EFFECTS OF CARBON NANOPARTICLES ON BIOMEMBRANES: A LANGMUIR MONOLAYER EXPERIMENT

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1. Introduction

The extent of the production and use of nanomaterials is rapidly growing. Carbon nanomaterials, such as fullerenes and nanotubes, are among the most extensively studied nanomaterials. Although the effects of nanoparticles on health and environment are becoming more of a concern, studies on toxicology and the environmental impact of nanoparticles are still scarce [1]. Inhaled ultrafine carbon particles deposit in the lungs and translocate into the brain into the olfactory bulb, by means of the olfactory nerves and the blood. The mechanism of nano-C_{60} penetration through a lipid membrane has not yet been established. The mechanism of cell membrane disruption is also not well understood. It has been reported that the toxicity of carbon nanoparticles (CNPs) depends on their solubility in water; e.g. the cytotoxicity of pristine fullerene is seven orders of magnitude higher than that for functionalized fullerenes with higher solubility [2].

In this communication, we focused on CNPs acting on lipid monolayers. We described the changes caused by the presence of fullerene molecules in a monolayer of dipalmitoyl phosphatidylcholine and evaluated mechanical as well as thermodynamic properties of the system in terms of excess of the area per a molecule and the Gibbs free energy. This enables to monitor the intermolecular interactions in this two-dimensional molecular system and thus provide insight into the thermodynamics of fullerene intercalation in a lipid environment and permeation through cell membranes as well as the effect of fullerene on the structural and elastic properties of a lipid monolayer.

2. Materials

Fullerene (PCBO) - [6,6]-Phenyl C_{61} butyric acid octyl ester, 99% - was purchased from Sigma-Aldrich Co. Lipid (DPPC) - $C_{40}H_{80}NO_8P.H_2O$ 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine monohydrate, 99% - was a product of Fluka BioChemika. Both materials were dissolved in chloroform at a concentration of 0.5 mmol/l. The subphase used in monolayer experiments was bidistilled deionised water (18 MΩ.cm).

3. Method

Monolayer measurements were performed using the Langmuir trough, model 611M (NIMA Technology, Coventry, UK). Surface pressure was monitored by the Wilhelmy balance attached with a filter paper plate. The system was thermostated using FL 300 cooler (Julabo Labortechnik, Germany). After spreading the material to the subphase/air interface the solvent was left to evaporate for 15 minutes to reach stability of the monolayer. Compression speed was kept at 2 cm^2/min. The experiments were carried out at temperatures of 22 ^{0}C.

4. Results

The standard way of determining mechanical properties of Langmuir monolayers is accomplished by measuring the isotherm ($\pi - A$) of continuously compressed 2D film of the substance spread over the surface of water. A shape of the isotherm is determined by material phase transitions during the compression process. The measured curves for mixtures contaning various molar ratios of DPPC and PCBO are presented in Fig. 1.

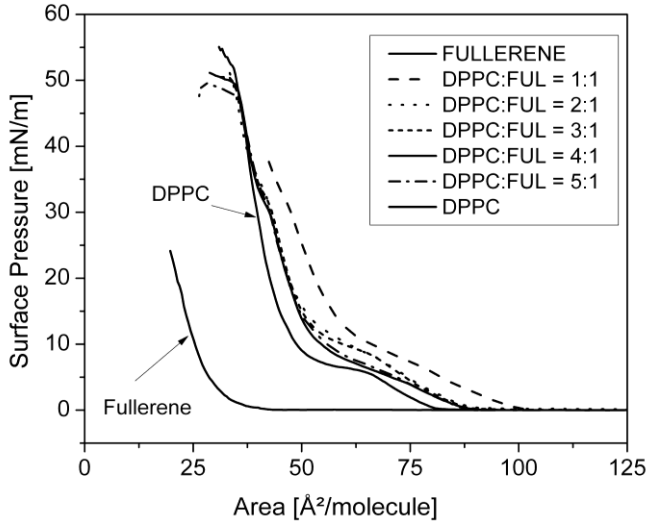

Fig.1: Pressure-area compression isotherms of mixtures with various DPPC/PCBO molar ratios

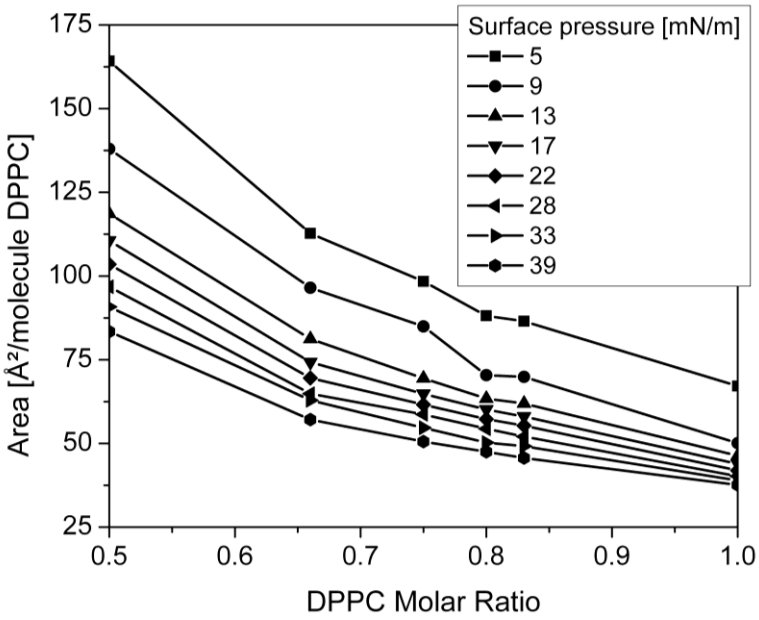

Fig.2: Area per one molecule of DPPC as a function of DPPC/PCBO molar ratio at various levels of surface pressures

The isotherm of pure C_{60} shown in Fig. 1 indicates that a compression of the monolayer results in the formation of a multilayered film, the limiting area is ca. 30 $Å^2$ whereas the estimated nearest-neighbour distance in a hexagonal 2D lattice of C_{60} is approx. 10 Å which yields the area of 87 $Å^2$

An important parameter for possible domain formation inside the mixture is represented by miscibility of components. This can be obtained from values of the area per molecule for various molar ratios at the particular surface pressure. Therefore, we express area per one molecule of DPPC. The area per single molecule of DPPC as a function of the DPPC/PCBO molar ratios for several surface pressures, are shown in Fig. 2. In all cases the area is monotonously growing up with increased amount of PCBO (pure PCBO is not shown). This indicates that the fullerene balls are embedded in the hydrophobic interior of the lipid monolayer. The differences in the area for pure DPPC and the 0.5 molar ratio mixture at lower surface pressure (below 10 mN/m) reveal that the monomeric fullerene molecules form a single sheet immersed in the lipid acyl chain array. At higher surface pressures approx. two fullerene molecules on average are situated within the thickness of the monolayer.

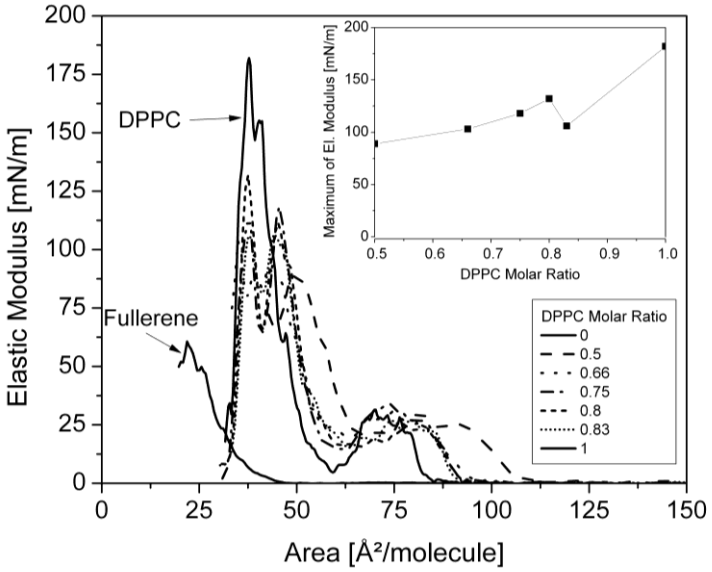

Fig.3: Elastic modulus of mixtures with various DPPC molar ratios. Inset shows maximum of elastic modulus for different ratios.

The presence of the fullerene molecules in the interior of the monolayer affects its mechanical properties, namely the elastic modulus (see the insert in Fig. 3). The elastic modulus represents mechanical behavior of a monolayer during isothermal compression; it is defined as

$$| E |= -A\left(\frac{\partial \pi}{\partial A}\right)_T$$

This parameter describes rigidity of the film and is important with regard to material deposition on solid substrates.

The molecular interactions in a two-component monolayer can be evaluated by a more detailed examination of the thermodynamics of the system. Such an analysis originated from Goodrich [35]. The variations of the Gibbs' free energy of a system containing a monolayer is given by

$$\left(\frac{\partial G}{\partial A}\right)_{T,P,n} = \gamma$$

where γ is the surface tension and A is the interfacial area. If we have a mixed monolayer with two components (1 and 2) constrained to remain in the surface, and variations of surface

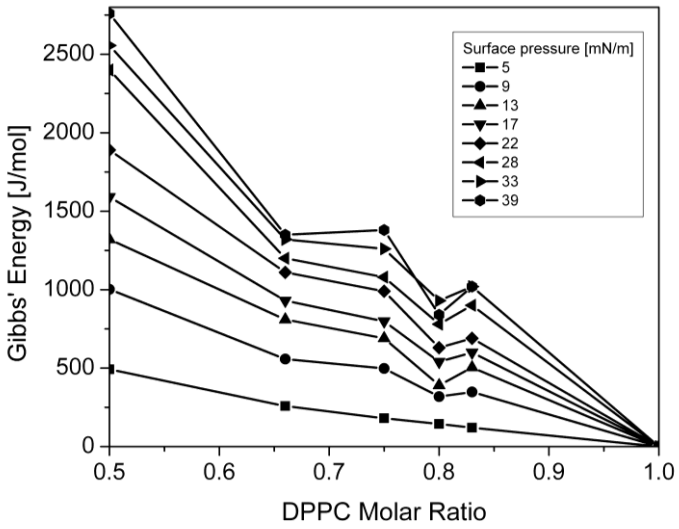

Fig.4 Gibbs' energy as a function of DPPC molar ratio for various values of surface pressure.

pressure are achieved by moving a barrier, at constant T and P, it is often useful to consider the excess Gibbs' free energy of mixing, above that found for an ideal mixed film

$$\Delta G = \int_0^{\pi} \left(A_{12} - x_1 A_1 - x_2 A_2 \right) \mathrm{d}\pi$$

A_{12} is the molecular area in the mixed monolayer at temperature T and surface pressure π, whereas A_1 and A_2 are molecular areas in the two single component monolayer, and x_1 and x_2 are molar ratios of the pure components in the mixture ($x_1 + x_2 = 1$). The value of ΔG provides information whether the particular interaction is energetically favorable (ΔG<0) or not (ΔG>0), while for ΔG=0 ideal mixing takes place. The value of ΔG as a function of the DPPC/PCBO molar ratio for various values the surface pressure is presented in Fig. 4.

The perturbation of monolayer properties upon insertion of fullerene molecules is evident. Although fullerenes have a tendency to aggregate in water (this was documented in case of pure C_{60} monolayer), they do not aggregate in the lipid monolayer even at high concentrations of up to one fullerene per one lipid. The presence of fullerene molecules in the monolayer results in the increase of the area per one lipid molecule and in disordering effect of the lipid tails. Also dynamic and elastic properties are affected by the nanoparticles to a large extent. A fullerene concentration of 25 % reduces the area compressibility modulus (elastic modulus) by 30 %. This decrease indicates a slight softening of the membrane. However, no noticeable mechanical damage (monolayer rupture, micellization, formation of pores) occurred during the monolayer treatment.

Acknowledgement
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