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Influence of Cu^{2+} ions on monolayer stability in an aqueous subphase and vesicle self-organization on the basis of the phosphorylated methanofullerene–lecithin system

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The influence of Cu^{2+} ions on the state of mixed phosphorylated methanofullerene–lecithin monolayers and vesicles has been estimated.

Methanofullerenes are applicable to the production of new materials and in the synthesis of biologically active compounds.[1–4] Possible applications depend on the capability to form self-organised assemblies, stable Langmuir monolayers and three-dimensional networks.

Although the spreading of fullerenes on a water subphase in a Langmuir trough was reported,[5–8] the structure and stability of the films remained not optimal.

In the development of biosensors, it is very important to combine fullerene C_{60} derivatives with well-known self-organising amphiphilic compounds such as lecithins. Lecithins form stable monolayers and lipid vesicles composed of membrane-like lipid layers surrounding aqueous compartments. Therefore, the addition of lecithin to the methanofullerene system is an effective method for constructing mixed monolayers and vesicles.

We used methoxycarbonyl (dimethoxyphosphoryl) methanofullerene and its mixtures with lecithin to study the stability of monolayers and self-organization in the presence of Cu^{2+} ions.

The synthesis and structure of phosphorylated methanofullerene (P-methano-C_{60}) were described elsewhere:[9] analytical grade 1-palmitoyl-2-oleylglycero-sn-phosphatidylcholine (Sigma) was used. Surface pressure–area isotherms were measured in a rectangular trough made of polytetrafluoroethylene (200×137×3 mm). Measurements of pressure–area (Π–A) isotherms were made using a computer-controlled film balance system. The concentration of P-methano-C_{60} was 1×10^{-4} mol dm^{-3} in chloroform and the spreading amount of P-methano-C_{60} solution was 30 μl. Lecithin was dissolved in chloroform–ethanol. After solvent evaporation, a monolayer was compressed at a speed of 14 mm min^{-1}. The average velocity of compression is 1.2 Å^2 min^{-1} molecule^{-1}. In the compression-decompression processes, hystheresis was not observed.

The molecular structures of P-methano-C_{60} and lecithin are shown in Figure 1.

The isotherms showed an initial increase in the surface pressure in the region 2–5 mN m^{-1} for the liquid phase (Figure 2, curve *2*). The lines drawn to $\Pi = 0$ indicate the collapse area of the condensed monolayer and correspond to surface molecular area per molecule for close packed monolayers.

The limiting area per molecule, extrapolated to $\Pi = 0$, is 1.04±0.04 nm^2 (Figure 2). Assuming a close-packed hexagonal lattice, this value corresponds to an inter-headgroup spacing of 10.75 Å.

Lecithin, which exists in a zwitterionic form and produces a condensed film in the monolayer under water subphase, is a gel phase including associated water and producing the limiting mean molecular area 0.54±0.2 nm^2 (Figure 2, curve *1*). The Π–A isotherms of the mixed monolayers of P-methano-C_{60}–

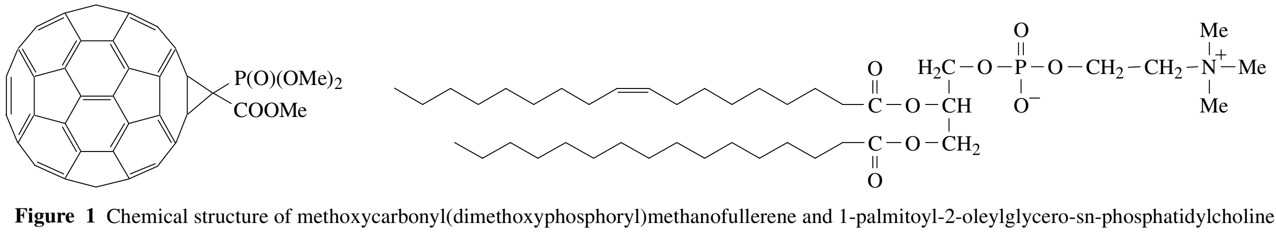

Figure 1 Chemical structure of methoxycarbonyl(dimethoxyphosphoryl)methanofullerene and 1-palmitoyl-2-oleylglycero-sn-phosphatidylcholine.

lecithin at molar ratios from 1:2 to 1:20 on a water subphase are shown in Figure 2. All the mixtures of P-methano-C_{60}–lecithin form stable monolayers with a collapse pressure of 62–66 mN m^{-1}. The limiting mean molecular area per molecule depends on the molar ratio of components in the mixed monolayers (Figure 2). According to ideal two-dimensional solution models, the theoretical molecular area A_0 per molecule can be calculated by the equation:

$$A_{0,\mathrm{calc}} = \varphi_1 A_{0,1} + \varphi_2 A_{0,2}, \tag{1}$$

where φ_1 and φ_2 are the molar fractions of components 1 and 2 in the mixed monolayers, respectively; $A_{0,1}$ and $A_{0,2}$ are the surface molecular areas per molecule of pure components in Langmuir monolayers; P-methano-C_{60} and lecithin are designated by 1 and 2, respectively.

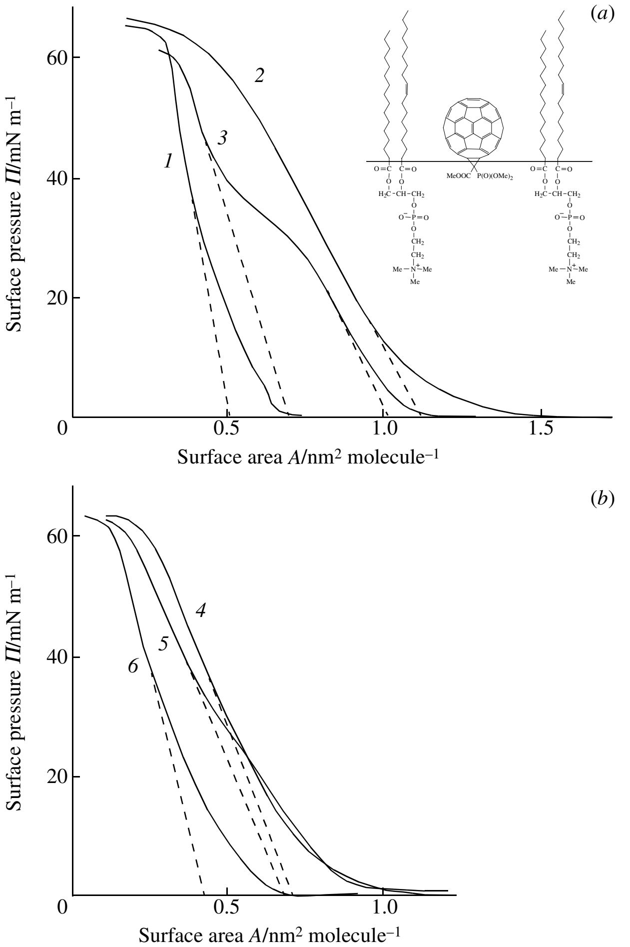

Figure 2 Surface pressure (Π) *vs.* surface area (A) isotherms of (*1*) lecithin, (*2*) P-methano-C_{60} and P-methano-C_{60}–lecithin mixtures at molar ratios of (*3*) 1:2, (*4*) 1:5, (*5*) 1:7 and (*6*) 1:20 at the air–water interface.

Table 1 shows that changes in the experimentally observed and calculated [according to equation (1)] mean molecular surface areas per molecule are insignificant. Considerable deviations of A_0 are noted for the mixed monolayers at a low molar ratio of lecithin to P-methano-C_{60} (2:1 and 5:1) but with 20:1 the difference is minor: $A_{0,\mathrm{calc}} = 0.58$ nm², $A_{0,\mathrm{observed}} = 0.50$ nm². The results shown in Table 1 may be explained by the intermolecular interactions between P-methano-C_{60} and lecithin in the mixed monolayers at the molar ratios 1:2 and 1:5. At the molar ratio 1:2, the changes in the surface pressure–mean molecular area per molecule isotherm are shown in Figure 2 (curve *3*). Compression of the P-methano-C_{60}–lecithin mixtures in a Langmuir trough showed reproducible transitions from a 'gaseous phase' ($\Pi \approx 0$ mN m^{-1}) through a short-range 'liquid-like' phase ($\Pi < 10$ mN m^{-1}) to a somewhat condensed phase. There are two types of the condensed solid films: the first type is characterised by a large slope of $d\Pi/dA_0$ and a mean molecular area of 0.74 nm², while the second-type 'solid-like' phase is characterised by the mean molecule area A_0 of 0.97 nm². The transient region between the 'solid-like' phase and the solid condensed state of the monolayer may be considered as a provisional phase transition. The transient region in the mixed monolayers decreases, if the molar ratio P-methano-C_{60}:lecithin increases and at 1:20 it disappears.

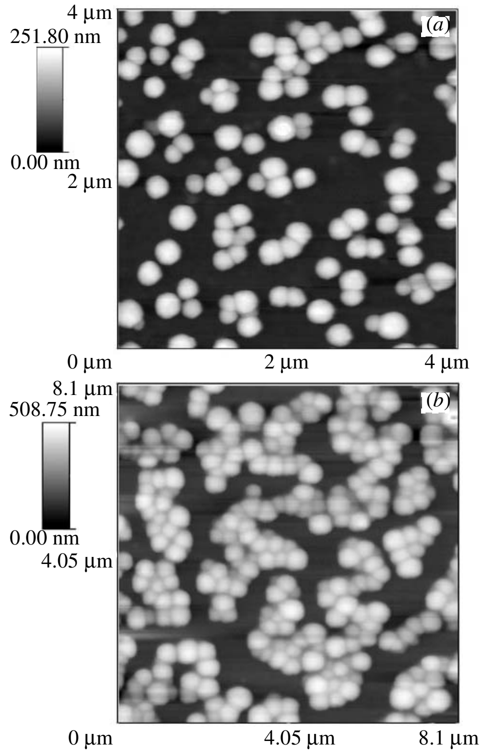

Figure 3 AFM images of the vesicles produced from the thin film of lecithin–P-methano-C_{60} at a molar ratio of 1:5. The subphase: (*a*) pure water, (*b*) 1.3×10^{-5} M $Cu(MeCOO)_2$ solution.

The interaction of the monolayers of P-methano-C_{60} and their mixtures with lecithin in a Cu^{2+}-containing aqueous solution leads to a decrease in the surface molecular area A_0 (Table 1). This fact may be interpreted as the complexation of Cu^{2+} ions with monolayer components.

The thin films of the mixed P-methano-C_{60}–lecithin system at a molar ratio of 1:5 are capable of organising vesicles after water treatment. AFM images show a regular structure of the vesicles (Figure 3). The average size of the vesicles is 0.2–0.3 μm. The analysis of chloroform extracts of the water solution after water treatment of the coated glass slide points to the implantation of P-methano-C_{60} in inclusion vesicles.

Figure 3 indicates that the average size of vesicles produced from the same P-methano-C_{60}–lecithin system after copper acetate solution treatment is twice larger than that after water treatment: 0.4–0.2 μm. Another feature of vesicles obtained in

Table 1 Comparative data of surface molecular area A_0 (collapse area) characterising mixed monolayers under an aqueous subphase in the presence of copper ions. A 1.3×10^{-5} M $Cu(MeCOO)_2$ solution was used.

Molar ratio P-methano-C_{60}: lecithin	Subphase	Surface molecular area/nm² Solid	Solid-like	Calculated	Π/mN m^{-1}
P-methano-C_{60}	H_2O	1.02	—	1.02	66
P-methano-C_{60}	Cu^{2+}	0.78	—	—	64
1:2	H_2O	0.74	0.97	0.72	65
1:2	Cu^{2+}	0.72	1.04	—	63
1:5	H_2O	0.67	—	0.64	66
1:5	Cu^{2+}	0.63	—	—	64
1:7	H_2O	0.68	—	0.62	66
1:7	Cu^{2+}	0.63	—	—	64
1:20	H_2O	0.50	—	0.58	65
1:20	Cu^{2+}	0.45	—	—	62
lecithin	H_2O	0.56	—	0.56	64
lecithin	Cu^{2+}	0.50	—	—	63

the presence of Cu^{2+} ions is their aggregation into clusters. This phenomenon is probably similar to that of liposome fusion under the influence of Ca^{2+} ions.

Thus, phosphorylated methanofullerene and its mixtures with lecithin in a wide range of molar ratios (1:2–1:20) can form stable monolayers at the air–water interface. Mixed phosphorylated-methanofullerene-with-lecithin thin films are able to produce self-organising vesicles after aqueous solution treatment. The aggregation of vesicles into clusters under the influence of Cu^{2+} ions was found.

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