



SURFACE POTENTIALS OF CHOLESTEROL AND DIMYRISTOYL PHOSPHATIDYLCHOLINE MONOLAYERS AT THE AIR/WATER INTERFACE

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Received June 4, 2010

The behaviour of cholesterol (CHO) and dimyristoyl phosphatidylcholine (DMPC), as well as of their mixed monolayers of various compositions has been investigated by surface potential (ΔV) measurements at the air/water interface for different surface pressures, using Langmuir technique coupled with the vibrating plate method. Isotherms of ΔV versus mean molecular area, A , at different cholesterol mole fractions, x_{CHO} , were recorded, and ΔV versus monolayer composition curves were constructed for various constant lateral surface pressures. From ΔV data, the component of the dipole moment perpendicular to the monolayer plane was estimated, and its variation with A and x_{CHO} was investigated. Our results indicate strong interactions between DMPC and CHO molecules, possibly leading to well defined nanostructures, plausible to occur in natural membranes. Besides hydrogen bonds between DMPC carbonyl and CHO hydroxyl groups, electrostatic and van der Waals interactions increase the ability of these biomolecules to generate nanostructured domains at fluid interfaces.

INTRODUCTION

An important question in membrane biology and biophysics is the role of lipids in cell membrane organization. In this respect, Langmuir monolayers (films) can offer important models for investigation of the existing molecular forces in oriented lipid arrangements¹⁻³ at fluid interfaces.

Thus, the mixed cholesterol (CHO) and phospholipids (e.g. dimyristoyl phosphatidylcholine: DMPC) monolayers make up such structured systems, appropriate for modelling the membrane organization and its properties. Molecular interactions based on the van der Waals and electrostatic forces, and on the hydrogen bonds between the cholesterol OH-group and carbonylic oxygen atoms of the DMPC should play an important role in this regard.⁴ Such monolayers can be formed by spreading and compressing the biomolecules at the air/water interface.⁵ The DMPC is a typical phospholipid forming highly

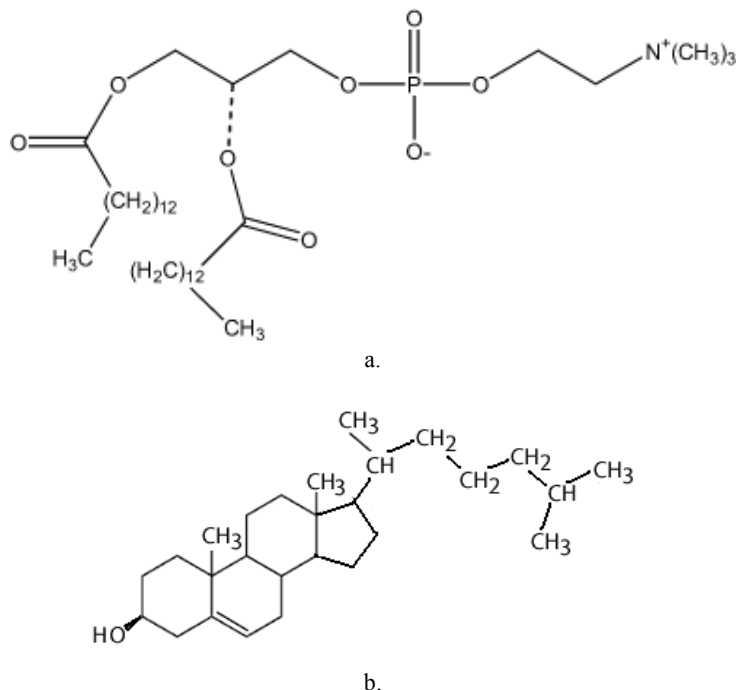
ordered monolayers.⁶⁻⁹ The DMPC molecules can be spread, compressed and highly oriented at the air/water interface at various lateral surface pressures to form monolayers of close-packed polar headgroups within the interfacial water having the hydrocarbon chains oriented into the air phase. The interaction of phospholipids with various sterols was studied both in films at the air/water interface,^{4, 9-13} and on solid support, by AFM techniques.^{9, 14-16}

We have previously investigated pure CHO and DMPC (Scheme 1) monolayers and their mixtures as Langmuir monolayers at the air/water interface and as Langmuir-Blodgett films transferred on solid support.^{9,16} The compression isotherms (surface pressure *versus* mean molecular area) and the deduced isobars (mean molecular area *versus* mixed monolayer composition at different constant surface pressures) evidenced the condensing effect of cholesterol in the mixed films with DMPC and confirmed the increased stability for mixed DMPC

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and CHO films particularly at nearly their equimolar ratio. From the surface compressional moduli of the monolayers, obtained by the derivation of the compression isotherms, the physical states of the spread Langmuir monolayers

(liquid expanded and liquid condensed) were assigned at the air/water interfaces. AFM images of the films transferred on solid surface near their collapse pressure revealed the formation of some characteristic nanostructured domains.



Scheme 1 – Chemical structures of DMPC (a) and of CHO (b).

The main aim of this work is to determine the role of the polar headgroup conformation of DMPC molecules on the behavior of the mixed DMPC and CHO monolayers at the air/water interface using surface potential measurements coupled with those of lateral surface pressures. The polar headgroup conformations of phospholipids, proposed by us previously,¹⁷ were utilized for the assessment of high stability of mixed monolayers made of DMPC and CHO at the air/water interface.

RESULTS AND DISCUSSION

The values of the surface potential, ΔV , measured at different surface pressures, π , for monolayers of pure DMPC and pure CHO and of their mixed monolayers at different cholesterol mole fractions, x_{CHO} , were represented against the mean area per molecule, A , in Fig. 1.

Comparing the data given in Fig. 1 with the compression isotherms (surface pressure, π , versus mean molecular area, A) of this system,¹⁶ the DMPC and CHO molecules both in single component (pure) monolayers and in binary monolayers bring a large modification of surface

potentials distribution at the interface, that begins at the A values significantly higher than those where the change in corresponding compression isotherms begins. For instance, these A values for DMPC are about 120 \AA^2 in the ΔV - A isotherm, against some 90 \AA^2 in the compressional π - A isotherm. For cholesterol the values are about 75 and 42 \AA^2 , respectively, and for the mixed 1:1 DMPC-CHO film the values are about 85 against 47 \AA^2 . The variation of surface potentials with the lateral surface compression of monolayers is therefore highly sensitive to the orientation of the molecules at the air/water interface, in good agreement with other reported systems.¹⁸

At the same chosen A value, pure DMPC monolayer presents the highest surface potential and pure cholesterol the lowest value (Fig. 1). Clearly, the surface potential is increasing with diminishing average molecular area, i.e. with increasing lateral surface pressures. For mixed DMPC and CHO monolayers, the high surface potentials are reached with an increase of x_{DMPC} content. For higher cholesterol mole fractions ($x_{\text{CHO}} > 0.5$), the surface potentials (not shown in the Fig. 1) are rather close to the ΔV - A curve corresponding to the pure CHO monolayer.

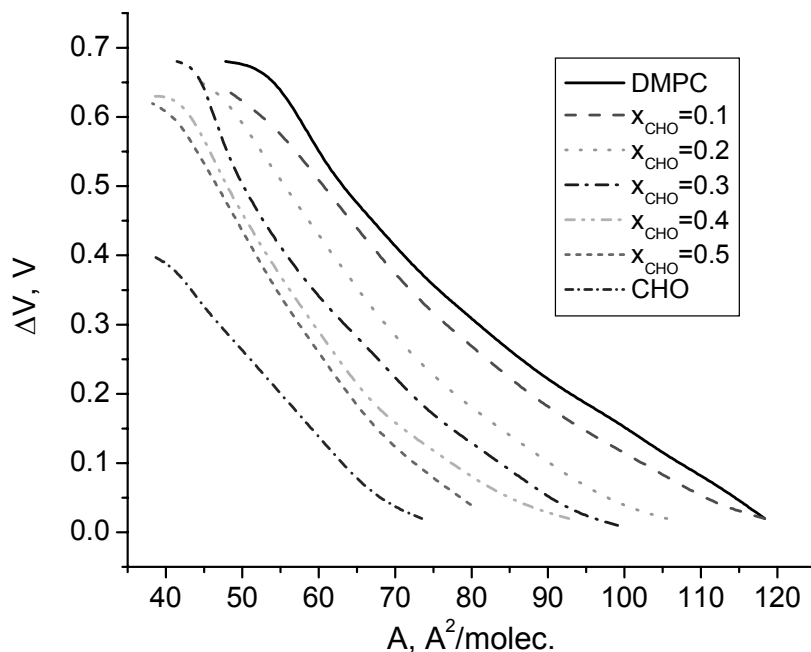


Fig. 1 – Representative isotherms of surface potentials, ΔV , versus mean area per molecule, A , for DMPC and CHO and for their mixed monolayers, at different cholesterol mole fractions, x_{CHO} , given in the insert.

These trends are more visible in the plots of the surface potentials against the cholesterol mole fraction, x_{CHO} , at constant lateral surface pressures (isobars), presented in Fig. 2, for pure DMPC and CHO monolayers and for their mixed monolayers with x_{CHO} varying from 0.1 to 1.0. The ΔV versus x_{CHO} isobars are represented for different lateral

surface pressures from 5 to 10, 15, 20, 25, 30, 35 and 40 mN/m.

The lack of linearity in the variation of surface potentials with cholesterol content in the mixed DMPC and CHO monolayers could be a consequence of the formation of localized domains, as suggested for the similar behaviour of mixed phospholipids and usnic acid monolayers.⁷

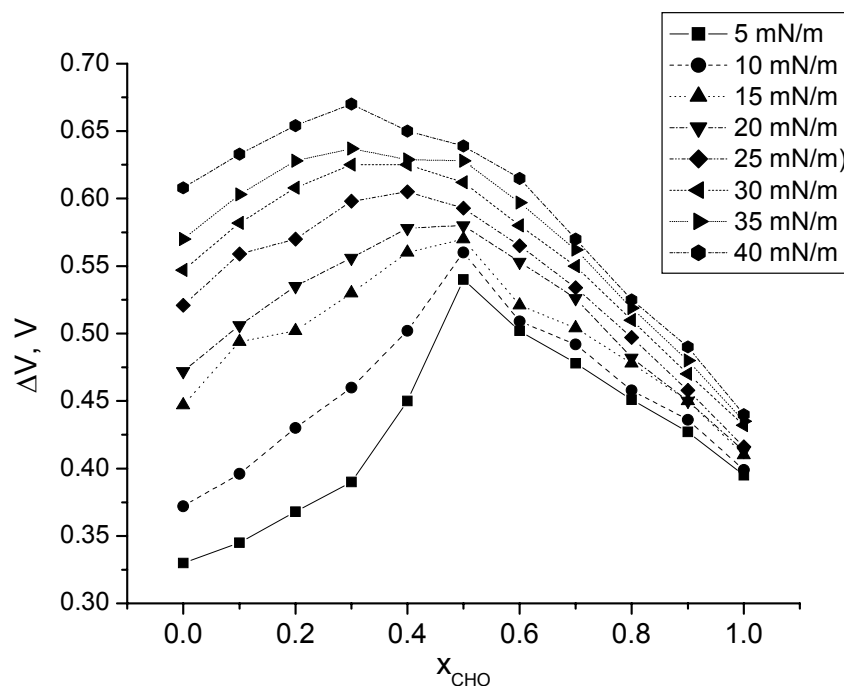


Fig. 2 – Surface potentials, ΔV , for DMPC, cholesterol and mixed DMPC-CHO monolayers against cholesterol mole fractions (x_{CHO}), at constant lateral surface pressures (given in the insert).

As seen from the ΔV versus x_{CHO} curves (Fig. 2), the surface potential rises with increasing x_{CHO} values, but after attaining a maximum value it diminishes toward the values for pure cholesterol monolayer. The maximum is reached at a molar ratio DMPC:CHO of about 1:1 at lower surface pressures, and it is shifted towards mixtures with lower CHO amounts at higher surface pressures of these monolayers. The values of the surface potential practically meet for the DMPC-CHO mixtures at the highest cholesterol amounts.

Further, the component of the molecular dipole moment in vertical direction to the monolayer plane, denoted μ_v , was estimated from the plots of the surface potentials, ΔV versus $1/A$, for each of the monolayers of DMPC and CHO and of the DMPC-CHO mixtures up to 0.5 in cholesterol mole fraction. According to Brookman,¹⁹ such a plot, ΔV versus $1/A$, should be described by the following formula

$$\Delta V = \Delta V_0 + 37.70\mu_v \cdot \frac{1}{A} \quad (1)$$

where ΔV and ΔV_0 are given in volts, μ_v is expressed as debyes (D)^{18, 19} and A is specified in $\text{\AA}^2/\text{molecule}$.¹⁹ This plot, ΔV versus $1/A$, is not a straight line, since the μ_v value varies with the surface pressure and the mean molecular area.

Using Eq. (1), the μ_v values were determined by plotting ΔV vs. $1/A$ for each of the investigated

monolayers up to 0.5 in CHO mole fractions and by the graphical derivation of these plots. The values of the dipole moment vector perpendicular to the monolayer plane are given in Fig. 3.

Starting with high average molecular areas, A , (low lateral surface pressures), the μ_v value initially increases for all monolayers with decreasing A values (Fig. 3), reflecting the change from the liquid expanded (LE) to the liquid condensed (LC) state¹⁶ within the monolayers. For the DMPC monolayer, this corresponds to a modification in the orientation of the polar headgroup of the DMPC molecules at the water/air interface from a horizontal arrangement (polar headgroup parallel oriented to the air/water interface) to a vertical one (polar headgroup oriented perpendicular to the interface).

Furthermore, Fig. 4 presents the two models proposed previously by us¹⁷ for the phosphatidylcholine moiety of phospholipids in its zwitterion form, namely oriented in its extended shape (Fig. 4a) and as its internal salt (Fig. 4b), both conformations perpendicular to the air/water interface. The notations a_h and b_h represent respectively the length and the width of the horizontal cross section of the polar headgroup, while c_w is the height of the polar part of the DMPC molecule, presumed to be anchored in the water phase.

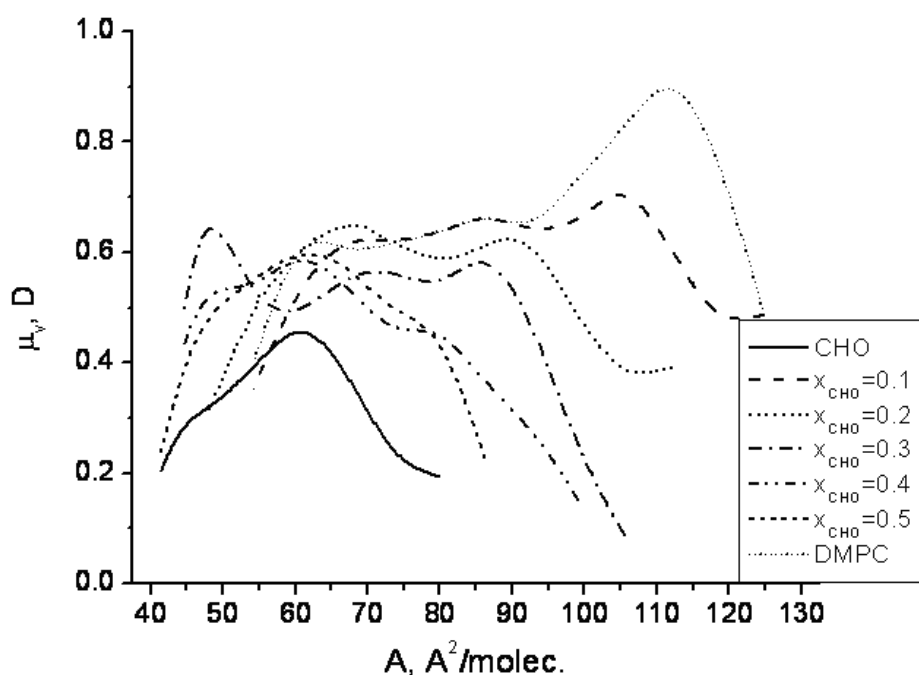


Fig. 3 – Dipole moment, μ_v , perpendicular to the monolayer plane against mean molecular area, for pure DMPC and CHO monolayers and for their mixtures. Symbols are given in the insert.

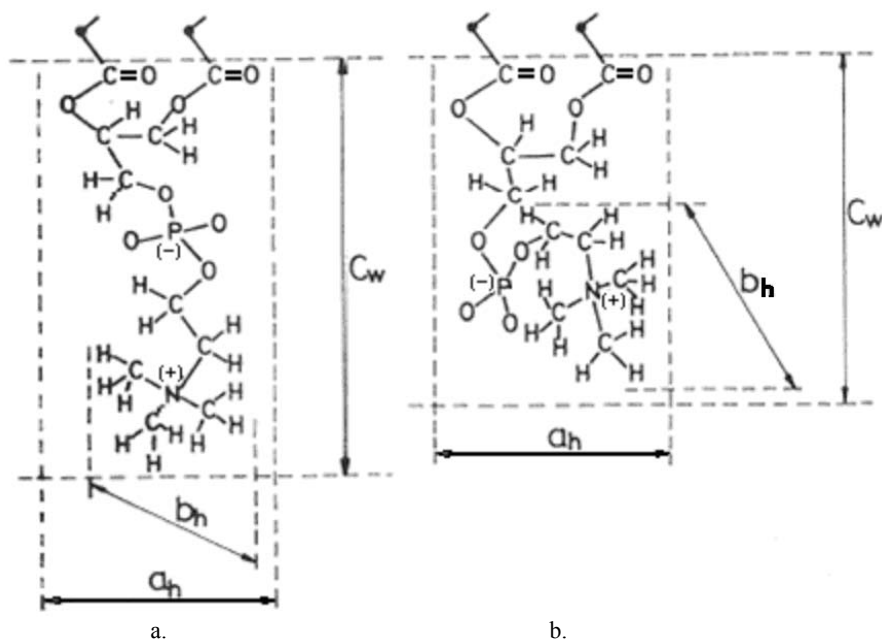


Fig. 4 – Models of the polar headgroup of DMPC molecule, for two conformations of the phosphatidylcholine moiety¹⁷, namely in its extended form (a) and in its internal salt form (b), both oriented perpendicular to the air/water interface. $(\mu_v)_a > (\mu_v)_b$. For symbols see the text.

For the pure DMPC monolayer, a maximum in the μ_v versus A curve (Fig. 3) is observed and it might correspond to an extended predominant conformation of the DMPC polar headgroup, as given in Fig. 4a, within monolayer. After the maximum the observed values of μ_v decrease to an apparent plateau. The smaller μ_v values suggest that an internal salt conformation (Fig. 4b) would be preferred within the monolayer. This fact is in substantial agreement with the dipole moment μ_v value of the extended *a* conformation (Fig. 4a) which is bigger than its corresponding value for the *b* conformation of the internal salt (Fig. 4b).

In addition, we mention that the compression isotherm of the pure DMPC monolayer corresponds to the LE state for the mean molecular areas of the plateau at the air/water interface (Fig. 3), where both conformations of phosphatidylcholine moiety could exist.

For the DMPC and CHO mixed monolayers, the aspect of the μ_v versus A curves is rather similar, but the curves are shifted toward lower A values (Fig. 3). This situation is more pronounced for the higher cholesterol content within the monolayer. Further, the plateau region is diminished for high x_{CHO} values, such as for x_{CHO} of about 0.4 and 0.5, but the highest values of μ_v are closed to each other and to the pure DMPC monolayer, suggesting that the internal salt conformation of phosphatidylcholine moiety might also be favoured in mixed DMPC and CHO monolayers.

These observations can be correlated with those recorded on the compression isotherms of mixed DMPC and cholesterol monolayers at the air/water interface,¹⁶ where a condensing effect of cholesterol was evidenced on DMPC monolayer. This effect might be ascribed to the attracting van der Waals forces and the hydrogen bondings between the phospholipids and cholesterol, stabilizing the mixed structures^{10, 20-22} and inducing the hydrocarbon chains ordering. In the compact monolayers, the penetration of water into the lipid monolayers is also reduced.⁴ We also found¹⁶ that the maximum collapse pressure and the highest stability of the mixed films occurs for a DMPC:CHO mole ratio of about 1:1. For higher cholesterol content ($x_{\text{CHO}} > 0.5$) the mixed DMPC and CHO monolayers pass into the liquid condensed state, which could explain the diminution of the dipole moment μ_v values being somehow similar in behavior with those for pure CHO monolayers.

EXPERIMENTAL

1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and β -cholesterol (CHO) were purchased from Sigma (Saint Louis, USA) and used without further purification. Benzene (>99.8%) was a product of Lach-Ner (Czech Republic), hexane (>99%) and ethanol (>99.5%) were purchased from Merck (Germany). All organic solvents used were analytical grade reagents. Ultra pure deionized water with a resistivity greater than 18 M Ω cm, obtained from an Elga apparatus was

used in all experiments as subphase. Its pH was 5.5 and its surface tension was superior to 71.8 mN/m at 25°C. All glassware was cleaned with sulfochromic mixture and then abundantly rinsed with distilled water.

The spreading solutions used were made in benzene (CHO, ca. 1 mg/mL), ethanol/hexane mixture (2:98, v/v) for DMPC, and by mixing DMPC and CHO solutions at different molar ratios. The organic solutions were spread with a Hamilton syringe. After spreading, the solution was left for 10 min for solvent evaporation. We used a KSV LB Standard Trough (KSV Ltd., Finland) controlled by KSV-5000 software and equipped with two movable barriers. The Teflon trough had an effective area of 765 cm²; the volume of the subphase was 1500 cm³. Before each measurement, the subphase surface was cleaned by sweeping and suction processes. The surface potential was measured by the vibrating plate method^{23, 24} under the same experimental conditions as in the case of the surface pressure measurements.¹⁶ It was assumed that the steady state was established when the ΔV values remained unchanged during more than 12 min. All reported ΔV results are the average values of at least five independent measurements.

CONCLUSIONS

Cholesterol, DMPC and their mixtures containing the two biomolecules in ratios from 9:1 to 1:9 were studied as spread monolayers at the air/water interface. These monolayers are important as membrane models, and their investigation is a step towards the understanding of membrane structure and properties.

The experiments performed along the present investigation reveal that surface potentials, ΔV , vary with the lateral compression of the monolayer as a function of the mean areas per molecule, and therefore, ΔV values are highly sensitive to the orientation of the molecules at the air/water interface.

This work completes our previous investigation on compression isotherms and surface compressional moduli for the DMPC and CHO system. The surface potential decreases with increasing cholesterol content, especially for $x_{\text{CHO}} > 0.5$, in the mixed monolayers, but not linearly, suggesting the formation of localized mixed domains.

Further, the plots of the dipole moment μ_v as function of mean molecular area, A , were constructed. The μ_v variation with the mean molecular surface, i.e. with the lateral compression, corresponds to changes in the orientation of the polar headgroup of the molecules, and reflects also the change between the physical states (LE to LC) of the monolayers at the air/water interface.

From structural perspective, the polar headgroup of DMPC, particularly its carbonyl groups, and the hydroxyl group of CHO can play a key role in the formation of hydrogen bonds

connecting these biomolecules in an interfacial lattice at the air/water interface. Besides these hydrogen bonds, the electrostatic interactions within the phosphatidylcholine moiety of DMPC polar headgroup cannot be neglected, and the possibility of an internal salt conformation within the DMPC monolayer is plausible. Moreover, the electrostatic interactions among the extended conformation of phosphatidylcholine moiety of DMPC molecules appear to stabilize the monolayers. These molecular interactions together with the van der Waals interactions in mixed DMPC and CHO monolayers are also favorable and lead to an increased ability of these biomolecules to generate nanostructured domains at fluid interfaces in substantial agreement with AFM observations.^{9, 16} These results indicate the existence of strong interactions between DMPC and CHO molecules, possibly leading to well defined interfacial nanostructures, which are plausible to appear even in the natural membranes.

Acknowledgements: One of us (R.-D. Pasca) received financial support from the Babeş-Bolyai University of Cluj-Napoca, Roumania (Programme No. 32/2009).

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