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Interfacial tension of the lipid membrane formed from lipid-fatty acid and lipid-amine systems

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Abstract

Interfacial tension has been determined for phosphatidylcholine–stearic acid and phosphatidylcholine–stearylamine membranes. Phosphatidylcholine, stearic acid and stearylamine were used in the experimental. The interfacial tension values of the pure components are 1.62×10^{-3} N/m, -1.54×10^{-2} N/m and 4.40×10^{-3} N/m (hypothetical values), respectively. The 1:1 complexes were formed during formation of phosphatidylcholine–stearic acid and phosphatidylcholine–stearylamine membranes. The following parameters describing the complexes were determined: the surface concentrations of the lipid membranes formed from these complexes, A_3^{-1}, the interfacial tensions of such membranes, γ_3 and the stability constants of these complexes, K.

Keywords: Interfacial tension; Phosphatidylcholine; Stearic acid; Stearylamine; Complex 1:1

1. Introduction

The physico–chemical studies of the phospholipids-fatty acid mixture may have an additional significance other than the interest in relation to the alteration of membrane function caused by fatty acid. Phospholipids — the major building blocks of most biomembranes have two fatty acids themselves, which are esterified to glycerol. The interaction between different acyl chains within a phospholipid molecule or among the different phospholipids molecules in the bilayer should determine the physical properties of biomembranes. The study of the phase behaviour of the hydrated bilayer, composed of a phospholipid-fatty acid mixture would be useful to understand the acyl–acyl interactions playing such an important role in phospholipids bilayers [1].

The phase behaviour of the phospholipid-fatty acid mixtures most extensively studied so far are concerned with the mixture of diacylphosphatidylcholine and saturated fatty acids with C_{14}–C_{18} chain lengths, in which the phase diagrams over the whole composition range have been reported for some mixture systems [2–6]. All the phase diagrams have exhibited the formation of a molecular compound (or phase compound) in the gel phase with the stoichiometry of diacylphosphatidylcholine: fatty acid = 1:2, which means that a strong attractive interaction acts between the two components in the gel phase bilayer. Although an agreement is documented for complex formation, there seems to be a discrepancy in a reported phase diagram for the composition ranges of both low and high fatty acid concentrations [2,3,5].

The mixture of phospholipid and fatty acid was examined as well as three-dimensional (3D) system as monolayers. The observed acid–base equilibria are very sensitive on the change of many parameters such as: temperature, concentration, ionic strength, electric field, molecular interaction and dynamics of phases [7]. Torosian and Lemberger investigated mixed monolayers of fatty acids with phosphatidylcholine at the air/

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water interface. They stated that both substances mix completely when subphase pH was in the range of 5.5–6.0. Then, carboxylic acids were mainly protonated [8].

The mixtures of lecithins with acids of different chain length (C12–C20) were examined by calorimetric method and X-ray diffraction as three-dimensional system [9,10]. The existence of 1:2 complex (L:FA) was proved as a result of strong hydrogen bonding between chains and polar group of lecithin [11]. There is a minimum of Gibbs's energy at such composition of complex.

In this work, the interfacial tension of the phosphatidylcholine (PC, lecithin)–stearic acid (SA) and phosphatidylcholine–stearylamine (ST) membranes were determined within all the composition range where the bilayer formation was possible.

The aim of these investigations were to study the mixed phosphatidylcholine–stearic acid and phosphatidylcholine–stearylamine bilayer, characterize the molecular interaction between phospholipids and fatty acids and between phospholipids and amine plus a comparison of the properties of these systems: stability constants of the formed complexes and surface areas occupied by pure membrane components. We would like to emphasize that the values of the stability constants of lipid-fatty acid and lipid–amine complexes are reported for the first time.

2. Theory

In the case where the membrane components form a 1:1 complex (compound 3), interaction in the membrane can be described by the following system of equations [12–14]:

$$\gamma_1 a_1 A_1 + \gamma_2 a_2 A_2 + \gamma_3 a_3 A_3 = \gamma$$

$$K = \frac{a_3}{a_1 \cdot a_2}$$

$$\frac{a_1 + a_3}{a_1 + a_2 + 2a_3} = x_1$$

$$x_1 + x_2 = 1$$

where:

A_1, A_2, A_3 [m^2] — area occupied by compound 1, 2 and complex 3, respectively;
a_1, a_2, a_3 [mol m^{-2}] — surface concentration of 1, 2 and complex 3, respectively;
γ_1, γ_2, γ_3 [N m^{-1}] — interfacial tension of the membrane built of component 1, 2 and complex 3, respectively;
γ [N m^{-1}] — measured interfacial tension of the membrane;
x_1, x_2 — molar fraction of component 1 and 2 in the solution forming membrane, respectively;
K — stability constant of compound 3.

Elimination of a_1, a_2, a_3 yields the basic equation:

$$\begin{aligned}&[(\gamma-\gamma_1)B_2x_1 + (\gamma-\gamma_2)B_1x_2][(\gamma_3-\gamma_1)B_2x_1\\ &\quad+(\gamma_3-\gamma_2)B_1x_2 + (\gamma_1-\gamma_2)(x_1-x_2)]\\ &\quad= KA_3^{-1}B_1B_2[(\gamma-\gamma_1)(x_2-x_1)\\ &\quad\quad+(\gamma_3-\gamma)B_1x_2][(\gamma-\gamma_2)(x_1-x_2) + (\gamma_3-\gamma)B_2x_1]\end{aligned} \tag{1}$$

where:

$$B_1 = \frac{A_3}{A_1};$$

$$B_2 = \frac{A_3}{A_2}.$$

Eq. (1) is the equation of second degree with respect to γ, to the complex composition as well as with respect to the constants: γ_1, γ_2, γ_3, B_1, B_2. Opening of parentheses results in a great complexity of the equation, and is troublesome when directly applied to the determination of constants. The constants mentioned above can be determined in individual cases using simplified forms of this equation.

Eq. (1) may be simplified taking into account the high stability constant of the complex. With this assumption, it represents a straight line for small x_2 values ($x_2<x_1$):

$$(\gamma_1-\gamma)\frac{x_1-x_2}{x_2} = -B_1\gamma_3 + B_1\gamma \tag{2}$$

while for the high x_2 ($x_2>x_1$) values it can represent another straight line:

$$(\gamma_2-\gamma)\frac{x_2-x_1}{x_1} = -B_2\gamma_3 + B_2\gamma \tag{3}$$

Eq. (1) can be simplified in some other way. In the case where $x_1=x_2$, it assumes the form [13,14]:

$$\begin{aligned}&K(A_1^{-1})^2(A_2^{-1})^2(A_3^{-1})^{-1}(\gamma-\gamma_3)^2\\ &\quad= [\gamma_2A_1^{-1} + \gamma_1A_2^{-1}-\gamma(A_1^{-1} + A_2^{-1})](\gamma_2A_1^{-1} + \gamma_1A_2^{-1})\\ &\quad\quad-[\gamma_2A_1^{-1} + \gamma_1A_2^{-1}-\gamma(A_1^{-1} + A_2^{-1})](A_1^{-1} + A_2^{-1})\gamma_3\end{aligned} \tag{4}$$

The parameters describing the complex determined from Eqs. (1) and (4) could be applied to present the agreement of the Eq. (4) with the experimental data using Eq. (5):

$$\begin{aligned}&KA_1^{-1}A_2^{-1}(a_1+a_2)(a_3-a_1)\gamma^2 + [KA_1^{-1}A_2^{-1}(\gamma_1a_1-\gamma_3a_3)(a_1 + a_2)\\ &\quad-KA_1^{-1}A_2^{-1}(\gamma_2a_1-\gamma_3a_2)(a_3-a_1) + a_4A_3^{-1}(a_3 + a_2)]\gamma\\ &\quad+KA_1^{-1}A_2^{-1}a_3\gamma_3(\gamma_3a_2 + \gamma_1a_2)-KA_1^{-1}A_2^{-1}a_1\gamma_1(a_1\gamma_2 + a_2\gamma_3)\\ &\quad-a_4A_3^{-1}(\gamma_2a_3 + \gamma_1a_2) = 0\end{aligned} \tag{5}$$

where:

$$a_1 = A_3^{-1}(x_2-x_1)$$

$$a_2 = A_2^{-1}x_1$$

$$a_3 = A_1^{-1}x_2$$

$$a_4 = [A_3^{-1}(\gamma_1-\gamma_2)(x_2-x_1) + (\gamma_1-\gamma_3)x_1A_2^{-1} + (\gamma_2-\gamma_3)x_2A_1^{-1}]$$

3. Materials and methods

The interfacial tension, γ, of the lipid bilayer was determined by measuring the curvature radius, R, of the convex surface formed by applying a pressure difference, Δp, on its sides. The method used was based on Young and Laplace's equation [15].

$$2\gamma = R\Delta p.$$

The apparatus and the measurement method were described in previous papers [12,14,16]. The lipid membranes were formed by the Mueller–Rudin method [17]. The interfacial tension was measured on a freshly created lipid bilayer membrane for 12–15 times for each concentration. For each membrane, about 10 instrument readings were made for each of the lipid spherical cap diameters, formed by a pressure difference applied on both sides. These measurements were made within the whole range: from very low values of the lipid spherical cap diameter to those almost equal to the Teflon element radius. From all of instrument readings (100–150), the arithmetic mean and standard deviation were enumerated. Measurements with preparation of the electrolyte solution were made 2–3 times in order to test the repeatability of these determinations.

The solution used to form the model membrane contained 20 mg/ml of choice substances (PC, stearic acid, stearylamine) in solution (*n*-decane, chloroform).

3-*sn*-phosphatidylcholine (99%) from Fluka was used in the experiment; it had been isolated from a hen egg yolk. Lecithin was dissolved in chloroform and the solvent was evaporated in an argon medium and the residue was dissolved in *n*-decane.

Stearic acid (97%) from Fluka and stearylamine (98%) made by Sigma were used in the experiment and were dissolved in chloroform.

0.1 mol dm^{-3} potassium chloride solutions were used as an electrolyte. The solutions were prepared using triply distilled water and KCl produced by POCh (Poland). KCl was analytical grade and was roasted prior to use at 600 °C for 2 h to remove organic impurities.

4. Results and discussion

The effect of the presence of stearic acid or stearylamine on interfacial tension of the membranes formed using phosphatidylcholine was studied. The resulting curves deviate from linearity indicating that some bonds are formed in the membrane. A 1:1 complex formation was assumed. The dependence of interfacial tension of the lipid membrane as a function of composition was studied at room temperature (293 ± 2 K) in all the feasible concentration range. The interfacial tension values reported in this paper refer to the two sides of the bilayer membrane surface area unit.

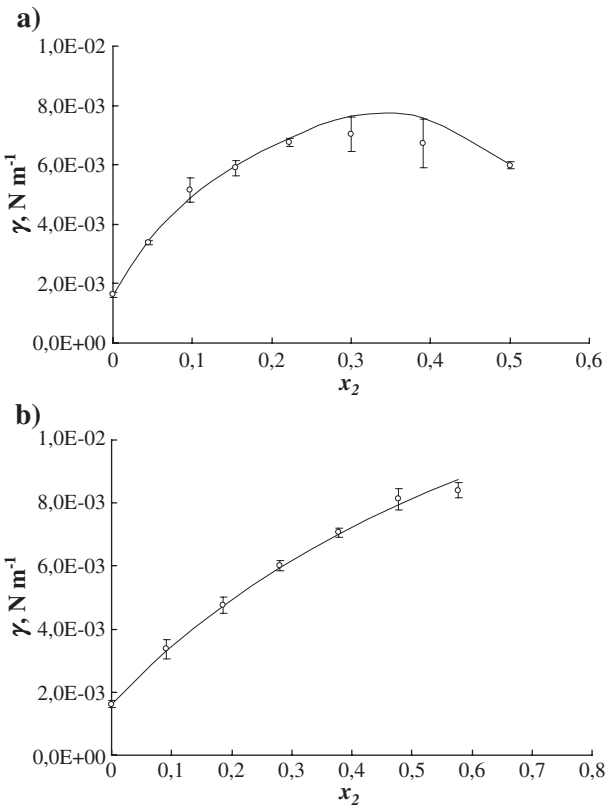

Fig. 1. The interfacial tension γ of the phosphatidylcholine–stearic acid (a) and phosphatidylcholine–stearylamine (b) membranes as a molar fraction of stearic acid or stearylamine x_2 (the experimental values are marked by points and the theoretical ones by curves).

The dependences of interfacial tension of the PC–SA and PC–ST membranes are presented in Fig. 1 as a molar fraction of stearic acid or stearylamine. The dependences of interfacial tension of lipid membranes formed from the PC–SA and PC–ST systems were evaluated as a function of the composition with up to 70% and 60% content of stearic acid and stearylamine, respectively. Only in range of concentration, in fact, the bilayer membrane formation was possible.

The interfacial tension value of pure lecithin membrane (component 1), γ_1 was measured directly and presented earlier [12], which is equal to 1.62×10^{-3} N m^{-1}. There is no accurate literature data on interfacial tension values for the pure component 2 (stearic acid or stearylamine), because these components do not form the bilayer membrane. However in order to characterize the course of the experimental curves, the γ_2 value for the pure components are necessary, which will be used in the calculations. In this case, the interfacial tension hypothetical values for membranes built from stearic acid and stearylamine were determined adjusting the experimental curve with the polynomial of the other mark extrapolating the $x_2=1$ value, which is presented in Fig. 2. The interfacial tension values obtained in this way for pure stearylamine and stearic acid are equal to 4.40×10^{-3} N m^{-1} and -1.54×10^{-2} N m^{-1}. A negative value of interfacial tension for the membrane built from the pure

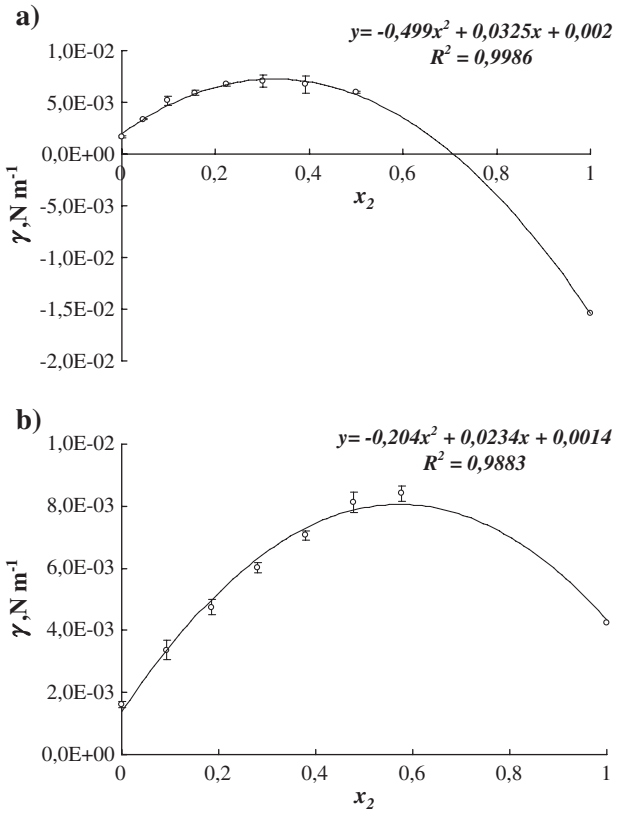

Fig. 2. A plot illustrating a method for evaluating γ_2 for phosphatidylcholine–stearic acid (a) and phosphatidylcholine–stearylamine (b) systems.

stearic acid is pointing to the fact that it is not possible to create the bilayer membrane from pure stearic acid. The thermodynamic potential for this bilayer would have a negative value, i.e. the bilayer is not forming.

The stearylamine membrane interfacial tension value is positive. However, due to the fact that the forming solution with more than 60% of stearylamine was granulated in the solution, it is not possible to create a bilayer lipid membrane from the pure component.

The other constants B_1, B_2, γ_3 were determined assuming that the value of the stability constant of the PC–SA and PC–ST complexes were sufficient to be simplified i.e. Eqs. (1), (2) and (3). Knowing the B_1, B_2 constants, which were determined from Eqs. (2) and (3) it was possible to calculate the interfacial tension values of the PC–SA and PC–ST complexes, γ_3. The mean values are equal to 7.16×10^{-3} N m^{-1} for PC–stearic acid and 6.04×10^{-3} N m^{-1} for PC–stearylamine.

The interfacial tension value determined as a function of the composition, it made it possible to determine the surface concentrations of the membranes composed of pure components. At least one of them is necessary for the determination of the A_3^{-1} value. The surface area occupied by a lecithin molecule equal to 85 Å^2 was determined in the previous work [18]. The surface area occupied by stearic acid and stearylamine is identical and equal to 19 Å^2 [19]. As mentioned earlier, the fatty acid forms a dimer [11,20,21]; therefore the surface area occupied by stearic acid is equal to 38 Å^2.

Knowing the A_1^{-1} and A_2^{-1} as well as B_1 and B_2 values, the surface concentration of the membrane composed of the lecithin–stearic acid and lecithin–stearylamine complexes could be determined. The resulting surface concentration value, A_3^{-1} for the PC–SA and PC–ST complexes were equal to 8.88×10^{-7} mol m^{-2} and 1.19×10^{-6} mol m^{-2}, respectively. It made it possible to determine the area occupied by one lecithin–stearic acid and lecithin–stearylamine complex, which were valued to be 187 and 140 Å^2, respectively. Values obtained by us from the present work of the surface occupied by similar complexes were 187 Å^2 for the PC–stearic acid complex and 140 Å^2 with the PC–stearylamine complex. These are much bigger than the amount of the surface area occupied by each component of the complexes. It is probably connected with the arrangement of lecithin molecules in such complex and also connected with the structural construction of such complexes. In this paper [22] we suggested the arrangement of the lecithin molecules in a bilayer membrane at pH>5. In these media, one particle from the lecithin molecules in the bilayer (orientated in this way), has two straightened chains; however, the next molecule of lecithin has one straightened and another chain fastened to the membrane surface. An association of ions occurs in such conditions with OH^- from the electrolyte solution. How these ions were characterized was previously reported [22]: these ions are strongly solvated and they produce a separation of lecithin particles in the bilayer which is an influence upon the increasing surface occupied by the single molecule of lecithin.

The only values to be determined were the stability constants of the PC–fatty acid and PC–amine complexes. It could be determined from Eq. (4) when $x_1=x_2=0.5$; these parameters amount to 2.18×10^9 and 1.38×10^7 m^2 mol^{-1}, respectively. During the course of our investigations, we assumed the formation of PC–stearic acid and PC–stearylamine complexes. These complexes arise by producing a connection between the $-N^{(+)}(CH_3)_3$group from the molecule of lecithin and $-COO^{(-)}$ groups from dimmer of stearic acid, in the case of the complex PC–stearic acid, and between the $-PO^{(-)}$ group from lecithin and $-N^{(+)}H_3$ group from stearylamine. The dissociation constants of the $-N^{(+)}(CH_3)_3$ group from PC and $-COO^{(-)}$ groups from the dimer of stearic acid are equal $10^{-5.7}$ [18] and about 10^{-5} [23], respectively. It should be noted that the dissociation constants of $--PO^{(-)}$ group from PC and $-N^{(+)}H_3$ group from stearylamine are equal $10^{-2.6}$ [18] and about 10^{-10} [23], respectively. Therefore the connection between PC and stearic acid will be stronger and it is possible to expect that the stability constant of the PC–stearic acid will be higher than the stability constant of the PC–stearylamine complex.

These parameters describing these complexes determined from Eqs. (1) and (4) were in agreement with Eq. (1); i.e. data (solid lines) with the experimental data (points) in Fig. 1 using Eq. (5). As it is a square equation, this equation can yield two solutions. The values yielding a better agreement of the experimental points with equations describing complex formation between membrane lipid components were chosen.

The experimental values in Fig. 1 are marked by points, and the theoretical ones obtained from Eq. (5) by lines. It can be seen from this figure that there is a good agreement between

experimental and theoretical points, which verifies the assumption of formation of a 1:1 phosphatidylcholine–stearic acid and phosphatidylcholine–stearylamine complex in the lipid membrane. Good agreement of the experimental and theoretical points verifies the assumption of the correct choice of the γ_2 values for components of the membrane.

5. Conclusion

The following conclusions can be drawn on the ground of the parameters describing the complexes studied:

1. The stability constant of the PC–stearic acid complex is 2.18×10^{9} m^2 mol^{-1}, whereas the stability constant of the PC–stearylamine complex is equal 1.38×10^{7} m^2 mol^{-1}. High values confirm the legitimacy of simplifying Eq. (1). The values of the stability constants of the lipid-fatty acid and lipid–stearylamine complexes are reported for the first time. It can be observed that the stability constants of the fatty acid-containing complex are higher. Thus, the PC–stearic acid complex is more stable than the PC–stearylamine complex.
2. The experimental area occupied by one PC–stearic acid complex is 187 $Å^2$, whereas the area occupied by the PC–stearylamine complex is equal to 140 $Å^2$.
3. Good agreement of the experimental and theoretical points verifies the assumption of formation of a 1:1 complex in the lipid membrane. A lack of variances between points indicates that complexes at different stoichiometries or associates are not possible in the PC–stearic acid or PC–stearylamine membranes.

References

[1] T. Inoue, S. Yanagihara, Y. Misono, M. Suzuki, Effect of fatty acids on phase behaviour of hydrated dipalmitpylphosphatidylcholine bilayer: saturated versus unsaturated fatty acids, Chem. Phys. Lipids 109 (2001) 117–133.
[2] S. Mabrey, J.M. Stutevant, Incorporation of saturated fatty acids into phosphatidylcholine bilayers, Biochim. Biophys. Acta 486 (1977) 444–450.
[3] S.E. Schullery, T.A. Seder, D.A. Weinstein, D.A. Bryant, Differential thermal analysis of dipalmitylphosphatidylcholine–fatty acid mixture, Biochemistry 20 (1981) 6818–6824.
[4] A.B. Kohn, S.E. Schullery, Dipalmitpylphosphatidylcholine-palmitic acid phase diagram studied by ^{13}C nuclear magnetic resonance, Chem. Phys. Lipids 37 (1985) 143–153.
[5] R.D. Koynova, A.I. Boyanov, B.G. Tenchov, Gel-state metastability and nature of the azeotropic points in mixtures of saturated phosphatidylcholines and fatty acids, Biochim. Biophys. Acta 903 (1987) 186–196.
[6] R.D. Koynova, B.G. Tenchov, P.J. Quinn, P. Laggner, Structures and phase behaviour of hydrated mixture of L-dipalmitpylphosphatidylcholineand palmitic acid. Correlations between structural rearrangements, specific volume changes and endothermic events, Chem. Phys. Lipids 48 (1988) 205–214.
[7] G. Torosian, A.P. Lemberger, Surface films of soybean lecithin. II. Interactions between lecithin and lipid substances in mixed monomolecular films, J. Pharm. Sci. 57 (1968) 17–22.
[8] A. Ortiz, J.C. Gomez-Fernandez, A differential scanning calorimetry study of the interaction of free fatty acids with phospholipid membranes, Chem. Phys. Lipids 45 (1987) 75–91.
[9] J.M. Seddon, R.H. Templer, N.A. Warrender, Z. Huang, G. Cevc, D. Marsh, Phosphatidylcholine–fatty acid membranes: effects of headgroup hydration on the phase behaviour and structural parameters of the gel and inverse hexagonal (H(II)) phases, Biochim. Biophys. Acta 1327 (1997) 131–147.
[10] J.M. Boggs, G. Rangaraj, K.M. Koshy, Effect of hydrogen-bonding and non-hydrogen-bonding long chain compounds on the phase transitin temperatures of phospholipids, Chem. Phys. Lipids 40 (1986) 23–34.
[11] I. Brzozowska, Z.A. Figaszewski, Palmitic acid dimer formation in the monolayers at the air/aqueous solution interface, Colloids Surf., B Biointerfaces 30 (2003) 187–192.
[12] A.D. Petelska, Z.A. Figaszewski, Interfacial tension of the two-component bilayer lipid membrane modelling of cell membrane, Bioelectrochem. Bioenerg. 46 (1998) 199–204.
[13] M. Naumowicz, A.D. Petelska, Z.A. Figaszewski, Impedance analysis of phosphatidylcholine–cholesterol system in bilayer lipid membrane, Electrochim. Acta 50 (2005) 2155–2161.
[14] A.D. Petelska, M. Naumowicz, Z.A. Figaszewski, Physicochemical insights into equilibria in bilayer lipid membranes, in: H.T. Tien, A. Ottova (Eds.), Advances in Planar Lipid Bilayers and Liposomes, vol. 3, Elsevier, Amsterdam, 2006, pp. 125–187 (Chapter 5).
[15] A.W. Adamson, Physical Chemistry of Surfaces, Interscience Publishers, Inc., New York, 1960, pp. 4–9.
[16] A.D. Petelska, Z.A. Figaszewski, The effect of pH on the interfacial tension of bilayer lipid membranes formed from phosphatidylcholine or phosphatidylserine, Biochim. Biophys. Acta 1561 (2002) 135–146.
[17] P. Mueller, D.O. Rudin, H.T. Tien, W.C. Wescott, Methods for the formation of single bimolecular lipid membranes in aqueous solution, J. Phys. Chem. 67 (1963) 534–535.
[18] A.D. Petelska, Z.A. Figaszewski, Effect of pH on the interfacial tension of bilayer lipid membrane, Biophys. J. 78 (2000) 812–817.
[19] D. Vaknin, Structure–function relations in self-assembled C18- and C20-sphingosines monolayers at gas/water interfaces, J. Am. Chem. Soc. 125 (2003) 1313–1318.
[20] F. Kaneko, J. Yano, K. Sato, Diversity in the fatty-acid conformation and chain packing of *cis*-unsaturated lipids, Curr. Opin. Struct. Biol. 8 (1998) 417–425.
[21] J. Zhao, S.V. Olesik, Separation of dimer acids using enhanced-fluidity liquid chromatography, Anal. Chim. Acta 449 (2001) 221–236.
[22] A.D. Petelska, Z.A. Figaszewski, Acid–base equilibria at interface separating electrolyte solution and lipid bilayer formed from phosphatidylcholine, Biophys. Chem. 104 (2003) 13–19.
[23] Engineers Handbook, WNT, Warszawa, 1974.