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# Effect of pH on the interfacial tension of bilayer lipid membrane formed from phosphatidylcholine or phosphatidylserine

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**Abstract**

The effect of pH of an electrolyte solution on the interfacial tension of lipid membrane formed from phosphatidylcholine (PC) or phosphatidylserine (PS) was studied. The relationships were well described by an equation presented earlier based on the Gibbs isotherm but only in the proximity of the isoelectric point. Therefore, in this work models have been derived to describe the adsorption of the $H^+$ and $OH^-$ ions at lipid surfaces formed from PC or PS, which would reproduce changes in interfacial tension more correctly, particularly in the ranges distant from the isoelectric point. In one model, the surface is continuous with uniformly distributed functional groups constituting the centres of $H^+$ and $OH^-$ ion adsorption while in the other the surface is built of lipid molecules, free or with attached $H^+$ and $OH^-$ ions. In both models, the contributions of the individual lipid molecule forms to the interfacial tension of the bilayer were assumed to be additive. 

*Keywords:* Interfacial tension; Effect of pH on interfacial tension; Bilayer lipid membrane; Phosphatidylcholine; Phosphatidylserine

## 1. Introduction

A cell membrane is a very complex system composed first of all of lipids and proteins. For this reason, experiments are usually done with simple models of the membrane, e.g. with artificial phospholipid membranes [1–4]. Numerous functions of biological membranes were reproduced and explained using the model membranes [5]. It was demonstrated by numerous experiments that the properties of the lipid membranes formed of artificial components were very similar to those of natural cell membranes. Various parameters of lipid bilayers were determined, e.g. thickness, potential difference, electric capacitance, resistance, and so on [6,7]. In some conditions, hydrophilic pores, e.g. caused by voltage, appear in bilayer lipid membranes and this phenomenon was applied in practice. The entire cells subjected to short-duration high voltage pulses undergo poration, making it possible to introduce a determined substance to the cell, e.g. an alien DNA or a protein, to cause a flow of plasma from the cell or the fusion of neighbouring cells [8–10].

An important characteristic of a biological membrane is its interfacial tension, which determines its rigidity and as a result affects its stability. The interfacial tension of lipid bilayer has been determined before [11–14]. Reported values ranged from 0.2 to 6.0 mN/m [2,15]. The interfacial tension of lipid bilayer was also determined by measuring the energy

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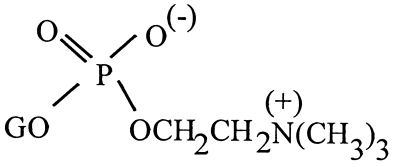
of the membrane formation. It was independent of the nature and concentration of the electrolyte, and had a value of $3.4 \pm 0.6$ mN/m [16].

The compound used most frequently for studying the properties of lipid layers is phosphatidylcholine (PC).

Its molecule is presented in the figure:




In the above figure, **G** is the doubly acylated glycerol group [17,18].

The PC molecule is an electrically neutral, but zwitterionic lipid which can be in equilibrium with $H^+$ as well as with $OH^-$ ions. Adsorption of the $H^+$ and $OH^-$ ions at the lecithin layer surface was described earlier [19]. The effect of pH on the interfacial tension of the lecithin membrane was described earlier in terms of the Gibbs equation. The maximal interfacial tension of the PC membrane was found to be $3.53 \times 10^{-3}$ N/m at pH equal to 4.15, in good agreement with the value given by Coster and Simons [16]. However, the dependence presented in their paper is correct in a narrow pH range only in the proximity of the isoelectric point of the membrane. For this reason, it is the aim of this work to describe the adsorption of the $H^+$ and $OH^-$ ions on the lecithin layer in the whole pH range. This will allow to explain the influence of the pH of an electrolyte solution on the interfacial tension of the phosphatidylcholine membrane.

## 2. Theory: phosphatidylcholine

The phospholipid layer observed from the aqueous solution side has uniformly distributed $-PO^{(-)}$ and $-N^{(+)}(CH_3)_3$ groups because it is built of molecules each having one $-PO^{(-)}$ group and one $-N^{(+)}(CH_3)_3$ group. Therefore, two descriptions of the lecithin surface can be adopted. In Description I, the membrane surface is continuous with uniformly distributed functional groups being the active centres of adsorption of the $H^+$ and $OH^-$ ions. In Description II the lecithin surface is composed of nonbonded lecithin molecules and of the molecules with bonded $H^+$ and $OH^-$ ions. A model is presented in Fig. 1.

### 2.1. Description I

Uniformly distributed active centres at which the $H^+$ and $OH^-$ ions can be adsorbed are present at the aqueous solution side.

They are schematically described with Eqs. 1 and 2:

$$\underset{\mathbf{(A^-)}}{-PO^{(-)}} + H^{(+)} \Leftrightarrow \underset{\mathbf{(AH)}}{-PO^{(-)} - H^{(+)}} \tag{1}$$

$$\underset{\mathbf{(B^+)}}{-\overset{(+)}{N}(CH_3)_3} + OH^{(-)} \Leftrightarrow \underset{\mathbf{(BOH)}}{-\overset{(+)}{N}(CH_3)_3 - OH^{(-)}} \tag{2}$$

Thus, four groups, $A^-$, AH, $B^+$ and BOH, are present at the layer surface.

The dependence of the interfacial tension of lipid membranes on the pH solution can be described in terms of acid–base equilibrium. Let us assume that $H^+$ and $OH^-$ ions are adsorbed on the phospholipid surface. The adsorption equilibria are described by the equations:

$$A^- + H^+ \Leftrightarrow AH \tag{1a}$$

$$B^+ + OH^- \Leftrightarrow BOH \tag{2a}$$

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

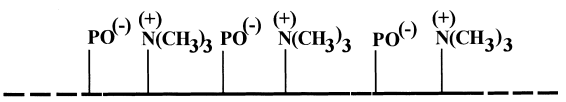



Fig. 1. Model of the bilayer lipid surface presenting the equilibria between the $H^+$ and $OH^-$ ions from the solution and the functional groups distributed on its surface.

$$K_A = \frac{a_{AH}}{a_{A^-} \cdot a_{H^+}} \quad (3)$$

$$K_B = \frac{a_{BOH}}{a_{B^+} \cdot a_{OH^-}} \quad (4)$$

The surface concentration of the lipid is denoted by $s$; the following equations can be written depending on the form of Eq. 1a or Eq. 2a of the acid–base equilibria:

$$a_{AH} + a_{A^-} = s \quad (5)$$

$$a_{BOH} + a_{B^+} = s \quad (6)$$

where $a_{A^-}$, $a_{AH}$, $a_{B^+}$ and $a_{BOH}$ (mol/m$^2$) are the concentrations on the membrane surface of the membrane components, respectively.

Assuming the contributions of the individual forms to the interfacial tension to be additive, the following equation can be written:

$$\gamma = \gamma_{A^-} + \gamma_{AH} + \gamma_{B^+} + \gamma_{BOH} \quad (7)$$

The expressions describing the contributions of the individual forms of the lecithin molecule to the interfacial tension are the following:

$$\gamma_{A^-} = \gamma^0_{A^-} \cdot \frac{a_{A^-}}{s} \quad (8)$$

$$\gamma_{AH} = \gamma^0_{AH} \cdot \frac{a_{AH}}{s} \quad (9)$$

$$\gamma_{B^+} = \gamma^0_{B^+} \cdot \frac{a_{B^+}}{s} \quad (10)$$

$$\gamma_{BOH} = \gamma^0_{BOH} \cdot \frac{a_{BOH}}{s} \quad (11)$$

Eqs. 3–11 will form an equation system and the $a_{A^-}$, $a_{AH}$, $a_{B^+}$ and $a_{BOH}$ values will be eliminated.

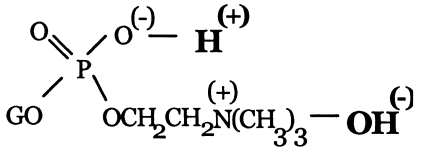
We therefore have:

$$\gamma = \gamma^0_{A^-}\left(\frac{1}{1 + K_A a_{H^+}}\right) + \gamma^0_{AH}\left(\frac{K_A a_{H^+}}{1 + K_A a_{H^+}}\right) + \gamma^0_{B^+}\left(\frac{1}{1 + K_B a_{OH^-}}\right) + \gamma^0_{BOH}\left(\frac{K_B a_{OH^-}}{1 + K_B a_{OH^-}}\right) \quad (12)$$

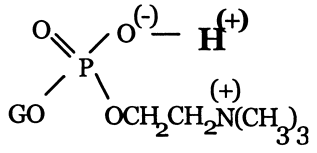
Eq. 12 presents the dependence of the interfacial tension of the lipid membrane on the pH of the electrolyte solution. Here $\gamma$ (N/m) is the interfacial tension of the lipid membrane, and $\gamma^0_{A^-}$, $\gamma^0_{AH}$, $\gamma^0_{B^+}$ and $\gamma^0_{BOH}$ (N/m) are the specific interfacial tensions of the membrane components, respectively.

### 2.2. Description II

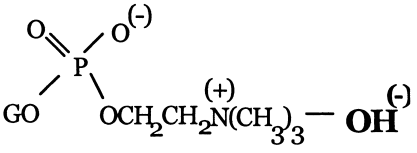
The adsorption of the $H^+$ and $OH^-$ ions at the lecithin layer surface can result in the presence of the forms:



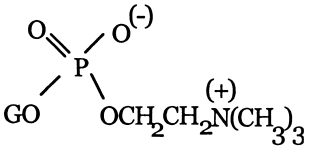

**(LHOH)**




**(LH$^+$)**




**(LOH$^-$)**




**(L)**

The sum of the contributions of the forms composing the lecithin surface in Description II is as follows:

$$\gamma = \gamma_{LHOH} + \gamma_{LH^+} + \gamma_{LOH^-} + \gamma_L \quad (13)$$

The surface concentrations of the groups postulated in Description I which take part in the equilibria can also be presented in terms of the surface concentrations of the forms of the lecithin molecule appearing in Description II:

$$a_{A^-} = a_L + a_{LOH^-} \quad (14)$$

$$a_{AH} = a_{LH^+} + a_{LHOH} \quad (15)$$

$$a_{B^+} = a_L + a_{LH^+} \quad (16)$$

$$a_{BOH} = a_{LOH^-} + a_{LHOH} \quad (17)$$

where $a_{LHOH}$, $a_{LH^+}$, $a_{LOH^-}$ and $a_L$ (mol/m$^2$) are the concentrations on the membrane surface of the membrane components, respectively.

The equilibrium constant of the association of groups L and $LOH^-$ with the hydrogen ion (Eq. 4) can be presented by the equation:

$$K_A = \frac{a_{LH^+} + a_{LHOH}}{(a_L + a_{LOH^-})a_{H^+}} \quad (18)$$

where the equilibrium constant of the association of groups L and $LH^+$ with the hydroxyl ion (Eq. 5) can be presented by the equation:

$$K_B = \frac{a_{LOH^-} + a_{LHOH}}{(a_L + a_{LH^+})a_{OH^-}} \quad (18)$$

The surface concentration of the lipid is denoted by $s$; the following equations can be written depending on the form of Eq. 14 or Eq. 17 of the association equilibria:

$$a_{LHOH} + a_{LH^+} + a_{LOH^-} + a_L = s \quad (20)$$

Assuming that the individual forms constituting the lecithin membrane contribute to the interfacial tension additively and taking Eq. 13 into consideration results in:

$$\gamma = \gamma^0_{LHOH}\cdot\frac{a_{LHOH}}{s} + \gamma^0_{LH^+}\cdot\frac{a_{LH^+}}{s} + \gamma^0_{LOH^-}\cdot\frac{a_{LOH^-}}{s} + \gamma^0_L\cdot\frac{a_L}{s} \quad (21)$$

Assuming that the individual forms constituting the lecithin membrane contribute to the interfacial tension additively and taking Eq. 13 into consideration results in:

$$\frac{a_{LHOH}}{s} = \frac{a_{AH}}{s}\cdot\frac{a_{BOH}}{s} \quad (22)$$

$$\frac{a_{LH^+}}{s} = \frac{a_{AH}}{s}\cdot\left(1-\frac{a_{BOH}}{s}\right) \quad (23)$$

$$\frac{a_{LOH^-}}{s} = \left(1-\frac{a_{AH}}{s}\right)\cdot\frac{a_{BOH}}{s} \quad (24)$$

$$\frac{a_L}{s} = \left(1-\frac{a_{AH}}{s}\right)\cdot\left(1-\frac{a_{BOH}}{s}\right) \quad (25)$$

The expressions:

$$a_{AH} = a_{LH^+} + a_{LHOH} = \frac{K_A\cdot s\cdot a_{H^+}}{1 + K_A a_{H^+}} \quad (26)$$

$$a_{BOH} = a_{LOH^-} + a_{LHOH} = \frac{K_B\cdot s\cdot a_{OH^-}}{1 + K_B a_{OH^-}} \quad (27)$$

can be written using Eq. 18–20, respectively.

Substituting Eqs. 26 and 27 to Eqs. 22–25 yields the equation:

$$\gamma = \gamma^0_{LHOH}\left(\frac{K_A a_{H^+}}{1 + K_A a_{H^+}}\right)\left(\frac{K_B a_{OH^-}}{1 + K_B a_{OH^-}}\right) + \gamma^0_{LH^+}\left(\frac{K_A a_{H^+}}{1 + K_A a_{H^+}}\right)\left(\frac{1}{1 + K_B a_{OH^-}}\right) + \gamma^0_{LOH^-}\left(\frac{1}{1 + K_A a_{H^+}}\right)\left(\frac{K_B a_{OH^-}}{1 + K_B a_{OH^-}}\right) + \gamma^0_L\left(\frac{1}{1 + K_A a_{H^+}}\right)\left(\frac{1}{1 + K_B a_{OH^-}}\right) \quad (28)$$

Eq. 28 presents the dependence of the interfacial tension of the lipid membrane on the pH of the electrolyte solution. Here $\gamma$ (N/m) is the interfacial tension of the lipid membrane, and $\gamma^0_{LHOH}$, $\gamma^0_{LH^+}$, $\gamma^0_{LOH^-}$ and $\gamma^0_L$ (N/m) are the specific interfacial tensions of the membrane components, respectively.

## 3. Theory: phosphatidylserine

The $-PO^{(-)}$, $-N^{(+)}H_3$ and the $-COO^{(-)}$ groups are present at the phospholipid layer surface at the aqueous solution side; the surface is built of molecules

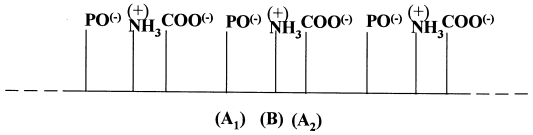



Fig. 2. Model I of the bilayer lipid surface, which presents the equilibria between the $H^+$ and $OH^-$ ions from the solution and the functional groups distributed on its surface.

each of them containing one $-PO^{(-)}$, one $-N^{(+)}H_3$, and one $-COO^{(-)}$ group. Various models of membrane surface structure can be adopted to analyse and describe the equilibria between the bilayer and the solution ions.

### 3.1. Model I

In this model, the surface is continuous with uniformly distributed functional groups constituting the active centres of $H^+$ and $OH^-$ ion adsorption. The scheme of the membrane surface in this model is presented in Fig. 2. The system is schematically presented by Eqs. 29a–31a:

$$-PO^{(-)} + H^{(+)} \Leftrightarrow -PO^{(-)} - H^{(+)} \quad (29a)$$

($A_1^-$) ($A_1H$)

$$-\overset{(+)}{N}H_3 + OH^{(-)} \Leftrightarrow -\overset{(+)}{N}H_3 - OH^{(-)} \quad (30a)$$

($B^+$) (BOH)

$$-COO^{(-)} + H^{(+)} \Leftrightarrow -COO^{(-)} - H^{(+)} \quad (31a)$$

($A_2^-$) ($A_2H$)

Thus, six kinds of groups are present on the layer surface: $A_1^-$, $A_1H$, $B^+$, BOH, $A_2^-$ and $A_2H$.

The dependence of the interfacial tension of lipid membranes on the pH solution can be described in terms of acid–base equilibria. Let us assume that $H^+$ and $OH^-$ ions are adsorbed on the phospholipid surface. The adsorption equilibria are described by the equations:

$$A_1^- + H^+ \Leftrightarrow A_1H \quad (29b)$$

$$B^+ + OH^- \Leftrightarrow BOH \quad (30b)$$

$$A_2^- + H^+ \Leftrightarrow A_2H \quad (31b)$$

The lipid is present in the membrane only. Therefore, the surface concentration of the lipid is equal to its amount related to the membrane surface area. These concentrations, and the concentration ions, determine the association acid and base constants according to the relationships:

$$K_{A_1} = \frac{a_{A_1H}}{a_{A_1^-} \cdot a_{H^+}} \quad (32)$$

$$K_B = \frac{a_{BOH}}{a_{B^+} \cdot a_{OH^-}} \quad (33)$$

$$K_{A_2} = \frac{a_{A_2H}}{a_{A_2^-} \cdot a_{H^+}} \quad (34)$$

The surface concentration of the lipid is denoted by $s$; the following equations can be written depending on the form of Eq. 29a or Eq. 30a of the acid–base equilibria:

$$a_{A_1H} + a_{A_1^-} = s \quad (35)$$

$$a_{BOH} + a_{B^+} = s \quad (36)$$

$$a_{A_2H} + a_{A_2^-} = s \quad (37)$$

where $a_{A_1H}$, $a_{A_1^-}$, $a_{BOH}$, $a_{B^+}$, $a_{A_2H}$ and $a_{A_2^-}$ (mol/m$^2$) are the concentrations on the membrane surface of the membrane components, respectively.

The following equation can be written assuming that contributions of the individual forms are additive:

$$\gamma = \gamma_{A_1^-} + \gamma_{A_1H} + \gamma_{B^+} + \gamma_{BOH} + \gamma_{A_2^-} + \gamma_{A_2H} \quad (38)$$

The expressions describing the interfacial tension values of the individual forms of the phosphatidyl-

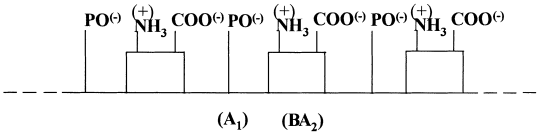



Fig. 3. Model II of the bilayer lipid surface, which presents the equilibria between the $H^+$ and $OH^-$ ions from the solution and the species containing $A_1$ and $BA_2$ groups.

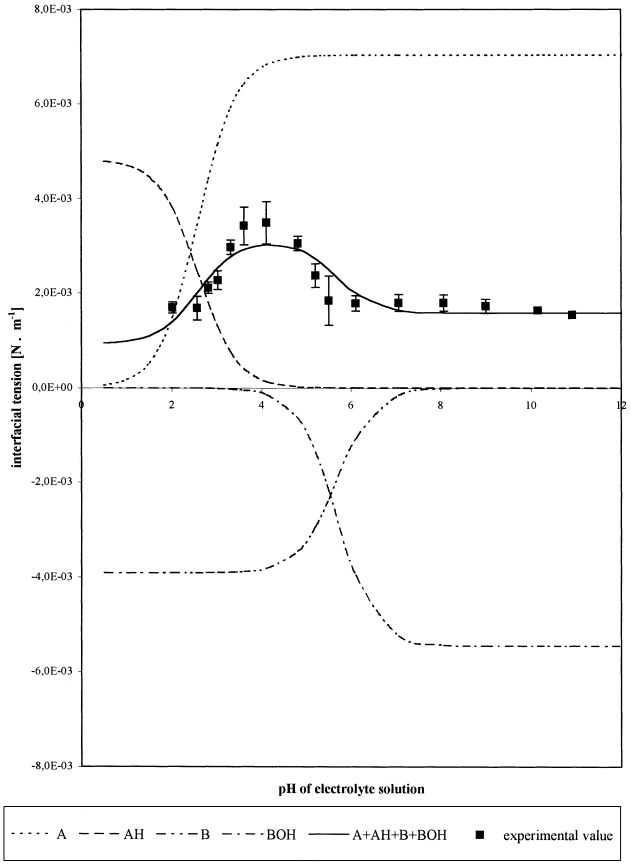



Fig. 4. The participation of the A and B groups, calculated for Description I, in dissociated and associated forms in the interfacial tension of the bilayer formed from PC, as a function of pH of the electrolyte solution.

serine (PS) molecule considered can then be written:

$$\gamma_{A_1^-} = \gamma^0_{A_1^-} \cdot \frac{a_{A_1^-}}{s} \tag{39}$$

$$\gamma_{A_1H} = \gamma^0_{A_1H} \cdot \frac{a_{A_1H}}{s} \tag{40}$$

$$\gamma_{B^+} = \gamma^0_{B^+} \cdot \frac{a_{B^+}}{s} \tag{41}$$

$$\gamma_{BOH} = \gamma^0_{BOH} \cdot \frac{a_{BOH}}{s} \tag{42}$$

$$\gamma_{A_2^-} = \gamma^0_{A_2^-} \cdot \frac{a_{A_2^-}}{s} \tag{43}$$

$$\gamma_{A_2H} = \gamma^0_{A_2H} \cdot \frac{a_{A_2H}}{s} \tag{44}$$

Eqs. 32–44 form an equation system and the $a_{A_1H}$, $a_{A_1^-}$, $a_{BOH}$, $a_{B^+}$, $a_{A_2H}$ and $a_{A_2^-}$ values will be eliminated.

We therefore have:

$$\gamma = \gamma^0_{A_1^-}\left(1-\frac{K_{A_1}a_{H^+}}{1+K_{A_1}a_{H^+}}\right) + \gamma^0_{A_1H}\left(\frac{K_{A_1}a_{H^+}}{1+K_{A_1}a_{H^+}}\right) + \gamma^0_{B^+}\left(1-\frac{K_B a_{OH^-}}{1+K_B a_{OH^-}}\right) + \gamma^0_{BOH}\left(\frac{K_B a_{OH^-}}{1+K_B a_{OH^-}}\right) + \gamma^0_{A_2^-}\left(1-\frac{K_{A_2}a_{H^+}}{1+K_{A_2}a_{H^+}}\right) + \gamma^0_{A_2H}\left(\frac{K_{A_2}a_{H^+}}{1+K_{A_2}a_{H^+}}\right) \tag{45}$$

Eq. 45 presents the dependence of the interfacial tension of the lipid membrane on the pH of the electrolyte solution. Here $\gamma$ (N/m) is the interfacial tension of the lipid membrane, and $\gamma^0_{A_1H}$, $\gamma^0_{A_1^-}$, $\gamma^0_{BOH}$, $\gamma^0_{B^+}$, $\gamma^0_{A_2H}$ and $\gamma^0_{A_2^-}$ (N/m) are the specific interfacial tensions of the membrane components, respectively.

### 3.2. Model II

In this model, the acid equilibrium between the $-PO^{(-)}$ group and the $H^+$ ion and the species containing the $-N^{(+)}H_3$ and the $-COO^{(-)}$ group with the $H^+$ and $OH^-$ ions of the solution are distinguished. The model is presented in Fig. 3.

The dependence of the interfacial tension of lipid membranes on the pH solution can be described in terms of acid–base equilibria. Let us assume that $H^+$ and $OH^-$ ions are adsorbed on the phospholipid surface. The adsorption equilibria are described by the

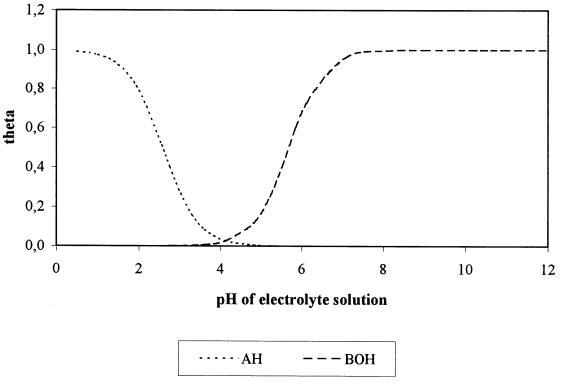



Fig. 5. $\theta$, calculated for Description I, in the associated A and B groups in the interfacial tension of the bilayer formed from PC, as a function of pH of the electrolyte solution.

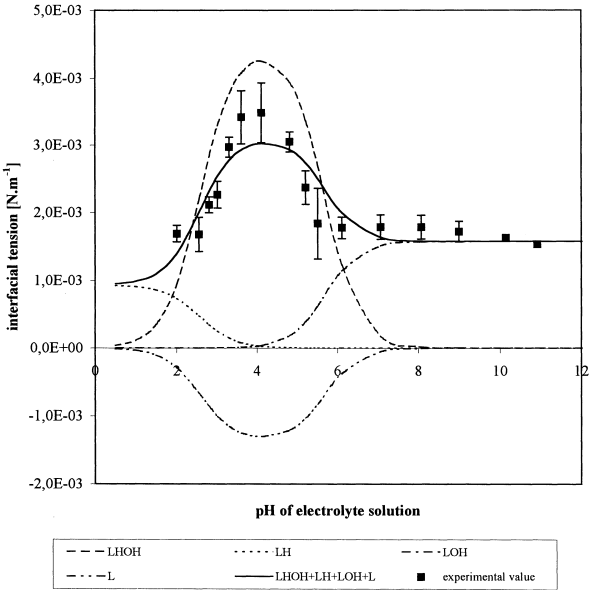



Fig. 6. The participation of the individual forms of the lecithin molecules, calculated for Description II, in the interfacial tension of the bilayer formed from PC, as a function of pH of the electrolyte solution.

equations:

$$A_1^- + H^+ \Leftrightarrow A_1H \tag{46}$$

$$B^+A_2^- + OH^- \Leftrightarrow BOHA_2^- \tag{47}$$

$$B^+A_2^- + H^+ \Leftrightarrow B^+A_2H \tag{48}$$

$$BOHA_2^- + H^+ \Leftrightarrow BOHA_2H \tag{49}$$

Thus, six groups, $A_1^-$, $A_1H$, $B^+A_2^-$, $BOHA_2^-$, $B^+A_2H$ and $BOHA_2H$, can be distinguished at the layer surface.

The lipid is present in the membrane only. Therefore, the surface concentration of the lipid is equal to its amount related to the membrane surface area. These concentrations, and the concentration ions, determine the association acid and base constants according to the relationships:

$$K_1 = \frac{a_{A_1H}}{a_{A_1^-} \cdot a_{H^+}} \tag{50}$$

$$K_2 = \frac{a_{BOHA_2^-}}{a_{B^+A_2^-} \cdot a_{OH^-}} \tag{51}$$

$$K_3 = \frac{a_{B^+A_2H}}{a_{B^+A_2^-} \cdot a_{H^+}} \tag{52}$$

$$K_4 = \frac{a_{BOHA_2H}}{a_{BOHA_2^-} \cdot a_{H^+}} \tag{53}$$

The surface concentration of the lipid is denoted by $s$; the following equations can be written depending on the form of Eq. 29a or Eq. 30a of the acid–base equilibria:

$$a_{A_1H} + a_{A_1^-} = s \tag{54}$$

$$a_{B^+A_2^-} + a_{BOHA_2^-} + a_{B^+A_2H} + a_{BOHA_2H} = s \tag{55}$$

where $a_{A_1^-}$, $a_{A_1H}$, $a_{B^+A_2^-}$, $a_{BOHA_2^-}$, $a_{B^+A_2H}$ and $a_{BOHA_2H}$ are the concentrations on the membrane surface of the membrane components, respectively.

Assuming that the contributions of the individual forms to the interfacial tension are additive, the following equation can be written:

$$\gamma = \gamma_{A_1^-} + \gamma_{A_1H} + \gamma_{B^+A_2^-} + \gamma_{BOHA_2^-} + \gamma_{B^+A_2H} + \gamma_{BOHA_2H} \tag{56}$$

The expressions describing interfacial tension values of the individual forms of the phosphatidylserine molecule considered can then be written:

$$\gamma_{A_1^-} = \gamma^0_{A_1^-} \cdot \frac{a_{A_1^-}}{s} \tag{57}$$

$$\gamma_{A_1H} = \gamma^0_{A_1H} \cdot \frac{a_{A_1H}}{s} \tag{58}$$

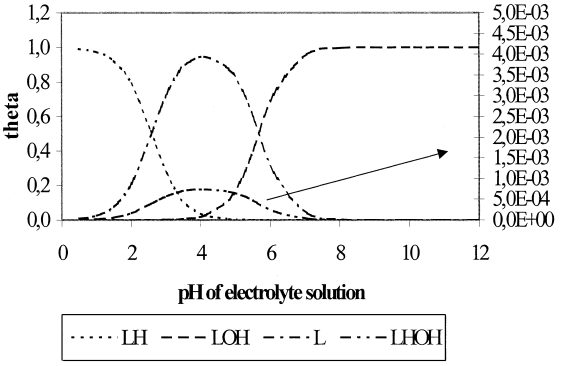



Fig. 7. $\theta$, calculated for Description II, for the individual form of the lecithin molecule, in the interfacial tension of the bilayer formed from PC, as a function of pH of the electrolyte solution.

$$\gamma_{B^+A_2^-} = \gamma^0_{B^+A_2^-} \cdot \frac{a_{B^+A_2^-}}{s} \quad (59)$$

$$\gamma_{BOHA_2^-} = \gamma^0_{BOHA_2^-} \cdot \frac{a_{BOHA_2^-}}{s} \quad (60)$$

$$\gamma_{B^+A_2H} = \gamma^0_{B^+A_2H} \cdot \frac{a_{B^+A_2H}}{s} \quad (61)$$

$$\gamma_{BOHA_2H} = \gamma^0_{BOHA_2H} \cdot \frac{a_{BOHA_2H}}{s} \quad (62)$$

Eqs. 50–62 form an equation system and the $a_{A_1^-}$, $a_{A_1H}$, $a_{B^+A_2^-}$, $a_{BOHA_2^-}$, $a_{B^+A_2H}$ and $a_{BOHA_2H}$ values will be eliminated.

We therefore have:

$$\gamma = -m_1 a^2_{H^+}\gamma - m_2 a_{H^+}\gamma - m_3 a_{OH^-}\gamma + m_4 a^2_{H^+} + m_5 a_{H^+} + m_6 a_{OH^-} + b \quad (63)$$

Here:

$$m_1 = \frac{K_1 K_3}{M}$$

$$m_2 = \frac{K_1 + K_3 + K_1 K_2 K_4 K_w}{M}$$

$$m_3 = \frac{K_2}{M}$$

$$m_4 = \frac{K_1 K_3 (\gamma^0_{A_1H} + \gamma^0_{B^+A_2H})}{M}$$

$$m_5 = \frac{K_3\gamma^0_{A_1H} + K_1K_2K_4K_w\gamma^0_{A_1H} + K_1\gamma^0_{B^+A_2^-} + K_1K_2K_4K_w\gamma^0_{BOHA_2H}}{M}$$

$$m_6 = \frac{K_2 \gamma^0_{A_1^-}}{M}$$

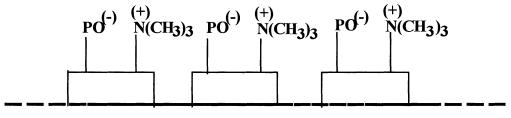



Fig. 8. Model of the bilayer lipid surface presenting the equilibria between the $H^+$ and $OH^-$ ions from the solution and the lecithin molecules.

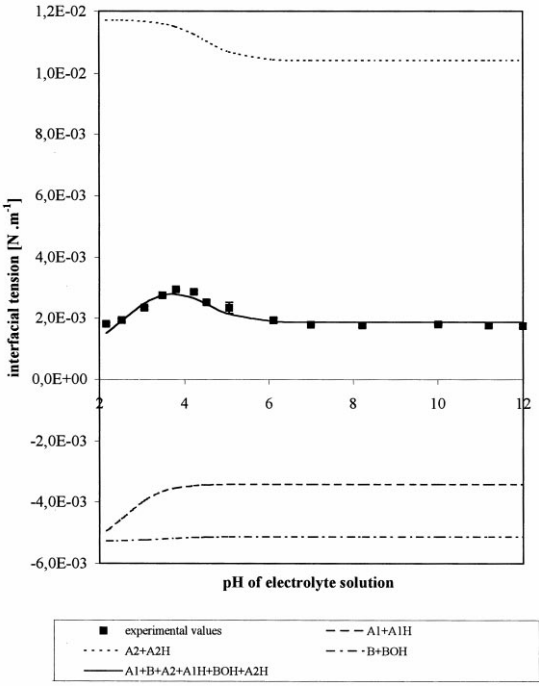



Fig. 9. The participation of the $A_1$, B and $A_2$ groups, calculated for Model I, in dissociated and associated forms in the interfacial tension of the bilayer formed from PS, as a function of pH of the electrolyte solution.

$$b = \frac{\gamma^0_{A_1^-} + \gamma^0_{A_1H} + \gamma^0_{B^+A_2^-} + \gamma^0_{BOHA_2^-} + \gamma^0_{B^+A_2H} + \gamma^0_{BOHA_2H}}{M} + \frac{K_2K_4K_w\gamma^0_{A_1^-} + K_1K_2K_w(\gamma^0_{A_1H} + \gamma^0_{BOHA_2^-})}{M}$$

$$M = 1 + (K_1 + K_4)K_2K_w$$

Eq. 63 presents the dependence of the interfacial tension of the lipid membrane on pH of electrolyte solution. Here $\gamma$ (N/m) is the interfacial tension of the lipid membrane, and $\gamma^0_{A_1^-}$, $\gamma^0_{A_1H}$, $\gamma^0_{B^+A_2^-}$, $\gamma^0_{BOHA_2^-}$, $\gamma^0_{B^+A_2H}$ and $\gamma^0_{BOHA_2H}$ (N/m) are the specific interfacial tensions of the membrane components, respectively.

## 4. Experimental

### 4.1. Methods

The interfacial tension, $\gamma$, of the lipid bilayer was determined by measuring the curvature radius, $R$, of

the convex surface formed by applying a pressure difference, $\Delta p$, on its sides. The method used was based on Young's and Laplace's equation [20]: $2\gamma = R\Delta p$.

### 4.2. Measurements

The apparatus and the measurement method have been described in a previous paper [19,21]. The lipid membranes were formed by the Mueller–Rudin method [22]. 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, as the spherical cap is hardly visible. While using yellow light its visibility gets better.

The interfacial tension was measured on freshly created lipid 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 instrument readings (100–150) the arithmetic mean and standard deviation were enumerated. Measurements with a 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 lipid in solution (*n*-decane, butanol).

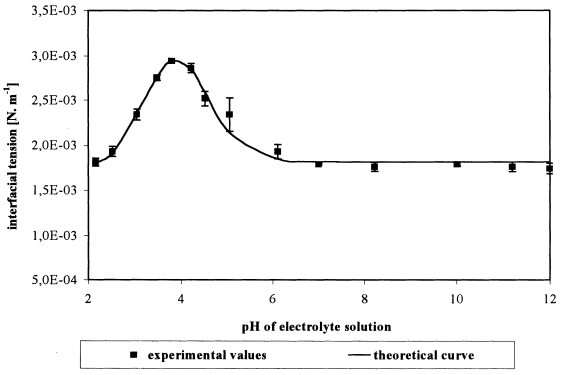



Fig. 10. The participation of the $A_1$ and $BA_2$ groups, calculated for Model II, in dissociated and associated forms in the interfacial tension of the bilayer formed from PS, as a function of pH of the electrolyte solution.

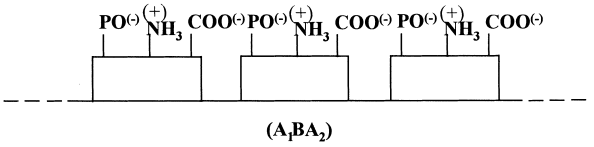



Fig. 11. Model III of the bilayer lipid surface, which presents the equilibria between the $H^+$ and $OH^-$ ions from the solution and the species containing $A_1BA_2$ groups.

### 4.3. Materials

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

3-*sn*-Phosphatidyl-L-serine from bovine brain (99%) from Fluka was used in the experiment.

Buffers of 2–12 pH ranges were prepared according to Britton and Robinson [23] and used as the electrolyte. They were prepared by adding 0.2 M sodium hydroxide to 100 ml of 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 sodium hydroxide added.

## 5. Results and discussions

The interfacial tension of the membrane formed from phosphatidylcholine is plotted in Fig. 4 vs. pH of the electrolyte solution. Points present the experimental values, the total values calculated from

Eq. 12 are presented by the continuous line and the interfacial tension values of lecithin membrane components are marked with broken lines. Fig. 4 refers to the above presented Description I where the distribution of the $-PO^{(-)}$ and $-N^{(+)}(CH_3)_3$ groups on the aqueous solution side of the lipid layer has been assumed to be uniform.

As seen in Eq. 12, the total interfacial tension value of the lecithin membrane is a sum of the interfacial tension values of its components, i.e. $A^-$, AH, $B^+$ and BOH.

Specific interfacial tension values of the individual components of the lecithin membrane were determined. The results were obtained by linear regression using the Excel 97 program. The $\gamma^0_{A^-}$, $\gamma^0_{AH}$, $\gamma^0_{B^+}$ and $\gamma^0_{BOH}$ values determined in this way are equal to 7.03 mN/m, 4.84 mN/m, −3.91 mN/m, and −5.46 mN/m, respectively.

Coverage of the lipid membrane surface by the $H^+$ and $OH^-$ ions vs. pH of the electrolyte solution is presented in Fig. 5. As can be seen, the lecithin membrane surface is not covered by the $H^+$ and $OH^-$ ions in the proximity of its isoelectric point, i.e. at pH equal to about 4.

The interfacial tension of the membrane formed from phosphatidylcholine is also plotted in Fig. 6 vs. the pH of the electrolyte solution. Points present the experimental values, the total values calculated from Eq. 28, which has been derived according to Description II, are presented by the continuous line and the interfacial tension values of lecithin membrane components are marked with broken lines. It is seen in Eq. 28 that the total interfacial tension value of the lecithin membrane is the sum of its individual components, i.e. LHOH, $LH^+$, $LOH^-$ and L.

Specific interfacial tension values of individual lecithin membrane components were determined by linear regression using the Excel 97 program. The $\gamma^0_{LHOH}$, $\gamma^0_{LH^+}$, $\gamma^0_{LOH^-}$ and $\gamma^0_L$ values determined in this way are equal to 5.743 mN/m, 9.35 mN/m, −1.58 mN/m, and −1.37 mN/m, respectively.

Coverage of the lipid membrane surface by the $H^+$ and $OH^-$ ions vs. pH of the electrolyte solution is presented in Fig. 7. Like in Description II, the L form predominates in the lecithin membrane surface in the proximity of its isoelectric point; its surface is not covered by the $H^+$ and $OH^-$ ions there. In both descriptions, coverage of the lipid membrane surface by the $H^+$ and $OH^-$ ions remains unchanged in the ranges below 1.5 and above 7.

Considerations of equilibria by considering the $H^+$ and $OH^-$ ions in terms of Model II presented in Fig. 8 and calculations of contributions of those forms to the interfacial tension of the lipid bilayer resulted in disagreement with the experiment.

The description presented above, in which the contributions of various forms of the lecithin molecule to the interfacial tension of the bilayer were assumed to be additive, resulted in a markedly better description of the dependence on pH than the description based on the Gibbs isotherm [19], particularly at distances far from the isoelectric point.

The interfacial tension of the membrane formed from phosphatidylserine is plotted in Fig. 9 vs. pH of the electrolyte solution. In this figure, the experimental values are marked by points and the theoretical ones obtained from Eq. 45 by lines. Broken lines present the interfacial tensions of the individual membrane components, i.e. $A_1^-$, $A_1H$, $B^+$, BOH, $A_2^-$ and $A_2H$. Fig. 9 refers to the earlier described structural Model I of a lipid membrane surface in which the functional groups have been assumed to be uniformly distributed on its surface on the aqueous solution side. As is seen in Eq. 45, the total interfacial tension value of the PS membrane is the sum of the interfacial tension values of its components, i.e. $A_1^-$, $A_1H$, $B^+$, BOH, $A_2^-$ and $A_2H$.

However, the acid–base constants of the PS membrane components were needed to calculate their interfacial tension contributions. Their determination is difficult because phosphatidylserine is insoluble in water. This was the reason why liposomes were used to calculate these magnitudes. In this way, a uniform distribution of phosphatidylserine functional groups in solution was assured in spite of its insolubility in water. It was assumed in the calculations that the PS molecules present in the outer layer of the liposome only take part in the acid–base equilibria. For this reason, the PS concentration used in the calculation is half of that introduced into the solution. The acid–base constants were determined by titration of liposomes previously obtained with hydrochloric acid and sodium hydroxide. Titrations were made with a 736GP Titrino apparatus from Metrohm (Switzerland) [20].

The acid–base equilibrium constants are $K_1 = 10^{3.36}$ and $K_2 = 10^{9.55}$. As two constants were determined and three were needed for the calculations, the association constant, $K_{A_1} = 10^{2.581}$, determined in [19], was assigned to the $-PO^{(-)}$ group. Thus, the $K_1$ constant determined by titration is the geometrical mean of the $K_{A_1}$ and $K_{A_2}$. In this way, it was possible to calculate $K_{A_2} = 10^{4.139}$. The $K_B$ was determined by titration and it was found to be equal to $10^{9.55}$. The specific interfacial tensions of individual forms of PS membrane can be calculated by substituting the $K_{A_1}$, $K_{A_2}$ and $K_B$ constants obtained in this way into Eq. 45.

Introducing the $K_{A_1}$, $K_{A_2}$ and $K_B$ values obtained in this way into Eq. 45 yielded specific interfacial tension values of the individual forms of the PS membrane. The results were obtained using the linear regression method of the Excel 97 program. The $\gamma_{A_1H}$, $\gamma_{A_1^-}$, $\gamma_{B^+}$, $\gamma_{BOH}$, $\gamma_{A_2H}$ and $\gamma_{A_2^-}$ values determined in this way were $-2\times10^{-3}$, $-4.4\times10^{-3}$, $-4.07\times10^{-3}$, $-2.69\times10^{-3}$, $7.81\times10^{-3}$, and $8.06\times10^{-3}$ N/m, respectively. When the interfacial tensions of bilayer lipid membranes formed from only individual forms have negative values, it is possible to suppose that no bilayer membranes formed from these forms do exist.

In Fig. 10, the experimental interfacial tension values are represented as points and those calculated for Model II from Eq. 63 as solid lines. Fig. 10 refers to Model II in which the phosphatidylserine surface is built of molecules. However, the determination of the interfacial tension of the individual components was difficult because the association constant values $K_1$, $K_2$, $K_3$ and $K_4$ were unknown. For this reason, the individual equation coefficients, $m_1$, $m_2$, $m_3$, $m_4$, $m_5$, $m_6$ and $b$, were obtained using the linear regression method of the Excel 97 program.

As can be seen in Fig. 10, Model II yields a much better agreement of calculated and experimental data in the whole pH range. This indicates that it is closer to reality.

Another structural model of the PS bilayer surface is presented in Fig. 11. This model was considered to explain acid equilibria between the functional groups of the PS molecule and the $H^+$ and $OH^-$ ions in solution. However, the presentation and the discussion of equilibria in the forms envisaged by this model and calculations of their contribution to the interfacial tension of the lipid bilayer result in disagreement with the experiment.

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