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The Equilibria of Phosphatidylethanolamine-Cholesterol and Phosphatidylcholine–Phosphatidylethanolamine in Monolayers at the Air/Water Interface

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Monolayers of phosphatidylethanolamine (PE), cholesterol (Ch), phosphatidylcholine (PC), and binary mixtures (PC-PE or PE-Ch) were investigated at the air/water interface. The surface tension values of pure and mixed monolayers were used to calculate π-A isotherms. The surface tension measurements were carried out at 20°C using an improved Teflon trough and a Nima 9000 tensiometer. The Teflon trough was filled with a subphase of triple-distilled water. Known amounts of lipid dissolved in 1-chloropropane were placed at the surface using a syringe. The interactions between phosphatidylethanolamine and cholesterol as well as phosphatidylcholine and phosphatidylethanolamine result in significant deviations from the additivity rule. An equilibrium theory to describe the behavior of monolayer components at the air/water interface was developed in order to obtain the stability constants of PC-PE and PE-Ch complexes. We considered the equilibrium between the individual components and the complex and established that phosphatidylethanolamine and cholesterol as well as phosphatidylcholine and phosphatidylethanolamine formed highly stable 1:1 complexes.

Keywords: Phosphatidylcholine, phosphatidylethanolamine, cholesterol, complex formation equilibria, monolayer, Langmuir trough

1 Introduction

The study of monolayers is a fascinating branch of physical chemistry, with considerable implications in fields including chemistry, physics, material science, and biology. A thorough understanding of monolayer behavior is essential to the Langmuir technique for generating monolayer films on water surfaces (1).

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. Monolayers were first used as membrane models in 1925, in the well-known experiment carried out by Görtel and Grendel (2). Since then monolayer techniques have been widely used to mimic the molecular organization of lipids in biomembranes. One particular monolayer compound that has received much attention over the years is phosphatidylcholine, the principal component of cell membranes (3–7).

The study of monolayers is of crucial importance in a great number of processes, including cell membrane modeling (8–12), breathing mechanics (13–14), vesicle formation (15–16), and optical and electronic device fabrication (17–18). Monolayer systems are often characterized by their surface pressure-area (π-A) isotherms, which provide useful information concerning molecular-level interactions between the components (1, 19–20).

Phospholipid-cholesterol interactions have been widely studied because of their biological importance (21). Cholesterol is an important component in the control of cell membrane properties and function (22).

Most studies of lipid monolayers concentrate on surface potential and surface pressure measurements, spectroscopy, or microscopic visualization of lateral domains (23). In spite of a wealth of experimental methods available for the study of lipid monolayers, a number of questions remain. One of them is the study of complex formation between phospholipids and cholesterol in monolayers at the air/water interface and in bilayers (11, 24). Interactions between phosphatidylcholine (PC) and cholesterol (Ch) appear as significant deviations from the additivity rule. The non-ideal behavior of the PC-Ch system has been explained by the condensing effect of cholesterol (23). The complexes

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most commonly found in monolayers or bilayers have stoichiometries of 1:1, 1:2, 2:1, 1:3, or 3:1 (24–26).

While most investigations of cholesterol behavior in model membranes have been undertaken using phosphatidylcholine, one should remember that biological membranes are composed of phospholipids with various polar head groups. Phosphatidylethanolamine (PE) is of interest because of its presence in natural membranes and its interesting phase properties. PE has a smaller head group that permits closer packing of the molecules compared to phosphatidylcholine. This leads to increased van der Waals interactions between the hydrocarbon chains and almost perpendicular packing relative to the membrane plane (27). The interactions between phosphatidylethanolamine and cholesterol were studied in Langmuir films using surface pressure isotherms and Brewster angle microscopy. The PE/Ch interactions were examined at various monolayer and subphase (Na^+, Ca^{2+}) compositions at 20, 25, and 30°C. PE and Ch are miscible for cholesterol mole fractions x_{Ch} between 0.3–0.5. Cholesterol induces condensation of the PE monolayers. The most significant condensation of the PE/Ch monolayers has been observed at $x_{Ch} = 0.4$ when Ca^{2+} ions are present in the subphase (27).

Two substances can form complexes of varying stoichiometry. However, due to the fact that the first stability constant in complexes is usually the largest (28), we assumed that 1:1 complexes were predominant.

The formation of complexes between egg lecithin and cholesterol in mixed monolayers was described in an earlier paper (11). This paper uses a new system of equations to describe the formation of 1:1 complexes in two other systems.

The Langmuir method is useful and relatively simple. However, it is difficult to obtain repeatable experimental results. The Langmuir trough has previously been improved by the introduction of a new method of depositing the solution on the subphase surface (in the form of a drop) (29), by a new method for uniform compression of the monolayers (30), and by the use of Teflon and Delrin barriers (31). Our work has led us to develop further improvements to the Langmuir method including the use of a glass barrier, a sensing device constructed of very thin glass, and the use of 1-chloropropane as a solvent. In this paper we present evidence for the formation of 1:1 PC-PE and PE-Ch complexes at the air/water interface and calculate their stability constants. We also describe the effect of the improvements on the repeatability of the technique.

1.1 Theory

During formation of a mixed two-component monolayer on a free electrolyte surface, the individual components (denoted by A and B) can form complexes. The equilibrium of such a system is described by the complexation reaction. Let us assume that a 1:1 complex is formed in a mixed monolayer at the air/water interface. The reaction:

$$A + B \Leftrightarrow AB$$

may be described by the system of equations:

$$c_{S_A} S_A + c_{S_B} S_B + c_{S_{AB}} S_{AB} = 1 \quad (1)$$

$$c_{S_A} + c_{S_{AB}} = c_A \quad (2)$$

$$c_{S_B} + c_{S_{AB}} = c_B \quad (3)$$

$$c_{S_{AB}} = K_{AB} c_{S_A} c_{S_B} \quad (4)$$

$$x_B(c_A + c_B) = c_B \quad (5)$$

Where c_{S_A}, c_{S_B}, and $c_{S_{AB}}$ [mol/m^2] are the surface concentrations of components A and B and complex AB; c_A and c_B [mol/m^2] are the total surface concentrations of components A and B and complex AB; S_A, S_B, and [m^2/mol] are the surface areas occupied by 1 mole of components A and B and complex AB; K_{AB} [m^2/mol] is the stability constant of complex AB, and x_A and x_B are the mole fractions of components A and B.

Attempts to solve this system of equations resulted in complicated expressions, so Eqs. (1)–(5) were differentiated with respect to x_B and approximated to low or high argument values. At $x_B \rightarrow 0.00$ (a monolayer formed from pure component A), the system of equations is simplified to:

$$c'_{S_A} S_A + c'_{S_B} S_B + c'_{S_{AB}} S_{AB} = 0 \quad (6)$$

$$c'_{S_A} + c'_{S_{AB}} = c'_{A(x_B=0)} \quad (7)$$

$$c'_{S_B} + c'_{S_{AB}} = c'_{B(x_B=0)} \quad (8)$$

$$c'_{S_{AB}} = K_{AB} \frac{1}{S_A} c'_{S_B} \quad (9)$$

At $x_B \rightarrow 1.00$ (a monolayer formed from pure component B), the system of equations after differentiation with respect to x_B is simplified to:

$$c'_{S_A} S_A + c'_{S_B} S_B + c'_{S_{AB}} S_{AB} = 0 \quad (10)$$

$$c'_{S_A} + c'_{S_{AB}} = c'_{A(x_B=1)} \quad (11)$$

$$c'_{S_B} + c'_{S_{AB}} = c'_{B(x_B=1)} \quad (12)$$

$$c'_{S_{AB}} = K_{AB}\left(- \frac{1}{S_B}\right) c'_{S_A} \quad (13)$$

In the above equations,

c'_{S_A}, c'_{S_B}, and $c'_{S_{AB}}$ are the derivatives of c_{S_A}, c_{S_B}, and $c_{S_{AB}}$ with respect to x_B.

The quantities c'_{S_A}, c'_{S_B}, and $c'_{S_{AB}}$ may be eliminated from the system of equations if the values of S_A and S_B are known. Suitable transformations lead to expressions for two quantities of interest: the stability constant of the complex K_{AB} and the surface area occupied by one molecule of the complex S_{AB}:

$$K_{AB} = \frac{S_B^3 c'_{B(x_B=1)} - 2S_A S_B - S_A^3 c'_{A(x_B=0)}}{S_B - S_A + S_A^2 c'_{A(x_B=0)} + S_B^2 c'_{B(x_B=1)}} \quad (14)$$

$$S_{AB} = \frac{\left(S_A S_B + c'_{A(x_B=0)} c'_{B(x_B=1)} S_A^2 S_B^2\right)(S_A + S_B)}{S_A^3 c'_{A(x_B=0)} + S_B^3 c'_{B(x_B=1)}} \quad (15)$$

The slopes of tangent lines at the points $x_B = 0.00$ (pure component A) and $x_B = 1.00$ (pure component B) may be calculated from the following equations:

$$c'_{A(x_B=0)} = \frac{K_{AB}(S_A - S_{AB}) - S_A S_B}{S_A^2(S_A + K_{AB})} \quad (16)$$

$$c'_{B(x_B=1)} = \frac{-K_{AB}(S_B - S_{AB}) - S_A S_B}{S_2^2(K_{AB} - S_B)} \quad (17)$$

Eqs. (16) and (17) may be used for verification of slopes obtained either from theory or by experiment. Agreement between the slopes indicates that the method of calculating K_{AB} and S_{AB} is correct.

2 Experimental

2.1 Measuring Apparatus and Measuring Procedures

Figure 1 depicts the homemade computer-controlled apparatus used for surface tension measurements. It consists of (1) a Nima 9000 tensiometer, (2) a Teflon trough of 648 cm^2 surface area, (3) a thin glass plate, (4) a glass barrier, (5) a moving glass barrier system, and (6) a control unit. The surface tension was recorded as a function of the monolayer surface area using the Nima Systems ST9002 computer program (7). The glass barrier was moved at a velocity of 0.03 cm s^{-1}. The apparatus was situated under an acrylic cover.

The surface tension measurements were carried out at the water/air interface at 22°C, and were expressed as surface pressure-area per molecule (π-A) isotherms. For all experiments, the trough was filled with triple-distilled water as the subphase. The monolayers were prepared by spreading a defined volume of a lipid solution in 1-chloropropane on the aqueous subphase using a Hamilton micro-syringe. Ten minutes were allowed for solvent evaporation and monolayer equilibration before an experiment was begun. The monolayer was continuously compressed to obtain the π-A isotherms using the glass barrier.The glass material allowed lipid molecules to pass under the barrier. This innovation considerably improved the reproducibility of the results (32).

The Nima ST9002 computer program was used to calculate the surface pressure of the monolayer π as a function of surface area per molecule A: $\pi = \gamma - \gamma_0 = f(A)$, where γ_0 is the surface tension of the lipid-covered surface and γ is the surface tension of the bare air/water interface.

Before each trial, the Teflon trough (trough size 648 cm^2) was washed and rinsed with purified water. The subphase surface was cleaned just prior to each measurement by suction with a vacuum pump until the surface tension was constant and equal to the surface tension value of pure water at 22°C (approximately 72 mN m^{-1}). All glassware in contact with the samples was cleaned with chromic acid and repeatedly rinsed with purified water before use.

The system was enclosed in an acrylic box to minimize water evaporation, to ensure high humidity, and to avoid contamination of the system.

All of the reported values are highly reproducible and represent the average of at least five experiments. The standard deviation for surface area measurements was less than 1%.

2.2 Reagents

Phosphatidylethanolamine (99%) and egg phosphatidylcholine (99%) were purchased from Fluka and were used as received. The phosphatidylcholine had the following fatty acid composition: 16:0∼33%, 18:0∼4%, 18:1∼30%, 18:2∼14%, 20:4∼4%. The molecular weights of the phospholipids were approximately 635.87 g $mol^{-1}$and 752.08 g mol^{-1}. Cholesterol (99%) was obtained from Sigma and was used without further purification. The molecular weight of cholesterol is 386.67 g mol^{-1}.

The 1-chloropropane solvent (> 98% pure) was supplied by Aldrich. Solutions were prepared by dissolving appropriate amounts of each material in 1-chloropropane at a concentration of 1 mg cm^{-3} and were stored at 4°C

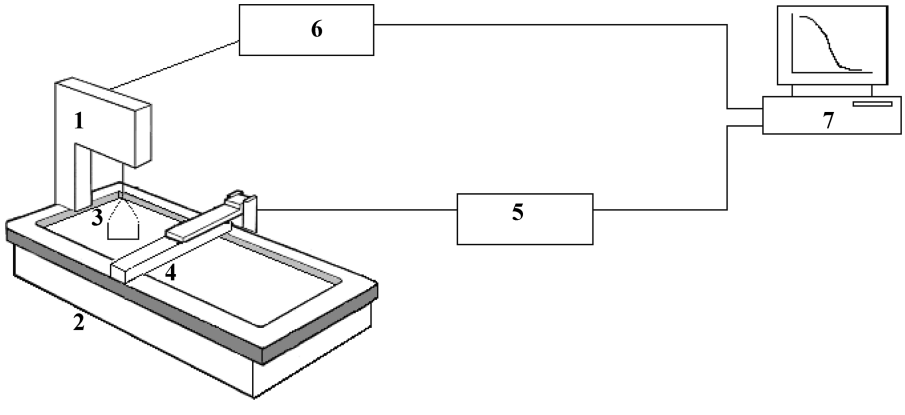

Fig. 1. Schematic diagram of the measurement apparatus: (1) Nima 9000 tensiometer, (2) Teflon trough of 648 cm^2 surface area, (3) thin glass plate, (4) glass barrier, (5) moving barrier system, (6) control unit of tensiometer, (7) Nima ST9002 computer program.

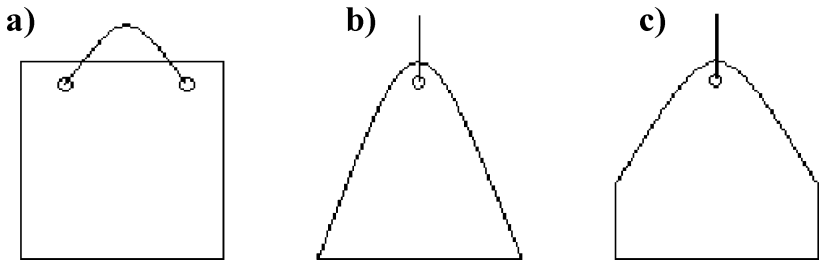

Fig. 2. Geometries of Wilhelmy plate for surface tension measurements.

(32). The water used in the experiments was prepared by triple distillation (the second distillation was performed over $KMnO_4$ and KOH to remove organic impurities).

3 Results and Discussion

In this paper, we present surface tension measurements of mixed monolayers obtained using an improved Langmuir method. The improvements include the use of a glass barrier, a sensing device formed from very thin glass, and the use of 1-chloropropane as a solvent.

3.1 Thin Glass Plate as a Tensiometer Element

A variety of sensing devices are applied to interfacial tension measurement. The most common sensing devices are a platinum ring (du Noüya), the Wilhelmy plate, and the Lenard frame. The first two sensing devices are particularly attractive; the Wilhelmy plate (usually platinum, glass, quantitative tissue paper) permits the simple determination of absolute values of surface tension, while the du Nouy'a method is useful for experiments employing surfactants.

Replacement of the tissue paper sensing element with a microscope slide (Fig. 2a) considerably improved the measurement repeatability due to more reliable contact of the slide with the subphase (32). The large disparity between the thickness of the plate and the length in contact with the water phase ensures the wetting angle is close to zero. This effect was more apparent with the rectangular microscope slide than with the element illustrated in Figure 2b. This shape reduced the mass of the sensor, but the excessively sharp corners caused frequent separation of the plate from the subphase surface. Cutting and polishing the microscope slide to the shape of Figure 2c eliminated problems with separation. The sensing element must be extremely clean, which is ensured by boiling for several minutes in chromic acid, followed by careful rinsing in hot triple-distilled water.

3.2 1-Chloropropane as a Solvent

In order to obtain repeatable effects consistent with the results of others, it is necessary to carefully select the lipid solvent. The physico-chemical parameters of common solvents for monolayer formation are listed in Table 1 (33).

The solvent must fulfill the following requirements (32):

1. The solvent should not react with the subphase, the subphase components or with the membrane lipids;
2. The solvent should not wet the material from which the trough is built. Wetting of the trough reduces the confinement of the membrane components, lowering the effective surface concentration and considerably increasing the uncertainty of the results.
3. The solvent should possess a high volatility to enhance the rate of evaporation. A good solvent will enable the membrane components to disperse quickly over the subphase surface and completely evaporate to avoid leaving traces in the monolayer. However, if the evaporation rate is greater than the speed with which the components are

Table 1. Physicochemical parameters of solvents commonly used for monolayer formation (33).

Solvent	*Boiling point [°C]*	*Density [g cm^{-3}]*	*Solubility in water [g per 1000 g of water]*	*Dipole moment [10^{-30} C m]*	*Teflon wettability*
Diethyl ether	35	0.72	7.5^{16}°C	1,15	Yes
Pentane	35–36	0.63	No	0.00	Yes
1-chloropropane	46–47	0.89	No	2.05	No
Chloroform	61	1.49	0.82^{210}°C	1.08	No
Hexane	69	0.66	0.014^{15}°C	0.00	Yes
Heptane	98	0.68	0.005^{16}°C	0.00	Yes
Octane	125–127	0.70	0.0015^{16}°C	0.00	Yes

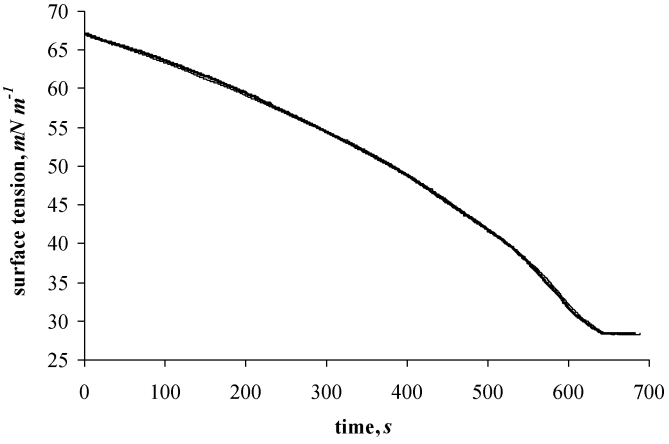

Fig. 3. Surface tension as a function of time for phosphatidylcholine. The plot was created as the barrier was traversed six times.

distributed, a 'patch' of material appears and the monolayer is heterogeneous.

4. The density of the solvent is also a consideration. Solvents heavier than the subphase will carry material to the bottom of the trough and reduce the surface concentration.

Common solvents such as chloroform (34), hexane, and chloroform mixtures with benzene (35), ethanol, methanol, or diethyl ether did not provide us with good quantitative results (32). The experimental reproducibility was low when hexane was used. Chloroform was effective in spreading the lipids on the water surface, but the high density carried a fraction of the monolayer constituents to the bottom of the trough.

From the above experience, it is clear that a good solvent should be immiscible with water, should have a boiling point between 40–60°C, should be slightly polar in character, should be of lower density than the subphase, and should not wet the Teflon components of the trough. Of the solvents listed in Table 1, only 1-chloropropane fulfills all the requirements of the ideal solvent.

Figure 3 depicts the surface tension as a function of time for phosphatidylcholine as the barrier was traversed six times. The excellent overlap of the six curves indicates the repeatability of the experimental process.

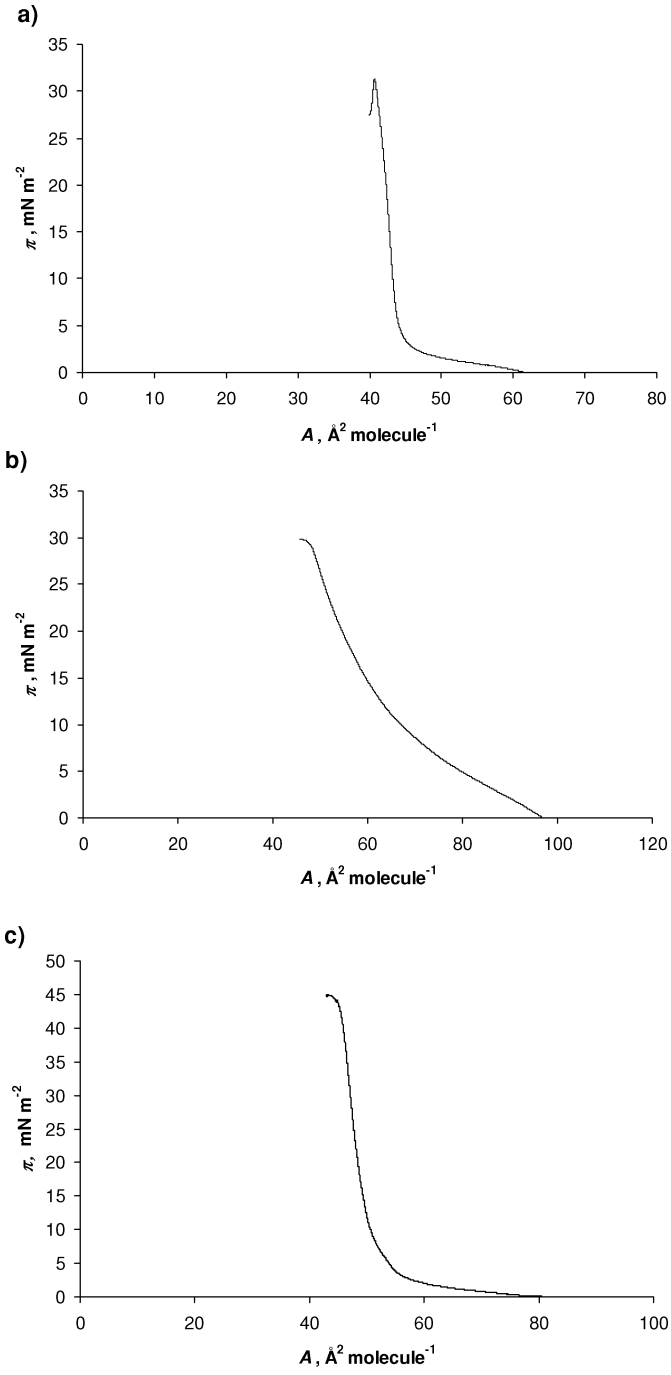

Fig. 4. Isotherms of cholesterol (a), phosphatidylcholine (b) and phosphatidylethanolamine (c).

3.3 Glass Barrier in Langmuir Method

In order to improve the repeatability of our surface concentration measurements, we examined each element of the experimental apparatus in an effort to isolate the major contributors to uncertainty in the measurements. Especially at higher values of surface pressure, it is imperative that the barriers completely confine the monolayer components. The traveling barrier should maintain a stable position upon stopping (36). Teflon barriers often do not provide repeatable results because the large polar head groups of phospholipids partially slide under the barrier. To prevent this effect, previous workers have described the use of a hydrophilic glass barrier instead (5, 11–12, 19, 32). This innovation was very effective at improving the repeatability (Fig. 3).

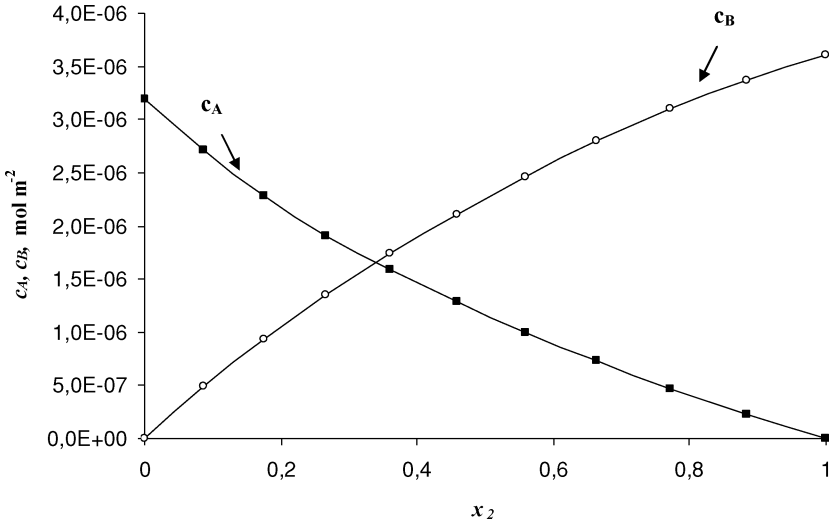

Fig. 5. The dependence of total surface concentration of phosphatidylethanolamine (c_A) and cholesterol (c_B) on the mole fraction of cholesterol (the experimental values are indicated by points and the theoretical values by the curve).

3.4 Phosphatidylethanolamine-cholesterol and Phosphatidylcholine–phosphatidylethanolamine Complexes

In this section we present evidence for the formation of 1:1 phosphatidylethanolamine-cholesterol and phosphatidylcholine-phosphatidylethanolamine complexes at the air/water interface, and develop a system of equations to describe the complex formation. Using these equations, the stability constants of the PE-Ch and PC-PE complexes were calculated.

Figure 4 presents π-A isotherms of cholesterol (a), phosphatidylcholine (b), and phosphatidyletaholamine (c). The slope of the cholesterol isotherm is very high, indicating a perpendicular orientation of the molecules at the interface with the hydrophilic OH group directed at the aqueous subphase. The surface area for the cholesterol molecule (45Å^2) was obtained experimentally by extrapolating the isotherm to $\pi = 0$. This is in agreement with the previously reported value of 45Å^2 (29).

The π-A isotherms of phosphatidylcholine and phosphatidylethanolamine are shaped differently. Phosphatidylethanolamine and phosphatidylcholine monolayers are examples of liquid-expanded membranes, with the hydrophilic head groups located in the aqueous subphase and the hydrophobic fatty acid tails oriented toward the air. The surface area per lipid molecule assumes various values depending on the length, conformation, and degree of unsaturation of the hydrocarbon chains. The surface areas for

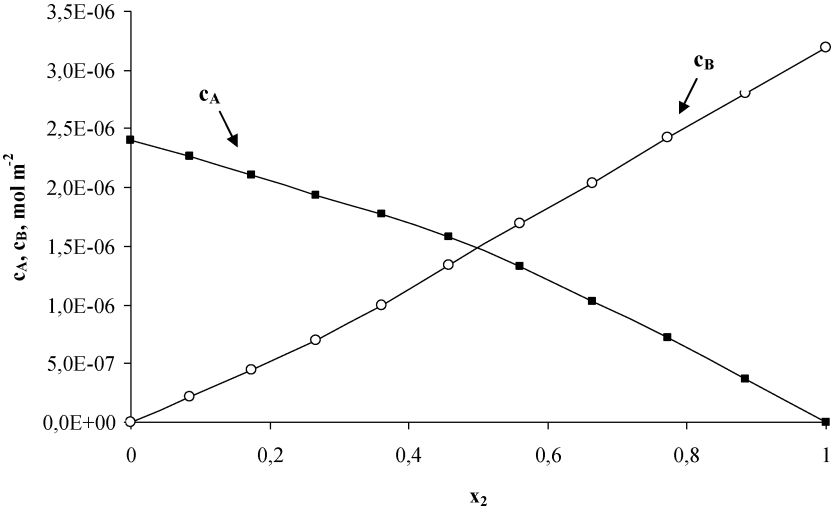

Fig. 6. The dependence of total surface concentration of phosphatidylcholine (c_A) and phosphatidylethanolamine (c_B) on mole fraction of phosphatidylethanolamine (the experimental values are illustrated with points and the theoretical values with curves).

the phosphatidylcholine and phosphatidylethanolamine molecules are 69 Å^2 and 52 Å^2. The literature values range from 54 Å^{2}to 96 Å^2 for lecithin (34, 37) and a few 4 Å^2 less for phosphatidylethanolamine (38).

3.5 Phosphatidylethanolamine-cholesterol Complex

The total surface concentrations of PE (c_A) and cholesterol (c_B) versus mole fraction of cholesterol are depicted in Figure 5. The nearly linear shape of the $c_B = f(x_B)$ function confirms the condensed character of the membrane (36). The condensation effect of cholesterol describes the decrease in surface area per phospholipid molecule in the monolayer in the presence of cholesterol (39). It is remarkable that the function $c_B = f(x_B)$ is almost linear for $x_B > 0.5$.

The 1:1 PE-Ch complex has been assumed to exist in monolayers composed of PE and Ch (Equations 1-3). It is characterized by the stability constant, K_{AB} (Eq. 4).

The area per PE-Ch complex, $S_{AB} = 5.66 \times 10^5$ m^2 mol^{-1} (94 Å^2 molecule^{-1}), and the stability constant, $K_{AB} = 1.69 \times 10^6$ were calculated by inserting the experimental data into equations (14) and (15). Using the values calculated for S_{AB} and K_{AB} in Equations (16) and (17), theoretical c'$_A$ and c'$_B$ values were calculated and compared with the slopes of lines tangent to the experimental data at points $x_B = 0$ and $x_B = 1$.

The S_{AB} value obtained this way is higher than the area of a PE molecule, $S_A = (52$ Å^2 molecule$^{-1})$, but slightly lower than the sum of the areas of phosphatidylethanolamine and cholesterol ($S_A + S_B = 97$Å^2).

3.6 Phosphatidylcholine-phosphatidylethanolamine Complex

The total surface concentration of PC (c_A) and PE (c_B) as a function of PE mole fraction is illustrated in Figure 6.

The area of the PC-PE complex was $S_{AB} = 7.27 \times 10^5$ m^2 mol^{-1} (121 Å^2 molecule^{-1}) and the stability constant K_{AB} was 3.18×10^5. The S_{AB} value is equal to the sum of the areas of phosphatidylcholine and phosphatidylethanolamine ($S_A + S_B = 121$Å^2).

The stability constant of the PE-Ch complex is 1.69×10^6 m^2 mol^{-1}, whereas the stability constant of the PC-PE complex is 7.27×10^5 m^2 mol^{-1}. The relatively high stability of PE-Ch provides additional evidence for the prevalence of the 1:1 complex in mixed phospholipid-cholesterol monolayers (23, 27). It should be emphasized that the stability constant is higher for complexes in bilayers ($K = 4.16 \times 10^{10}$ m^2 mol^{-1}) (40). A monolayer is a two-dimensional system forming a plane at the air/water interface, while a bilayer possesses a third dimension, and is additionally stabilized by hydrophobic interactions between the hydrocarbon chains.

In Figures 5 and 6, the experimental points are compared with the values calculated using Equations 1–3 (depicted as lines). The experimental results agree closely with the theoretical predictions, indicating that our theoretical model is sufficient to accurately describe mixed PE-Ch and PC-PE monolayers.

The experimental area occupied by one PE-Ch complex is 94 Å^2, while the area occupied by a PE-PC complex is 121 Å^2. The excellent agreement between the experimental and theoretical points validates the assumption of 1:1 complex formation in the lipid membrane. The homogeneity of the measurement results indicates that complexes of stoichiometries other than 1:1 do not play a significant role in these systems.

4 Conclusions

In conclusion we would like to emphasize that the stability constants for PE-Ch and PC-PE complexes in monolayers have been reported here for the first time. Interfacial tension measurements of mixed monolayers provide a quantitative description of equilibria in the monolayer as well as between the molecules in the monolayer and in the subphase. A system of equations may be developed to calculate and verify parameters such as the stability constant, molecular area, and interfacial tension of the complex. These mathematically derived and experimentally confirmed values are of great importance for the interpretation of phenomena occurring in lipid monolayers and bilayers. In our opinion, this information will be very helpful in understanding the transmembrane transport mechanisms for ions, toxins, and drugs.

References

1. Dynarowicz-Latka, P., Dhanabalan, A. and Oliveira Jr., O.N. (2001) *Adv. Colloid. Interface Sci.*, 91(2): 221–293.
2. Bringezu, F., Brezesinski, G. and Mohwald, H. (1998) *Chem. Phys. Lipids*, 94(2): 251–260.
3. Gzyl, B. and Paluch, M. (2004) *Prog. Colloid Polym. Sci.*, 126: 60–63.
4. Dynarowicz-Latka, P. and Hac-Wydro, K. (2004) *Colloids Surf. B*, 37(1–2): 21–25.
5. Brzozowska, I. and Figaszewski, Z.A. (2003) *Colloids Surf.* B 27(4): 303–309.
6. Gerber, F., Krafft, M.P. and Vandamme, T.F. (2007) *Biochim. Biophys. Acta*, 1768(3): 490–494.
7. Zhao, L. and Feng, S.S. (2005) *J. Colloid Interface Sci.*, 285(1): 326–335.
8. Sospedra, P., Espina, M., Alsina, M.A. and Haro, I., Mesteres, C. (2001) *J. Colloid Interface Sci.*, 244(1): 79–86.
9. Choi, S.Y., Oh, S.G. and Lee, J.S (2000) *Colloids Surf. B*, 17(4): 255–264.
10. Gaboriau, F., Saint-Pierre-Chazalet, M., Homand, J., Vaultier, M. and Moulinoux, J.P. (2002) *Colloids Surf. B*, 24(2): 133–143.
11. Brzozowska, I. and Figaszewski, Z.A. (2002) *Colloids Surf. B*, 23(1): 51–58.
12. Brzozowska, I. and Figaszewski, Z.A. (2002) *Biophys. Chem.*, 95(2): 173–179.

13. Wang, L., Lin, C., Cai, P., Galla, H.J., He, H., Huixin, F., Flach, C.R. and Mendelsohn, R. (2005) *Eur. Biophys. J*., 34(3): 243–254.
14. Sosnowski, T.R. and Radon, L.G. (1996) *J. Aerosol Science*, 27(1): S501-.
15. Gugliotti, M. and Politi, M.J., (2001) *Biophys. Chem*., 89(2–3) 243–251.
16. Gugliotti, M., Chaimovich, H. and Politi, M.J. (2000) *Biochim. Biophys. Acta*, 1463(2): 301–306.
17. Tojima, A., Matsuo, Y., Hiyoshi, R., Manaka, Manaka, Y. and Majima, M., (2001) *Thin Solid Films*, 393(1–2): 86–91.
18. Davies, F. and Higson, S.S.J., (2005) *Biosens. Bioelectron*., 21(1): 1–20.
19. Brzozowska, I. and Figaszewski, Z.A. (2003) *Colloids Surf. B*, 30(3): 187–192.
20. Gopal, A. and Lee, K.Y.C. (2001) *J. Phys. Chem. B*, 105(42): 10348–10354.
21. Regen, S.L. (2002) *Curr. Opin. Chem. Biol*., 6(6): 729–735.
22. Yagle, P.L. Biology of Cholesterol, Boca Raton: CRC Press, 121–130, 1988.
23. Presti, F.T., Pace, R.J. and Chan, S.I. (1982) *Biochemistry*, 21(16): 3831–3835.
24. Darke, A., Finer, E.G., Flook, A.G. and Phillips, M.C. (1971) *FEBS Lett*., 18(2): 326–330.
25. Anderson, T.G. and McConnell, H.M. (2001) *Biophys. J*., 81: 2774–2785.
26. Rog, T. and Pasenkiewicz-Gierula, M. (2004) *Biophys. Chem*., 107(2): 151–164.
27. Korchowiec, B., Paluch, M., Corvis, Y. and Rogalska, E. (2006) *Chem. Phys. Lipids*, 144(2): 127–136.
28. Inczedy, J. Analytical Applications of Complex Equilibria, Akademia Kiado: Budapest, Chapter 1, 1976.
29. Wege, H.A., Holgado-Terriza, J.A., Gálvez-Ruiz, M.J. and Cabrerizo-Vilchez, M.A. (1999) *Colloids Surf. B*, 12(3–6): 339–349.
30. Zhu, R., Lu, Z. and Wei, Y. (1996) *Thin Solid Films*, 284–285: 43–45.
31. Hardy, N.J., Richardson, T.H. and Grunfeld, F. (2006) *Colloids Surf. A,* 284–285: (2) 202–206.
32. Brzozowska, I. Ph.D. Thesis, Chemistry Department, University of Warsaw, Poland, 2003.
33. Engineering Handbook, WNT: Warsaw, 1974.
34. Joos, P. and Demel, R.A. (1969) *Biochim. Biophys. Acta*, 183(3): 447–457.
35. Ghosh, D. and Tinoco, J. (1972) *Biochim. Biophys. Acta*, 266(1): 41–49.
36. Birdi, K.S. Lipid and Biopolymer Monolayers at Liquid Interfaces, Plenum Press: New York, 1989.
37. Jain, M.K. The Biomolecular Lipid Membrane, Litton Educational Publishing, Inc.: New York, 90–91, 1972.
38. Vaughan, D.J. and Keough, K.M. (1974) *FEBS Lett*., 47(1): 158–161.
39. Yeagle, P.L. (1985) *Biochim. Biophys. Acta*, 822(3–4): 267–287.
40. Petelska, A.D., Naumowicz, M. and Figaszewski, Z.A. (2006) *Cell Biochem. Biophys.*, 44(2): 205–211.