Monolayers of lipids at the water–air interface

Introduction

Biological membranes are very complex structures. Lipids are the major constituents of biological membranes. Membranes are formed of a bilayer of lipid compounds in which the other compounds, such as proteins and sterols, are immersed or bound to the two interfaces [1]. Studies of lipid–lipid interactions in these membranes are important to understand their organisation. The results obtained from studies on isolated membranes are difficult to interpret; therefore, it is necessary to work on well-defined model membranes. Of the several model systems used to study lipid interactions, the monolayer technique has been applied extensively [2, 3, 4, 5]. Experiments with monolayers have the advantage that the arrangement of molecules can be easily controlled by changing the molecular area and the surface pressure of the monolayer. The study of monolayers is a useful means to learn the interfacial distribution of the molecules and the possible interactions among them in the two-dimensional state. Monolayers were first used as membrane models in 1925, in the well-known experiment carried out by Görtel and Grendel [6]. Since then monolayer techniques have been widely used to mimic the molecular organisation of lipids in biomembranes. One particular monolayer compound that has received much attention over the years is the phospholipid dipalmitoyl phosphatidylcholine (DPPC), the principle component of cell membranes [7, 8, 9].

In this paper we report a study of spread monolayers of dipalmitoyl phosphatidylcholine, methyl palmitate and their mixtures were studied. For this purpose, the surface pressure ($\pi$)-average molecular area ($A$) isotherms were recorded using a Langmuir film balance. From the $\pi$-$A$ results information on the mutual miscibility of the two components in the two-dimensional state was deduced. Then classical surface chemistry thermodynamic analysis was performed on these isotherms, which involved calculating the excess free energy of mixing, $\Delta G_{\text{mix}}^E$. The negative values of $\Delta G_{\text{mix}}^E$ indicate that the compounds in the two-dimensional state experience mainly attractive interactions.

Abstract Spread monolayers of dipalmitoyl phosphatidylcholine, methyl palmitate and their mixtures were studied. For this purpose, the surface pressure ($\pi$)-average molecular area ($A$) isotherms were recorded using a Langmuir film balance. From the $\pi$-$A$ results information on the mutual miscibility of the two components in the two-dimensional state was deduced. Then classical surface chemistry thermodynamic analysis was performed on these isotherms, which involved calculating the excess free energy of mixing, $\Delta G_{\text{mix}}^E$. The negative values of $\Delta G_{\text{mix}}^E$ indicate that the compounds in the two-dimensional state experience mainly attractive interactions.

Keywords Langmuir monolayer · Dipalmitoyl phosphatidylcholine · Methyl palmitate

Experimental

DPPC and MP were supplied by Aldrich Chemie, Germany, and were used without further purification. Chloroform (the spreading
solvent) from POCH, Poland, was distilled before use. The water used in all the experiments was distilled four times.

The $\pi$–$A$ isotherms were determined with a Langmuir trough (KSV 1000, Finland) at 20 °C. The subphase temperature was controlled with a U3 thermostat. Before each measurement, the water cleanliness was checked for possible adsorbed impurities by measuring the surface pressure in the entire area interval; this was always less than 0.1 mN m$^{-1}$ in the whole field. The monolayers were obtained by spreading the solution of lipids in chloroform with a 100-μl microsyringe (Hamilton). After deposition about 20 mm was allowed before compression to permit the solvent to evaporate and the film to spread homogeneously. The compression speed was 6 mm min$^{-1}$. The compression speed was fixed at that level, because below it no difference in the $\pi$–$A$ isotherms was observed. The measurement accuracy was ±0.01 mN m$^{-1}$ for the surface pressure, ±0.01 Å$^2$ per molecule for the area and ±0.05°C for temperature. Each isotherm was recorded at least twice.

### Results and discussion

The experimental isotherms corresponding to the monolayers formed by DPPC, MP and their mixtures in different molar fractions, at 20 °C, are presented in Fig. 1.

The surface parameters obtained from the isotherms of pure component are reported in Table 1. $A_{\lim}$ is the limiting area per molecule, i.e. the area extrapolated to zero surface pressure under maximum packing conditions, $\pi_{\text{coll}}$ is the collapse pressure determined from the change in the slope of the straight-line part of the isotherm in the high-pressure range and $C_s$ is the maximum value of the compressibility modulus defined as

$$C_s^{-1} = -A \left( \frac{\partial \pi}{\partial A} \right)_T.$$  

The values of $C_s^{-1}$ enable the monolayer phases to be defined according to the classification of Davies and Rideal [10].

![Fig. 1 $\pi$–$A$ isotherm of the dipalmitoyl phosphatidylcholine (DPPC)-methyl palmitate (MP) system on water at 20 °C](image)

<table>
<thead>
<tr>
<th></th>
<th>$A_{\lim}$ [Å$^2$ per molecule]</th>
<th>$\pi_{\text{coll}}$ [mN m$^{-1}$]</th>
<th>$C_s$ [mN m$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPC</td>
<td>48.87</td>
<td>59.31</td>
<td>293.6</td>
</tr>
<tr>
<td>MP</td>
<td>19.98</td>
<td>35.09</td>
<td>347.9</td>
</tr>
</tbody>
</table>

The isotherm of the DPPC monolayer is in satisfactory agreement with that previously reported [11]. The characteristic of this isotherm is a plateau representing the region of coexistence of two different phases, the liquid-expanded phase, $L_1$, and the liquid-condensed phase, $L_2$. The value of $A_{\lim} = 48.87$ Å$^2$ per molecule is higher than we could expect for the close-packed double-chain molecules, assuming vertical orientation of alkyl chains (around 2×20 Å$^2$=40 Å$^2$). This indicates a tilting of the hydrocarbon chains with respect to the normal to the surface. The value of $C_s$ is 293.6 mN m$^{-1}$ shows that the monolayer is in the liquid-condensed phase under maximum packing conditions. The collapse pressure is reached at 59.31 mN m$^{-1}$.

The $\pi$–$A$ isotherm for MP monolayers has a steep slope, which indicates that a small decrease in area causes a significant increase in pressure until $\pi_{\text{coll}} = 35.09$ mN m$^{-1}$. The shape of the curve and very low value of the limiting area, $A_{\lim} = 19.98$ Å$^2$ per molecule, imply that the MP molecules in the monolayer are arrayed with their hydrocarbon chains almost perpendicular to the interface. This is also confirmed by the value of $C_s$ higher than obtained for the DPPC monolayer, indicating more compact packing of hydrophobic chains.

The $\pi$–$A$ isotherms of mixed DPPC/MP monolayers, determined for different molar fractions of DPPC (0.2, 0.4, 0.5, 0.6, 0.8) are included between those of DPPC and MP monolayers in a regular sequence of mole fractions. The most striking features of these isotherms are the surface pressures at which the liquid-condensed state appears – they are lower than those observed for monolayers of pure components. This suggests the condensation of mixed monolayers. To validate this suggestion, the maximum compressibility modulus were calculated and compared with those of the pure components (Fig. 2).

For mixtures with similar content of both components $C_s$ is much higher than for monolayers of DPPC or MP, 704.8, 849.7 and 708.9 mN m$^{-1}$, for $x_{\text{DPPC}}$ (0.4, 0.5 and 0.6, respectively). Therefore, progressive condensation is observed as the equimolar mixture is approached. Low compressibility is a sign that the molecules are packed tightly and the cohesive forces between them are considerable. The values of the collapse pressures (Fig. 3) are included between those for DPPC and MP monolayers.