Effect of pH on the Interfacial Tension of Lipid Bilayer Membrane

Aneta D. Petelska and Zbigniew A. Figaszewski
Laboratory of Interfacial Electrochemistry, Faculty of Chemistry, University of Warsaw, 02-093 Warsaw, Poland

ABSTRACT The dependence of the interfacial tension of a lipid bilayer on the pH of the aqueous solution has been studied. A theoretical equation is derived to describe this dependence. Interfacial tension measurements of an egg phosphatidylcholine bilayer were carried out. The experimental results agreed with those derived from the theoretical equation obtained close to the isoelectric point within a range of three pH units. A maximum corresponding to the isoelectric point appears both in the theoretical equation and in the experimental data.

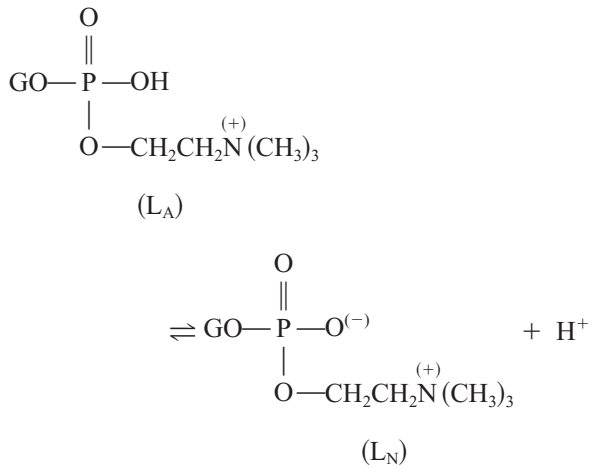
INTRODUCTION

Natural cell membranes have been studied by numerous techniques including physicochemical ones. An important property of a cell membrane is its interfacial tension, which determines its rigidity and, as a result, affects its stability.

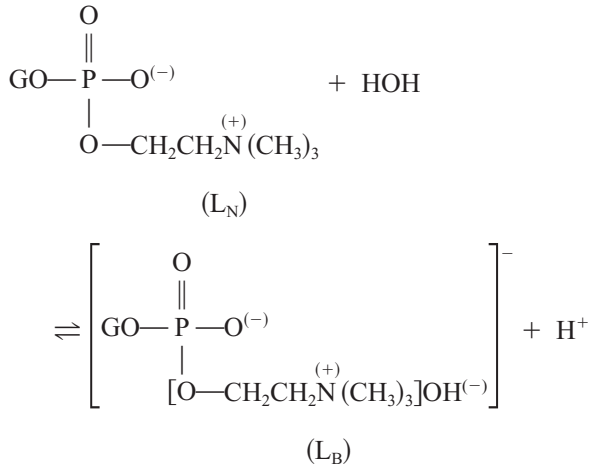
A cell membrane is a very complex system, and it contains various structural components that can influence its interfacial tension. Therefore, it is easier to study the effect of various factors; e.g., pH of the medium using artificial phospholipid bilayer model membranes. The properties of the artificial membrane should be well known and generally similar to the properties of the membranes of living cells. Lipid monolayers, lipid bilayers, collodion, cellophane, milipore, ion-exchanger, or other membranes have been used as artificial membranes (Przestalski, 1983), but the interfacial tension of a cell membrane is best measured by means of a bilayer lipid model membrane.

The specific purpose of this work is to investigate the influence of pH on the interfacial tension of a bilayer formed from egg phosphatydylcholine (PC). Our general research goal is to understand the physicochemical properties of lipid bilayer, including interfacial tension, in its dependence on the membrane–solution interface (Liu et al., 1994).

The PC molecule is an electrically neutral, but zwitterionic lipid that can be in equilibrium with H^+ as well as with OH^- ions. When hydrogen ions are in excess, equilibria 1 and 2 are shifted to the left, which means that PC cations L_A dominate in the solution. However, in basic solutions, the equilibria 1 and 2 are moved to the right and the solution is dominated by L_B anions.



(1)



(2)

In the above equilibria, G is the doubly acylated by glycerol group (Stace, 1965; Morison and Boyd, 1985).

THEORY

The dependence of interfacial tension of lipid membranes on the pH of the solution can be described in terms of acid–base equilibria. The phospholipid molecule is in various acid–base equilibria with the medium. The equilibria can be described as follows:

$$AH \Leftrightarrow A^- + H^+, \tag{3}$$

Received for publication 6 August 1998 and in final form 11 October 1999.

Address reprint requests to Zbigniew A. Figaszewski, Faculty of Chemistry, Laboratory of Interfacial Electrochemistry, University of Warsaw, Pasteura St. 1, 02-093 Warsaw, Poland. Tel.: +48-22-822-0211 x363; Fax: +48-22-822-5996; Email: figaszew@noc.uwb.edu.pl.

0006-3495/00/02/812/06 $2.00

$$B^+ + OH^- \Leftrightarrow BOH, \tag{4}$$

$$HOH \Leftrightarrow H^+ + OH^-. \tag{5}$$

It results from equilibria 4 and 5 that

$$B^+ + HOH \Leftrightarrow BOH + H^+. \tag{6}$$

The dissociation constants of the lipid membrane are presented as the equations,

$$K_a = \frac{a_{A^-} \cdot a_{H^+}}{a_{AH}}, \tag{7}$$

$$K_b = \frac{a_{BOH} \cdot a_{H^+}}{a_{B^+}}. \tag{8}$$

Let us assume that H^+ and OH^- ions are adsorbed on the phospholipid surface. The adsorption equilibria are described by the equations,

$$A^- + H^+ \Leftrightarrow AH, \tag{9}$$

$$B^+ + OH^- \Leftrightarrow BOH. \tag{10}$$

The lipid is present in the membrane only. Therefore, the volume or surface concentration of the lipid is equal to its amount related to the solution volume or to the membrane surface area. These concentrations, and the concentrations of hydrogen or hydroxyl ions, determine the acid–base constants according to the relationships,

$$K_1 = \frac{a_{AH}}{a_{A^-} \cdot a_{H^+}} = \frac{\Gamma_{AH}}{\Gamma_{A^-} \cdot a_{H^+}}, \tag{11}$$

$$K_2 = \frac{a_{BOH}}{a_{B^+} \cdot a_{OH^-}} = \frac{\Gamma_{BOH}}{\Gamma_{B^+} \cdot a_{OH^-}}. \tag{12}$$

The concentrations of the dissociated and nondissociated form concentrations of the lipid appear in the same power (one) in the above equations.

Let us write the Gibbs equation,

$$d\gamma = -\sum \Gamma_i d\mu_i. \tag{13}$$

In the case where the adsorbed molecules are electrically charged, the molar free energy of charged components depends on the internal potential of the considered phase. Therefore, the chemical potentials in Eq. 13 should be replaced by electrochemical potentials. The Gibbs equation assumes the form,

$$d\gamma = -\sum \Gamma_i d\bar{\mu}_i. \tag{14}$$

If the H^+ and OH^- ions are adsorbed, then the above equation assumes the form,

$$d\gamma = -\Gamma_{H^+} d\bar{\mu}_{H^+} - \Gamma_{OH^-} d\bar{\mu}_{OH^-}. \tag{15}$$

Taking advantage of the electrochemical potential of the hydrogen and hydroxide ions, we have

$$\bar{\mu}_{H^+} = \mu^0_{H^+} + RT \ln a_{H^+} + F\varphi, \tag{16}$$

$$\bar{\mu}_{OH^-} = \mu^0_{OH^-} + RT \ln a_{OH^-} - F\varphi. \tag{17}$$

The results are the following equations:

$$d\bar{\mu}_{H^+} = RTd \ln a_{H^+} + Fd\varphi, \tag{18}$$

$$d\bar{\mu}_{OH^-} = RTd \ln a_{OH^-} - Fd\varphi. \tag{19}$$

Substituting Eqs. 18 and 19 to 15 yields

$$d\gamma = -\Gamma_{H^+} RTd \ln a_{H^+} - \Gamma_{OH^-} RTd \ln a_{OH^-} - (\Gamma_{H^+} - \Gamma_{OH^-}) Fd\varphi. \tag{20}$$

The ionic product of water is

$$K_w = a_{H^+} \cdot a_{OH^-}. \tag{21}$$

Hence,

$$a_{OH^-} = \frac{K_w}{a_{H^+}}, \tag{22}$$

$$\ln a_{OH^-} = \ln K_w - \ln a_{H^+}. \tag{23}$$

Hence,

$$d \ln a_{OH^-} = -d \ln a_{H^+}. \tag{24}$$

It follows from Eqs. 20 and 24 that

$$d\gamma = -(\Gamma_{H^+} - \Gamma_{OH^-}) RTd \ln a_{H^+} - (\Gamma_{H^+} - \Gamma_{OH^-}) Fd\varphi. \tag{25}$$

It follows from Eqs. 8 and 21 that

$$\frac{K_b}{K_w} = \frac{a_{BOH}}{a_{B^+} \cdot a_{OH^-}}. \tag{26}$$

Therefore,

$$K_b = K_2 \cdot K_w. \tag{27}$$

The second term of Eq. 25 was neglected because the electric potential of the solution is assumed to be unchanged.

Let us write Eqs. 25, 11, and 12 in the form,

$$d\gamma = -(\Gamma_{AH} - \Gamma_{BOH}) RTd \ln a_{H^+}, \tag{28}$$

$$\Gamma_{AH} = K_1 \cdot \Gamma_{A^-} \cdot a_{H^+}, \tag{29}$$

$$\Gamma_{OH^-} = K_2 \cdot \Gamma_{B^+} \cdot a_{OH^-}. \tag{30}$$

The surface concentration of the lipid is denoted by s; the following equations can be written depending on the form of Eqs. 3 or 6 of the acid–base equilibria:

$$\Gamma_{AH} + \Gamma_{A^-} = s, \tag{31}$$

$$\Gamma_{BOH} + \Gamma_{B^+} = s. \tag{32}$$

Equations 31 and 32 can be considered as independent because they are connected by the water self-ionization equilibrium, which has not been considered in the deduction of the equation system.

Equations 28–32 will form an equation system, and the Γ_{AH}, Γ_{A^-}, Γ_{BOH}, and Γ_{B^+} values will be eliminated.

We therefore have

$$\gamma = -sRT\ln\left[\left(\frac{K_a}{a_{H^+}}+1\right)\left(\frac{a_{H^+}}{K_b}+1\right)\right] + \text{const.} \quad (33)$$

It follows from Eq. 33 that maximum interfacial tension occurs at

$$a_{H^+} = \sqrt{K_a K_b}. \quad (34)$$

Taking into account condition 34 and Eq. 33, we obtain the dependence of the interfacial tension on hydrogen ion activity, from

$$\gamma = \gamma_{max} + 2sRT\ln\left(\sqrt{\frac{K_a}{K_b}}+1\right) - sRT\ln\left[\left(\frac{K_a}{a_{H^+}}+1\right)\left(\frac{a_{H^+}}{K_b}+1\right)\right]. \quad (35)$$

Here, K_a and K_b = acid and base equilibrium constants, respectively; s [mol/m^2] = lipid surface concentration; R [J/mol · K] = the gas constant; T [K] = medium temperature; γ [mN/m] = interfacial tension of the lipid membrane; γ_{max} [mN/m] = maximal interfacial tension value of the lipid membrane; and Γ [mol/m^2] = the surface concentration of adsorbed ions.

Equation 35 presents the dependence of the interfacial tension of the lipid membrane on pH of electrolyte solution.

Dependence 35 is an equation of a straight line that makes it possible to easily assign the maximal interfacial tension value of the lipid membrane, γ_{max}, and the lipid surface concentration, s.

$$\gamma = \gamma_{max} + s \cdot f(a_{H^+}) \quad (36)$$

$$f(a_{H^+}) = 2RT\ln\left(\sqrt{\frac{K_a}{K_b}}+1\right) - RT\ln\left[\left(\frac{K_a}{a_{H^+}}+1\right)\left(\frac{a_{H^+}}{K_b}+1\right)\right] \quad (37)$$

EXPERIMENT

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's and Laplace's equation (Adamson, 1960),

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

Measurements

The apparatus and the measurement method were described in a previous paper (Petelska and Figaszewski, 1998). The lipid membranes were formed by the Mueller–Rudin method (Mueller et al., 1963). They were formed in a Teflon diaphragm of 1.5-mm outer diameter containing an orifice along its axis. An electrolyte solution was present on both sides of the orifice.

The convexity of the spherical cap was measured by means of a microscope with an objective equipped with a scale with 0.1-mm-interval scale marks. Therefore, the instrument readings of the lipid spherical cap were made with 0.05-mm precision. The convexity of the lipid membrane of the spherical cap, together with the Teflon element diameter corresponding to the lipid spherical cap diameter, yielded the radius of curvature. The measurement of the spherical cap was difficult because the spherical cap is hardly visible. While using yellow light its visibility gets better.

The interfacial tension was measured on freshly created egg PC bilayer membrane 12–15 times for each pH electrolyte solution. For each membrane, about 10 instrument readings of the lipid spherical cap diameter, formed by pressure difference applied on both sides, were made. These measurements were made in the whole range, from the 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, marked in Figs. 1 and 2. Measurements with preparation of the electrolyte solution were made 2–3 times to test the repeatability of these determinations.

The solution used to form the model membrane contained 20 mg/ml of egg PC in *n*-decane. The PC was dissolved in chloroform to prevent oxidation; the solvent was evaporated in an atmosphere of argon, and the residue was dissolved in *n*-decane, which had been additionally purified by distillation, yielding ε = 1.991 (293.15 K). The pH of electrolyte was carefully controlled during the measurements.

Lipid bilayer membranes in the form of liposomes were also used for these measurements. These can be formed because most phospholipids undergo spontaneous aggregation in water or in aqueous electrolyte solutions if shaken or subjected to ultrasound. Bubbles of spherical or cylindrical shape, having sizes ranging from less than 0.1 μm to a fraction of millimeter, are then formed (Jahnson et al., 1971; Ziętkiewicz and Słomski, 1984) as follows (Lasic, 1995): 10 mg lecithin (99%, Fluka) was dissolved in 1–2 ml chloroform, and the solvent was evaporated in the atmosphere of argon until 25–50 μm^3 lipid film remained in the beaker.

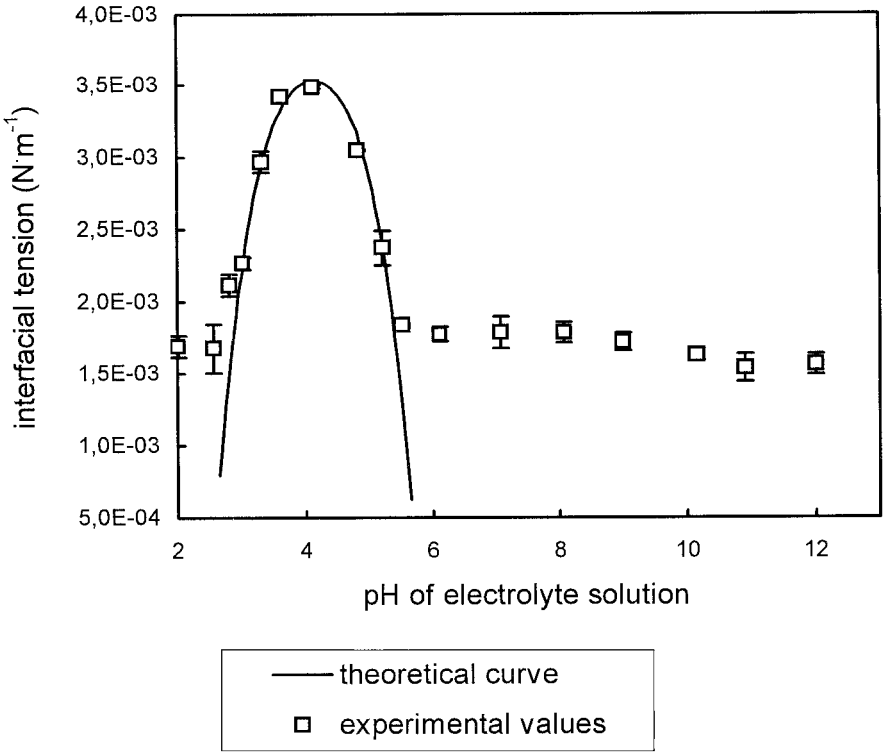


FIGURE 1 The dependence of the interfacial tension of a lipid membrane formed from PC of the pH of the electrolyte solution.

Fifteen milliliters of an aqueous solution of 0.9% NaCl was then added, and the beaker was placed in a water bath at ~280.15 K. The head of a UD-20 ultrasound generator was then immersed in the solution, and the solution was subjected to ultrasounds five times for 1.5 min each time. Liposomes of 10–20-nm diameters were obtained (Huang, 1963).

Materials

Egg PC (99%) from Fluka was used in the experiment; it had the following fatty acids composition: 16:0 ~ 33%, 18:0 ~ 4%, 18:1 ~ 30%, 18:2 ~ 14%, 20:4 ~ 4%.

Buffers of 2–12 pH ranges were prepared according to Britton and Robinson (Engineers Handbook, 1974) and

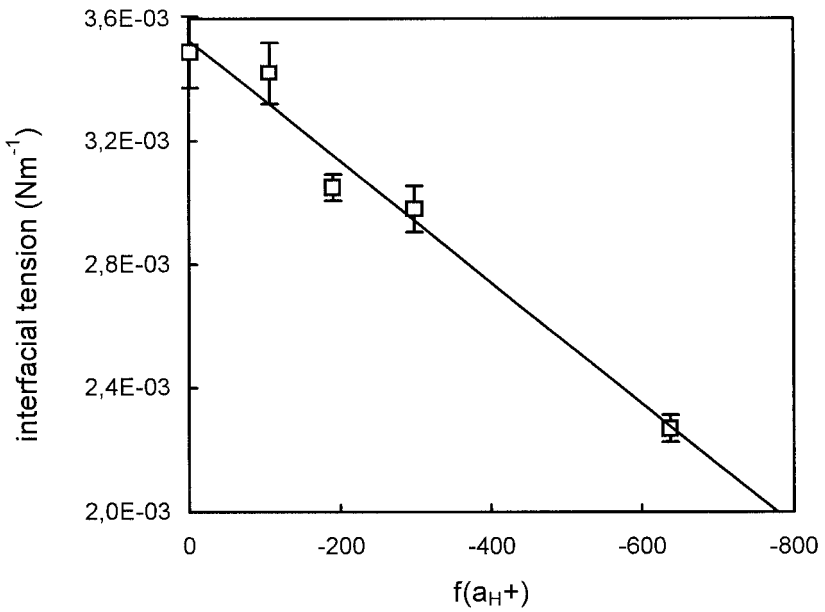


FIGURE 2 A plot illustrating Eq. 35 in the pH range 2.5–5.8.

used as the electrolyte. They were prepared by adding 0.2 M sodium hydroxide to a 100-ml solution having the following composition: 0.04 M acetic acid (80%, POCh), 0.04 M phosphoric acid (POCh), and 0.04 M boric acid (POCh). A suitable pH of the buffer was established depending on the amount of added sodium hydroxide.

RESULTS

The dependence of the interfacial tension of a lipid membrane formed from egg PC on the pH of the electrolyte solution is illustrated in Fig. 1. The maximal interfacial tension of the PC membrane was found to be 3.53 mN/m at pH = 4.15, in good agreement with the value given in Coster and Simons (1968). The interfacial tension of lipid bilayers has been determined before (Chiu et al., 1995; Roux, 1996; Jahnig, 1996; Feller and Pastor, 1996). Reported values ranged from 0.2 to 6.0 mN/m (Tien, 1974; Tieleman, 1996). The interfacial tension of lipid bilayers was also determined by measuring the energy of the membrane formation. Depending on the electrolyte composition, the reported value was 3.4 ± 0.6 mN/m (Coster and Simons, 1968).

The experimental values are marked in Fig. 1 by points and the theoretical ones obtained from Eq. 35 by lines. It can be seen from this figure that the theoretical and the experimental interfacial tension values of the lecithin membrane agree in the pH ranges 2.5–5.8 but they diverge in the 2.0–2.5 and 5.8–12 pH ranges. The deviations from the theoretical curve may be caused by the interaction of the PC membrane with the solution components other than H^+ and OH^-. The electrolyte contained orthophosphoric acid, boric acid, and sodium hydroxide. The interaction with those electrolyte solution components was not taken into account in Eq. 35; perhaps other ions like phosphate, acetate, or borate are adsorbed at the PC membrane in addition to the H^+ and OH^- ions.

The change in the pH of the solution induces changes in electrical charge of the membrane due to the variations in acid–base equilibrium of the groups present in the lipid molecule. At a certain pH value, the number of positive and negative groups is equal. The membrane then attains its isoelectric point in which the total electric charge density is zero. It is thus worthwhile to check whether the maximum interfacial tension of the egg PC bilayer appears at the isoelectric point of the membrane. The isoelectric point of a lipid membrane can be determined if the acid–base equilibrium constants are known. However, it was difficult to determine their magnitudes because PC is insoluble in water. For this reason, it was necessary to use liposomes to determine these values. It was accepted in the calculations that only the PC molecules present in the outer layer of liposome took part in acid–base equilibrium. Thus, the PC concentrations used in the equations were half of that introduced into the solution.

The acid–base equilibria 1 and 2, written in an abbreviated form, yield

$$L_A \Leftrightarrow L_N + H^+, \tag{38}$$

$$L_N \Leftrightarrow L_B + H^+, \tag{39}$$

where L_A = the acidic form of PC; L_B = the basic form of PC; and L_N = the neutral form of PC.

The dissociation constants of the membrane are presented by equations

$$K_a = \frac{[L_N][H^+]}{[L_A]}, \tag{40}$$

$$K_b = \frac{[L_B][H^+]}{[L_N]}. \tag{41}$$

They were determined by titration of the previously obtained liposomes with hydrochloric acid and with sodium hydroxide. A 736GP Titrino apparatus (Metrohm, Switzerland) was used in the titration. The acid–base equilibrium constants are $pK_a = 2.581$ and $pK_b = 5.687$.

Having determined the dissociation constants of the membrane, we can calculate its isoelectric point. The isoelectric point of an ampholyte like PC is the pH of its solution, meeting the relationship

$$[L_A] = [L_B]. \tag{42}$$

Substitution of the concentrations resulting from Eqs. 40 and 41 to Eq. 42 yields (Greenstein and Winitz, 1961; Sobczyk and Kisza, 1982)

$$[H^+] = \sqrt{K_a K_b}. \tag{43}$$

Thus,

$$pH_{isoel.} = \frac{pK_a + pK_b}{2}. \tag{44}$$

Equation 35 describes the dependence of the interfacial tension of PC membrane on the pH of an electrolyte solution. It allows one to determine the maximal interfacial tension value, γ_{max}, at pH = 4.15 and the PC surface concentration, s.

The dependence resulting from Eq. 35 is presented in Fig. 2 in the coordinate system, γ versus $f(a_{H^+})$ in which it should be a straight line. Equation 35 was plotted in the pH range 2.5–5.8 because the theoretical interfacial tension values of the PC bilayer agree then with the experimental ones. The PC surface concentration, s, amounting to 1.96×10^{-6} mol/m^2, was calculated from the slope of the line. The line determines the γ_{max} value equal to 3.53 mN/m on the abscissa; both values were determined by the least square method.

The surface area occupied by a PC molecule could be determined from the determined surface concentration, and was 85 Å^2. The surface occupied by an egg PC molecule,

depends on the way the phospholipid is prepared, because this affects the length of the fatty acids chains and degree of unsaturation. Therefore, the values of the literature range between 57 $Å^2$ and 96 $Å^2$ (Joos and Demel, 1969; Jain, 1972; Smondyrev and Berkowitz, 1999).

This work was supported by a grant from Polish Committee of Scientific Research No. 3TO9A00615.

We are indebted to Beata Magnuszewska for her assistance in determining the isoelectric point of PC bilayer.

REFERENCES

Adamson, A. W. 1960. Physical Chemistry of Surfaces. Interscience Publishers, Inc., New York, 4–9.

Chiu, S. W., et al. 1995. Incorporation of surface tension into molecular dynamics simulation of an interface: a fluid phase lipid bilayer membrane. *Biophys. J.* 69:1230–1245.

Coster, H. G. L., and R. Simons. 1968. Energy of formation of bimolecular lipid membranes. *Biochim. Biophys. Acta.* 163:234–239.

Engineers Handbook. 1974. Wydawniczwa Naukowo-Techniczne (WNT Press), Warsaw, Poland. 283–284.

Feller, S. E., and R. W. Pastor. 1996. On simulating lipid bilayers with an applied surface tension: periodic boundary conditions and undulations. *Biophys. J.* 71:1350–1355.

Greenstein, J. P., and M. Winitz. 1961. Chemistry of the Amino Acids. Vol. 1, John Wiley & Sons, Inc., New York. 482–484.

Huang, C. 1963. Studies on phosphatidylcholine vesicles. Formation and physical characteristics. *Biochemistry.* 8:344–352.

Jahnig, F. 1996. What is the surface tension of a lipid bilayer membrane? *Biophys. J.* 71:1348–1349.

Jahnson, S. M., A. D. Bagham, H. W. Hill, and E. D. Korn. 1971. Single bilayers liposomes. *Biochim. Biophys. Acta.* 233:820–826.

Jain, M. K. 1972. The Biomolecular Lipid Membrane. Litton Educational Publishing, Inc., New York. 90–92.

Joos, P., and R. A. Demel. 1969. The interaction energies of cholesterol and lecithin in spread mixed monolayers at the air–water interface. *Biochim. Biophys. Acta.* 183:447–457.

Lasic, D. D. 1995. Liposomes: from Physics to Application. Elsevier Science B.V., Amsterdam, The Netherlands. 63–100.

Liu, H., R. Z. Lu, J. G. Turcotte, and R. H. Notter. 1994. Dynamic interfacial properties of surface-excess films of phospholipids and phosphonolipid analogs. 1. Effects of pH. *J. Colloid Interface Sci.* 167: 378–390.

Morison, R. T., and R. N. Boyd. 1985. Organic Chemistry. 3rd edition. Allyn and Bacon, Inc., Boston, MA. 281–286.

Mueller, P., D. O. Rudin, H. T. Tien, and W. C. Wescott. 1963. Methods for the formation of single bimolecular lipid membranes in aqueous solution. *J. Phys. Chem.* 67:534–535.

Petelska, A. D., and Z. A. Figaszewski. 1998. Interfacial tension of the two-component bilayer lipid membrane modelling of the cell membrane. *Biolectrochem. Bioenerg.* 46:199–204.

Przestalski, S. 1983. Błony Biologiczne. WP, Warsaw, Poland. 48–54.

Roux, B. 1996. Commentary: surface tension of biomembranes. *Biophys. J.* 71:1346–1347.

Smondyrev, A. M., and M. L. Berkowitz. 1999. United atom force field for phospholipid membranes: constant pressure molecular simulation of dipalmitoylphosphatidicholine/water system. *J. Comp. Chem.* 20: 531–545.

Sobczyk, L., and A. Kisza. 1982. Eksperymentalna chemia fizyczna, Polish Science Publishers (PWN), Warsaw, Poland. 335–345.

Stace, C. A. 1965. A Guide to Subcellular Botany. Green & Co. Ltd., London, U.K. 50–51.

Tieleman, D. P., and H. J. C. Berendsen. 1996. Molecular-dynamics simulations of a fully hydrated dipalmitoyl phosphatidylcholine bilayer with different macroscopic boundary conditions and parameters. *J. Chem. Phys.* 105:4871–4880.

Tien, H. T. 1974. Bilayer Lipid Membrane (BLM). Marcel Dekker, New York. 31–41.

Ziętkiewicz, E., and R. Słomski. 1984. Liposomes as carriers in the transfer of substances of biological importance into cell. *Post. Biochem.* 30: 149–172.