chemicals are environmentally persistent and have been found in wildlife and humans across the entire globe [11]. According to animal studies performed so far, PFCs may affect such properties of cell membranes as their fluidity [12] and may cause developmental and reproductive toxicity [13].

The effect of perfluorinated compounds on model phospholipid membranes composed of different phospholipids has been already investigated by means of different techniques including Langmuir-Blodgett, NMR and theoretical modeling [14]. In the present study we summarize the influence of two selected perfluorinated compounds: perfluorooctanoic acid (PFOA) and perfluoro-octanesulphonic acid (PFOS) (Figure 1) on the properties of model biological membranes composed of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC). To achieve this goal, perfluorinated compounds were added to the subphase on which phospholipid monolayer was formed and the changes in the interfacial properties of the monolayers were observed and discussed. In order to observe the influence of PFCs on already formed phospholipid layers, DPPC monolayers were also transferred onto glassy carbon electrodes using Langmuir-Schaefer technique and cyclic voltammetry was employed to monitor the changes in the properties of the modified electrode after the exposure to PFOS solutions.

2. EXPERIMENTAL

DPPC was purchased from Avanti Polar Lipids Inc., PFOA was from Aldrich and PFOS from Apollo Scientific Ltd. Chloroform used for preparing DPPC solutions, sodium and potassium phosphates for buffer preparation and potassium hexacyanoferrate were purchased from POCh Gliwice, Poland. Menadione was from Aldrich. Distilled water was passed through a Milli-Q® water purification system (resistivity 18.2 MΩ/cm).

Surface pressure and surface potential vs. area per molecule isotherms were recorded using KSV LB Trough 5000 (KSV Ltd., Finland) controlled by KSV 5000 software and equipped with a Wilhelmy balance and 5000SP surface potential meter (vibrating capacitor method). Water with or without perfluorinated compound was used as the subphase. The spreading solutions were prepared by dissolving DPPC samples in chloroform. After spreading, the solution was left for 10 min to allow for solvent evaporation. Compression of the film was performed at a speed of 7.5 cm^2/min and temperature was kept constant at 22±1 ^{0}C if not indicated otherwise. Electrochemical experiments were

performed using AutoLab AUT 71819 with the GPES 4.9 software in three electrode cell with Ag/AgCl as a reference electrode and platinum foil as a counter electrode. The supporting electrolyte was 50 mM phosphate buffer.

3. RESULTS AND DISCUSSION

The isotherms of DPPC monolayers formed on subphases containing different concentrations of perfluorinated compounds were recorded and compared with the isotherm of DPPC monolayer formed on pure water (Figure 2A and B). Both PFOS and PFOA incorporate into the DPPC monolayer during its formation which results in the increase in the area per molecule in the organized monolayer. Moreover, the incorporation of PFCs causes changes in such surface properties of DPPC monolayers as their fluidity, which is reflected by the changes in the value of compression modulus. Compression modulus (reciprocal of compressibility) is defined as [15]:

$$Cs^{-1} = -A\,(d\pi/dA) \quad (1)$$

where A is area per molecule and π is surface pressure. Cs^{-1} coefficient gives the information on the phase, in which monolayer occurs. Generally, when Cs^{-1} is in the range of 50 to 100 mN/m, a liquid expanded film is indicated, while Cs^{-1} value higher than 100mN/m corresponds to the liquid condensed film [16]. The incorporation of both PFOS and PFOA into DPPC monolayers causes the decrease in the maximum value of compression modulus (Insets in Figure 2A and B). Although even in case of the highest concentration of PFCs the value remains higher than 100 mN/m, the decrease in Cs^{-1} suggests the formation of a more fluidic monolayer in the presence of perfluorinated compounds if compared to DPPC monolayer formed on pure water. The minimum in the compression modulus versus area per molecule plot observed approximately at 70 Å for DPPC isotherm corresponds to the phase transition from liquid expanded to liquid condensed. However, in the presence of perfluorinated compounds, this minimum becomes less developed and finally disappears for the highest investigated concentration of PFCs (Insets in Figure 2A and B). This observation proves the changes in the surface properties of DPPC monolayers caused by the incorporation of perfluorinated compounds. Interestingly, the influence of PFOS is significantly greater than that of PFOA, which might be explained by the stronger interactions between polar head group region of DPPC and sulphonic group of PFOS.

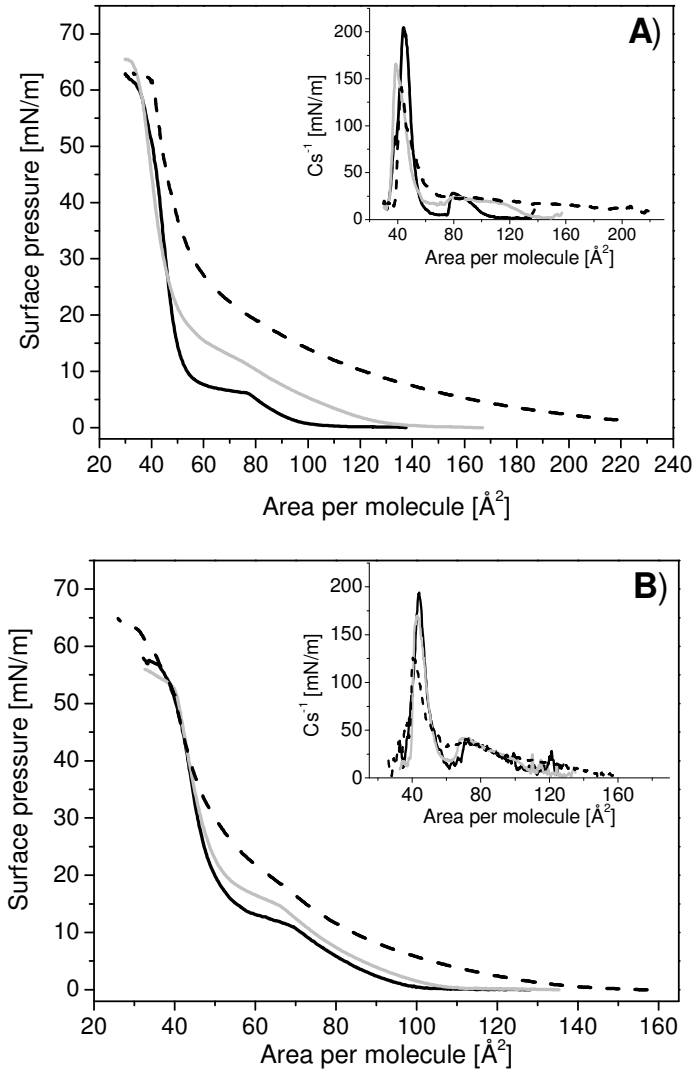

Fig. 2. Surface pressure – area per molecule (π - A) isotherms of DPPC monolayers on pure water subphase (black curve) and aqueous subphases containing 10^{-5}M PFCs solution (grey curve) and 10^{-4} M PFCs solution (dashed curve) Insets: Compression modulus versus area per molecule plots. A) subphase containing PFOS (T=22 ^{o}C); B) subphase containing PFOA (T=27 ^{o}C).

Additional information on the nature of the interactions of perfluorinated compounds with model biological membranes can be obtained from surface potential measurements. The observation of surface potential at early stages of

the monolayer formation allows one to extract the information on the changes in the orientation of molecules and the properties of the monolayer itself. In order to perform a semi-quantitative analysis of surface potential isotherm, Helmholtz equation has been used [17,18]:

$$\mu = \varepsilon\varepsilon_0 \Delta V \, A \qquad (2)$$

where μ is the vertical component of the dipole moment, A is the area per molecule and ε and ε_0 are the permittivities of the monolayer and of vacuum, respectively. The maximum apparent dipole moment is calculated as $\mu_A = \mu/\varepsilon$. In the presence of perfluorinated compounds in the subphase, the onset of surface potential is shifted towards greater areas per molecule when compared to DPPC monolayer formed on pure water (Figure 4A). This implies that the changes in the orientation of DPPC molecules at the air-water interface are promoted by the presence of PFCs and occur earlier, that is at larger areas per molecule. The changes in the surface potential are also reflected in the changes of dipole moment (Figure 4B). The maximum value of dipole moment is shifted toward larger areas per molecule in the presence of PFOA when compared to DPPC monolayer formed on pure water. It can be also observed that in the presence of PFCs in the subphase, the surface potential is higher than zero even at large areas per molecule. It might suggest that perfluorinated compounds themselves show some surface activity. However, neither PFOS nor PFOA is capable of forming a monolayer, since the fluorocarbon chain is too short. It has been reported that only perfluorinated carboxylic acids with fluorinated carbon chain containing at least 12 carbon atoms may form stable monolayers at the air-water interface [19].

Since Langmuir-Blodgett studies revealed that perfluorinated compounds incorporate into DPPC monolayer during its formation, it was also interesting to investigate, if those compounds may interact with already formed model biological membranes. Previously performed molecular dynamics simulations for DPPC bilayers surrounded with PFOS molecules showed the ability of PFCs to incorporate into preformed phospholipids layer [14]. Theoretical assumptions were also proved by the results of stability experiments, in which PFOS was injected into the water subphase on which DPPC monolayer was first compressed to a given surface pressure [14]. In order to investigate this phenomenon in a more detailed manner, DPPC monolayer compressed to 4 mN/m was transferred onto glassy carbon electrode by means of Langmuir – Schaefer technique. Then the modified electrode was brought into contact with PFOS solution and the changes in the properties of the DPPC monolayer at the electrode were monitored by cyclic voltammmograms recorded in phosphate buffer solution and in the presence of two electroactive probes: hexacyano-ferrates and menadione. Since electron transfer rate constant (k_s) can be treated

as a measure of the extent of electrode surface blocking, its value for the two probes was calculated by fitting the curves using the GPES 4.9 software and the changes with time of incubation in PFOS solution were observed (Figure 5). Transport of the two electroactive probes through the phospholipid layer is described by different mechanisms [20,21]. Hexacyanoferrates approach electrode surface using defects in the layer ("pinhole model"), while menadione penetrates directly through the phospholipid layer.

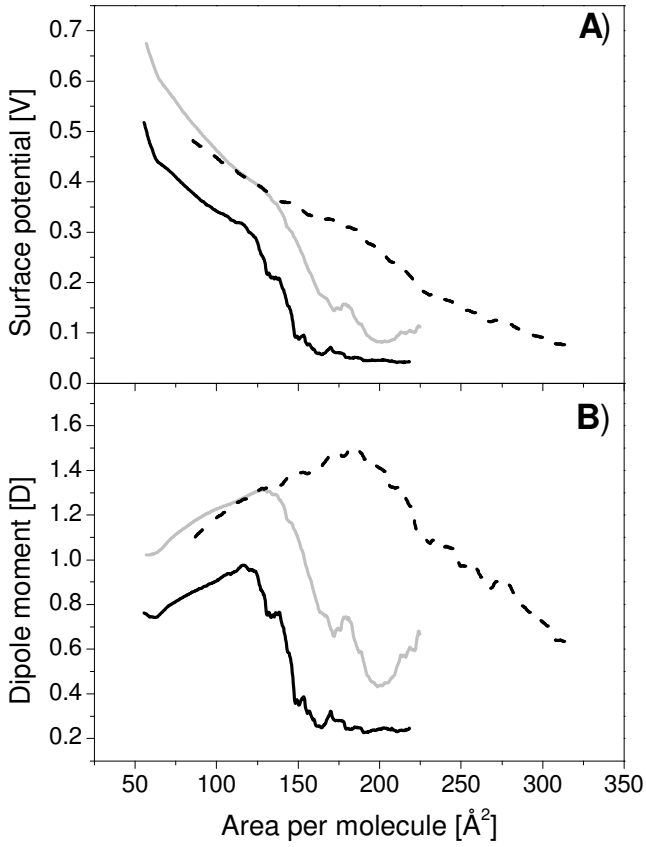

Fig. 4. A) Surface potential – area per molecule isotherms (V - A) and B) dipole moment – area per molecule isotherms (μ – A) for DPPC monolayers formed on pure water (black curve) and subphases containing 10^{-5}M PFOA (grey curve) and 10^{-4}M PFOA (dashed curve) solutions in the subphase.

In case of both probes, the k_s value decreases with time of incubation in PFOS solution, which might suggest that PFOS both blocks the defects in DPPC monolayer (decrease in hexacyanoferrates electron transfer rate constant) and incorporates into the DPPC monolayer (decrease in menadione electron transfer

rate constant). Therefore, PFC molecules present in the monolayer improve blocking properties of the monolayer towards electroactive probes and decrease the efficiency of their transport to the electrode surface.

The changes in the capacitance of the electrode modified with DPPC monolayer are consistent with the results of electrochemical experiments in the presence of electroactive probes and with Langmuir studies. As a result of the incorporation of perfluorinated compounds, the DPPC monolayer becomes more fluid, that is less packed, which is reflected by the increase in the electrode capacitance (Figure 6). Moreover, the observed changes depend also on the concentration of PFOS solution, in which the modified electrode was immersed.

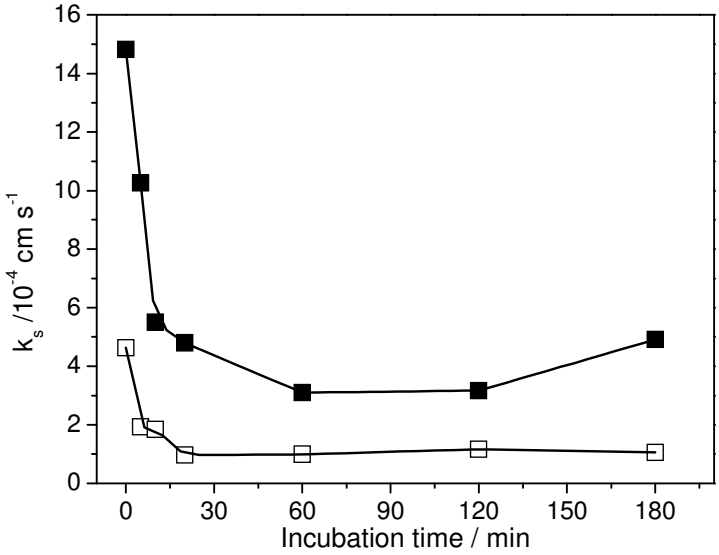

Fig. 5. Dependence of electron transfer rate constant (k_s) of two electroactive probes: $Fe(CN)_6^{3-/4-}$ (■) and menadione (□) on the incubation time in 1mM PFOS solution.

4. CONCLUSIONS

DPPC monolayers have been successfully used as simple models of biological membranes to study the influence of environmentally abundant pollutants such as perfluorinated compounds. Langmuir – Blodgett studies revealed that those chemicals incorporate into DPPC monolayers during their formation, which leads to the changes in such important properties of phospholipids layers as their fluidity. Moreover, the phase transition from liquid expanded to liquid condensed phase, characteristic for the DPPC isotherm, is also affected by the presence of PFCs in the subphase. The investigated compounds are also able to penetrate preformed phospholipid monolayers.

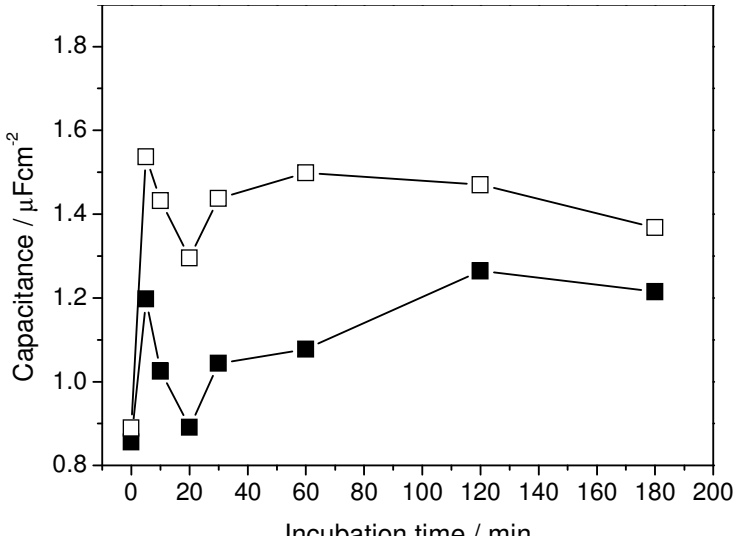

Fig. 6. Dependence of capacitance of GC electrode modified with DPPC monolayer on the incubation time in 1mM PFOS (■) and 10mM PFOS (□) solutions.

The results of electrochemical experiments proved that perfluorinated compounds incorporate into DPPC monolayer transferred onto the electrode surface, which causes changes in the modified electrode capacitance and in the electron transfer rate for solution - resident electroactive probes.

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