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# Interfacial tension of phosphatidylcholine–phosphatidylserine system in bilayer lipid membrane

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**Abstract**

The effect of pH of electrolyte solution on the interfacial tension of lipid membrane formed of phosphatidylcholine (PC, lecithin)–phosphatidylserine (PS) system was studied. In this article, three models describing the $H^+$ and $OH^-$ ions adsorption in the bilayer lipid surface are presented. In Model I and Model II, the surface is continuous with uniformly distributed functional groups constituting the centres of $H^+$ and $OH^-$ ions adsorption while in the other the surface is built of lipid molecules, free or with attached $H^+$ and $OH^-$ ions. In these models contribution of the individual lipid molecule forms to interfacial tension of the bilayer were assumed to be additive. In Model III the adsorption of the $H^+$ and $OH^-$ ions at the PC–PS bilayer surface was described in terms of the Gibbs isotherm. Theoretical equations are derived to describe this dependence in the whole pH range.


*Keywords:* Phosphatidylcholine–phosphatidylserine system; Interfacial tension; Effect of pH on interfacial tension; Phosphatidylcholine; Phosphatidylserine

## 1. Introduction

Biomembranes play an important role in all essential biological phenomena. They organize living matter in the cell, create a fluid two-dimensional matrix, and allow for the controlled transport of solution. 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. Numerous functions of biological membranes were reproduced and explained using the model membranes. It was demonstrated by numerous experiments that properties of the lipid membranes formed of artificial components were very similar to those of natural cell membranes [1,2].

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 reported values ranging from 0.2–6.0 mN $m^{-1}$ [3]. The interfacial tension of the lipid bilayer was also determined by measuring the energy of the membrane formation and had a value of $3.4 \pm 0.6$ mN $m^{-1}$ [4]. Interfacial tension is affected by such factors as medium pH or the presence of some substances incorporated in the lipid bilayer, for example cholesterol, other lipids, proteins [5–9]. The effect of pH of electrolyte solution on monolayer and bilayer lipid membranes built from different lipids was determined. The dependence of interfacial tension on effect of pH of the electrolyte solution was presented in earlier studies for the phosphatidylcholine [6,10–13], phosphatidylserine [13–15] and phosphatidylethanolamine (PE) [16,17], but only pure PC, PS or PE were used there. The curves obtained demonstrate the maximal interfacial tension values at the isoelectric point. Runs of these curves were well characterised by the simplified description based on the Gibbs isotherm, but only in proximity to the isoelectric point [6]. Using exactly the definition of surface excess in the Gibbs equation (taking into account volumes of adsorbed ions at the membrane surface) permits one to explain the run of experimental curves in whole pH range [18,19]. Also in earlier studies [13,16] proposed were the models derived to describe adsorption of the $H^+$ and $OH^-$ ions at lipid surfaces formed from phospholipids, which would reproduce changes in interfacial tension more correctly,

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particularly in the ranges distant from the isoelectric point. In these models, contribution of the individual lipid molecule forms to interfacial tension of the bilayer was assumed to be additive. It is very hard to say from which of the offered descriptions would be more appealing for further experiments. These papers concentrated especially on phospholipids because they are major fractions of lipids found in biological membranes: phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine were chosen. Phosphatidylcholine was a basic component of formed bilayers not only because it has been widely examined and described in the literature, but also because it creates permanent bilayers, of which one can easily build in other components. The interaction between PC and PS was studied using the NMR method [20], FRAP measurements [21], fluorimetry [22] and molecular dynamics simulation [23], but not as a function of pH of electrolyte solution.

As it was mentioned earlier, adsorption of the $H^+$ and $OH^-$ ions at the phosphatidylcholine or phosphatidylserine layer surface were described earlier; the effect of pH on the interfacial tension of PC or PS membranes were described earlier in terms of the Gibbs' equation and other models. These models make an assumption effect of pH on the interfacial tension of phosphatidylcholine or phosphatidylserine, where the existence of equilibria of uniformly distributed $-PO^{(-)}$ and $-N^{(+)}(CH_3)_3$ groups with the $H^+$ and $OH^-$ ions was assumed and experimentally confirmed. The PS has been chosen for further studies because of its molecular structure: it has the $-N^{(+)}H_3$ and the $-COO^{(-)}$ groups situated close to one another and the third one, $-PO^{(-)}$ spaced from them.

This work has been aimed at determination of the dependence of interfacial tension of the PC–PS system on pH of electrolyte solution in the whole experimental pH range (2–12).

## 2. Theory

The phospholipid system layer observed from the aqueous solution side hasuniformly distributed $-PO^{(-)}$, $-N^{(+)}(CH_3)_3$, $-COO^{(-)}$ and $-N^{(+)}H_3$ groups because it is built of the molecules each having $-PO^{(-)}$, and $-N^{(+)}(CH_3)_3$, groups and $-PO^{(-)}$, $-N^{(+)}H_3$ and $-COO^{(-)}$ groups. Therefore, two models of the PC–PS membrane surface can be adopted. In Model I, the membrane surface is continuous with uniformly distributed functional groups being the active centres of adsorption of the $H^+$ and $OH^-$ ions, which was presented in Fig. 1a. In Model II the acid equilibria between the $-PO^{(-)}$ group from PC and PS molecule and the $H^+$ ion and $-N^{(+)}(CH_3)_3$ group from PC molecule and the $OH^-$ ion and the species containing the $-N^{(+)}H_3$ and the $-COO^{(-)}$ group from PS molecule with the $H^+$ and $OH^-$ ion of solution are distinguished. This model is presented in Fig. 1b.

The dependence of the interfacial tension of the lipid membrane on pH of electrolyte solution were described in a previous article by the simplified description based on the Gibbs isotherm, but only in proximity of isoelectric point [6]. In Model III, the exact definition of the surface excess in the Gibbs equation (taking into account volumes of adsorbed ions at the membrane surface) permitted one to explain the run of experimental curves in whole pH range.

Thanks to the presented models below, we are able to better understand the interactions between biological molecules which make an easy understanding of the phenomena of more complicated systems, e.g. of cellular membranes [24–26].

### 2.1. Model I

Uniformly distributed active centres at which the $H^+$ and $OH^-$ ions can be adsorbed are present at the aqueous solution side. This model was presented in Fig. 1a. They are schematically described with Eqs. (1)–(4):

$$\underset{(A_1^-)}{-PO^{(-)}} + H^{(+)} \Leftrightarrow \underset{(A_1H)}{-PO^{(-)}-H^{(+)}} \quad (1)$$

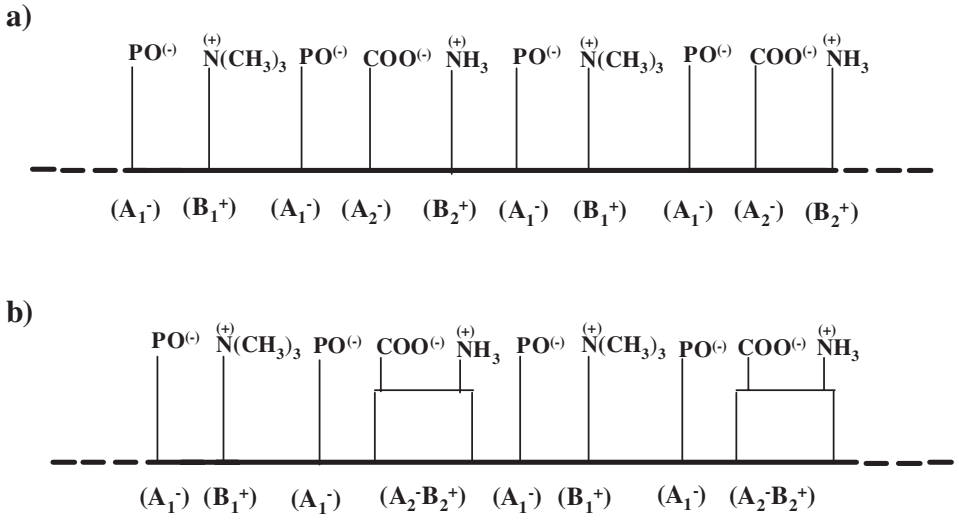


Fig. 1. The Model I (a) and Model II (b) of the bilayer lipid surface, which presents the equilibria between the $H^+$ and $OH^-$ ions from solution and the functional groups distributed on its surface ($-PO^{(-)}$, $-N^{(+)}(CH_3)_3$) groups from PC and $-PO^{(-)}$, $-COO^{(-)}$, $-N^{(+)}H_3$ groups from PS).

$$\underset{(B_1^+)}{-N^{(+)}(CH_3)_3} + OH^{(-)} \quad \Leftrightarrow \quad \underset{(B_1OH)}{-N^{(+)}(CH_3)_3 - OH^{(-)}} \tag{2}$$

$$\underset{(A_2^-)}{-COO^{(-)}} + H^{(+)} \Leftrightarrow \quad \underset{(A_2H)}{-COO^{(-)} - H^{(+)}} \tag{3}$$

$$\underset{(B_2^+)}{-N^{(+)}H_3} + OH^- \quad \Leftrightarrow \quad \underset{(B_2OH)}{-N^{(+)}H_3 - OH^{(-)}}. \tag{4}$$

Thus, eight groups: $A_1^-$, $A_1H$, $B_1^+$, $B_1OH$, $A_2^-$, $A_2H$, $B_2^+$, $B_2OH$ are present at the layer surface.

The dependence of interfacial tension of lipid membranes system 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 system. The adsorption equilibria are described by the equations:

$$A_1^- + H^+ \Leftrightarrow A_1H \tag{1a}$$

$$B_1^+ + OH^- \Leftrightarrow B_1OH \tag{2a}$$

$$A_2^- + H^+ \Leftrightarrow A_2H \tag{3a}$$

$$B_2^+ + OH^- \Leftrightarrow B_2OH. \tag{4a}$$

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. Surface concentrations of the bilayer membrane component ($a_{A_1^-}$, $a_{A_1H}$, $a_{B_1^+}$, $a_{B_1OH}$, $a_{A_2^-}$, $a_{A_2H}$, $a_{B_2^+}$, $a_{B_2OH}$) and the concentrations of hydrogen or hydroxyl ions determine the acid–base constants according to the relationships:

$$K_{A_1} = \frac{a_{A_1H}}{a_{A_1^-} \cdot a_{H^+}} \tag{5}$$

$$K_{B_1} = \frac{a_{B_1OH}}{a_{B_1^+} \cdot a_{OH^-}} \tag{6}$$

$$K_{A_2} = \frac{a_{A_2H}}{a_{A_2^-} \cdot a_{H^+}} \tag{7}$$

$$K_{B_2} = \frac{a_{B_2OH}}{a_{B_2^+} \cdot a_{OH^-}}. \tag{8}$$

The surface concentration of the lipid is denoted by $s$; the following equations can be written depending on the form of Eqs. (1a)–(4a) of the acid–base equilibria:

$$a_{A_1H} + a_{A_1^-} = s \tag{9}$$

$$a_{B_1OH} + a_{B_1^+} = s \tag{10}$$

$$a_{A_2H} + a_{A_2^-} = s \tag{11}$$

$$a_{B_2OH} + a_{B_2^+} = s \tag{12}$$

where: $a_{A_1^-}$, $a_{A_1H}$, $a_{B_1}+$, $a_{B_1OH}$, $a_{A_2^-}$, $a_{A_2H}$, $a_{B_2}+$, $a_{B_2OH}$, [mol $m^{-2}$] concentration on the membrane surface of the membrane component, respectively.

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

$$\gamma = \gamma_{A_1^-} + \gamma_{A_1H} + \gamma_{B_1^+} + \gamma_{B_1OH} + \gamma_{A_2^-} + \gamma_{A_2H} + \gamma_{B_2^+} + \gamma_{B_2OH}. \tag{13}$$

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

$$\gamma_{A_1^-} = \gamma^0_{A_1^-} \cdot \frac{a_{A_1-}}{s} \tag{14}$$

$$\gamma_{A_1H} = \gamma^0_{A_1H} \cdot \frac{a_{A_1H}}{s} \tag{15}$$

$$\gamma_{B_1^+} = \gamma^0_{B_1^+} \cdot \frac{a_{B_1^+}}{s} \tag{16}$$

$$\gamma_{B_1OH} = \gamma^0_{B_1OH} \cdot \frac{a_{B_1OH}}{s} \tag{17}$$

$$\gamma_{A_2^-} = \gamma^0_{A_2^-} \cdot \frac{a_{A_2^-}}{s} \tag{18}$$

$$\gamma_{A_2H} = \gamma^0_{A_2H} \cdot \frac{a_{A_2H}}{s} \tag{19}$$

$$\gamma_{B_2^+} = \gamma^0_{B_2^+} \cdot \frac{a_{B_2^+}}{s} \tag{20}$$

$$\gamma_{B_2OH} = \gamma^0_{B_2OH} \cdot \frac{a_{B_2OH}}{s}. \tag{21}$$

The Eqs. (5)–(21) will form an equation system and the $a_{A_1^-}$, $a_{A_1H}$, $a_{B_1^+}$, $a_{B_1OH}$, $a_{A_2^-}$, $a_{A_2H}$, $a_{B_2}+$, $a_{B_2OH}$ values will be eliminated.

We therefore have:

$$\gamma = \gamma^0_{A_1^-} \frac{1}{K_{A_1}a_{H^+} + 1} + \gamma^0_{A_1H} \frac{K_{A_1}a_{H^+}}{K_{A_1}a_{H^+} + 1} + \gamma^0_{B_1^+} \frac{1}{K_{B_1}a_{OH^-} + 1} + \gamma^0_{B_1OH} \frac{K_{B_1}a_{OH^-}}{K_{B_1}a_{OH^-} + 1} + \gamma^0_{A_2^-} \frac{1}{K_{A_2}a_{H^+} + 1} + \gamma^0_{A_2H} \frac{K_{A_2}a_{H^+}}{K_{A_2}a_{H^+} + 1} + \gamma^0_{B_2^+} \frac{1}{K_{B_2}a_{OH^-} + 1} + \gamma^0_{B_2OH} \frac{K_{B_2}a_{OH^-}}{K_{B_2}a_{OH^-} + 1}. \tag{22}$$

Eq. (22) presents the dependence of the interfacial tension of the lipid membrane on pH of electrolyte solution. where:$\gamma$ [N $m^{-1}$] — interfacial tension of the lipid membrane; $\gamma^0_{A_1}$-, $\gamma^0_{A_1H}$, $\gamma^0_{B_1}$+, $\gamma^0_{B_1OH}$, $\gamma^0_{A_2}$-, $\gamma^0_{A_2H}$, $\gamma^0_{B_2}$+, $\gamma^0_{B_2OH}$ [N $m^{-1}$] — specific interfacial tension of the membrane component, respectively.

### 2.2. Model II

In this model, which was presented in Fig. 1b, the acid equilibria between the $-PO^{(-)}$ group from PC and PS

molecule and the $H^+$ ion and $-N^{(+)}(CH_3)_3$ group from PC molecule and the $OH^-$ ion and the species containing the $-N^{(+)}H_3$ and the $-COO^{(-)}$ group from PS molecule with the $H^+$ and $OH^-$ ion of solution are distinguished.

Such a distribution of acidic–base groups of the PS molecule surface was chosen since it is conforming to reality, which was presented in earlier work [14].

The dependence of 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 system surface. The adsorption equilibria are described by the equations:

$$A_1^- + H^+ \Leftrightarrow A_1H \tag{23}$$

$$B_1^+ + OH_- \Leftrightarrow B_1OH \tag{24}$$

$$B_2^+A_2^- + OH^- \Leftrightarrow B_2OHA_2^- \tag{25}$$

$$B_2^+A_2^- + H^+ \Leftrightarrow B_2^+A_2H \tag{26}$$

$$B\pm_2OHA_2^- + H^+ \Leftrightarrow B_2OHA_2H. \tag{27}$$

Thus, eight groups: $A_1^-$, $A_1H$, $B_1^+$, $B_1OH$, $B_2^+A_2^-$, $B_2OHA_2^-$, $B_2^+A_2H$, $B_2OHA_2H$ 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. Surface concentrations of the bilayer membrane component, ($a_{A_1^-}$, $a_{A_1OH}$, $a_{B_1^+}$, $a_{B_1OH}$, $a_{B_2^+A_2^-}$, $a_{B_2OHA_2^-}$, $a_{B_2^+A_2H}$, $a_{B_2OHA_2H}$) and the concentrations of hydrogen or hydroxyl ions; determine the acid–base constants according to the relationships:

$$K_1 = \frac{a_{A_1H}}{a_{A_1^-}\cdot a_{H^+}} \tag{28}$$

$$K_2 = \frac{a_{B_1OH}}{a_{B_1^+}\cdot a_{OH^-}} \tag{29}$$

$$K_3 = \frac{a_{B_2OHA_2^-}}{a_{B_2^+A_2^-}\cdot a_{OH^-}} \tag{30}$$

$$K_4 = \frac{a_{B_2^+A_2H}}{a_{B_2^+A_2^-}\cdot a_{H^+}} \tag{31}$$

$$K_5 = \frac{a_{B_2OHA_2H}}{a_{B_2OHA_2^-}\cdot a_{H^+}}. \tag{32}$$

The surface concentration of the lipid is denoted by $s$; the following equations can be written depending on the form of Eqs. (23)–(27) of the acid–base equilibria:

$$a_{A_1H} + a_{A_1^-} = s \tag{33}$$

$$a_{B_1OH} + a_{B_1^+} = s \tag{38}$$

$$a_{B_2^+A_2^-} + a_{B_2OHA_2^-} + a_{B_2^+A_2H} + a_{B_2OHA_2H} = s \tag{35}$$

where $a_{A_1^-}$, $a_{A_1H}$, $a_{B_1^+}$, $a_{B_1OH}$, $a_{B_2^+A_2^-}$, $a_{B_2OHA_2^-}$, $a_{B_2^+A_2H}$, $a_{B_2OHA_2H}$ — concentration on the membrane surface of the membrane component, respectively.

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

$$\gamma = \gamma_{A_1^-} + \gamma_{A_1H} + \gamma_{B_1^+} + \gamma_{B_1OH} + \gamma_{B_2^+A_2^-} + \gamma_{B_2OHA_2^-} + \gamma_{B_2^+A_2H} + \gamma_{B_2OHA_2H}. \tag{36}$$

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

$$\gamma_{A_1^-} = \gamma^0_{A_1^-}\cdot\frac{a_{A_1^-}}{s} \tag{37}$$

$$\gamma_{A_1H} = \gamma^0_{A_1H}\cdot\frac{a_{A_1H}}{s} \tag{38}$$

$$\gamma_{B_1^+} = \gamma^0_{B_1^+}\cdot\frac{a_{B_1^+}}{s} \tag{39}$$

$$\gamma_{B_1OH} = \gamma^0_{B_1OH}\cdot\frac{a_{B_1OH}}{s} \tag{40}$$

$$\gamma_{B_2^+A_2^-} = \gamma^0_{B_2^+A_2^-}\cdot\frac{a_{B_2^+A_2^-}}{s} \tag{41}$$

$$\gamma_{B_2OHA_2^-} = \gamma^0_{B_2OHA_2^-}\cdot\frac{a_{B_2OHA_2^-}}{s} \tag{42}$$

$$\gamma_{B_2^+A_2H} = \gamma^0_{B_2^+A_2H}\cdot\frac{a_{B_2^+A_2H}}{s} \tag{43}$$

$$\gamma_{B_2OHA_2H} = \gamma^0_{B_2OHA_2H}\cdot\frac{a_{B_2OHA_2H}}{s}. \tag{44}$$

The Eqs. (28)–(44) form an equation system and the $a_{A_1^-}$, $a_{A_1H}$, $a_{B_1^+}$, $a_{B_1OH}$, $a_{B_2^+A_2^-}$, $a_{B_2^+A_2H}$, $a_{B_2OHA_2^-}$ and $a_{B_2OHA_2H}$ values will be eliminated.

We therefore have:

$$\gamma = -m_1a^2_{H^+}\gamma - m_2a_{H+}\gamma - m_3a^2_{OH^-}\gamma - m_4a_{OH^-}\gamma + m_5a^2_{H^+} + m_6a_{H^+} + m_7a^2_{OH^-} + m_8a_{OH^-} + b \tag{45}$$

where:

$$m_1 = \frac{K_1K_4}{M}$$

$$m_2 = \frac{K_1K_2K_4K_w + K_1 + K_1K_3K_5K_w + K_4}{M}$$

$$m_3 = \frac{K_2K_3}{M}$$

$$m_4 = \frac{K_1K_2K_3K_w + K_2 + K_2K_3K_5K_w + K_3}{M}$$

$$m_5 = \frac{K_1K_4\left(\gamma^0_{A_1H} + \gamma^0_{B_1^+} + \gamma^0_{B_2^+A_2H}\right)}{M}$$

$$m_6 = \frac{K_4\left(\gamma^0_{A_1^-} + \gamma^0_{B_1^+} + \gamma^0_{B_2^+A_2H}\right) + K_1\left(\gamma^0_{A_1H} + \gamma^0_{B_1^+} + \gamma^0_{B_2^+A_2^-}\right)}{M} + \frac{K_1K_2K_4K_w\left(\gamma^0_{A_1H} + \gamma^0_{B_1OH} + \gamma^0_{B_2^+A_2H}\right) + K_1K_3K_5K_w\left(\gamma^0_{A_1H} + \gamma^0_{B_1^+} + \gamma^0_{B_2OHA_2H}\right)}{M}$$

$$m_7 = \frac{K_2K_3\left(\gamma^0_{A_1^-} + \gamma^0_{B_1OH} + \gamma^0_{B_2OHA_2^-}\right)}{M}$$

$$m_8 = \frac{K_3\left(\gamma^0_{A_1^-} + \gamma^0_{B_1^+} + \gamma^0_{B_2OHA_2^-}\right) + K_2\left(\gamma^0_{A_1^-} + \gamma^0_{B_1OH} + \gamma^0_{B_2^+A_2^-}\right)}{M} + \frac{K_2K_3K_5K_w\left(\gamma^0_{A_1^-} + \gamma^0_{B_1OH} + \gamma^0_{B_2OHA_2^-}\right) + K_1K_2K_3K_w\left(\gamma^0_{A_1H} + \gamma^0_{B_1OH} + \gamma^0_{B_2OHA_2^-}\right)}{M} + \frac{K_1K_2K_3K_5K_w\gamma^0_{B_1OH}}{M}$$

$$b = \frac{K_1K_2K_3K_5K_w^2\left(\gamma^0_{A_1H} + \gamma^0_{B_2OHA_2H}\right) + K_1K_2K_w\left(\gamma^0_{B_1OH} + \gamma^0_{B_2^+A_2^-}\right) + K_1K_3K_w\left(\gamma^0_{A_1H} + \gamma^0_{B_1^+}\right)}{M} + \frac{K_3K_5K_w\left(\gamma^0_{A_1^-} + \gamma^0_{B_1^+} + \gamma^0_{B_2OHA_2H}\right) + K_2K_4K_w\left(\gamma^0_{A_1^-} + \gamma^0_{B_2^+A_2H} + \gamma^0_{B_1OH}\right) + \gamma^0_{A_1^-} + \gamma^0_{B_1^+} + \gamma^0_{B_2^+A_2^-}}{M}$$

$$M = 1 + K_1K_2K_w + K_1K_2K_3K_5K_w^2 + K_1K_3K_w + K_2K_4K_w + K_3K_5K_w.$$

Eq. (45) presents the dependence of the interfacial tension of the lipid membrane on pH of electrolyte solution.

where:

$\gamma$ [N m$^{-1}$] interfacial tension of the lipid membrane;

Kw ionic product of water;

$\gamma^0_{A_1^-}$, $\gamma^0_{A_1H}$, $\gamma^0_{B_1^+}$, $\gamma^0_{B_1OH}$, $\gamma^0_{B_2^+A_2^-}$, $\gamma^0_{B_2OHA_2^-}$, $\gamma^0_{B_2^+A_2H}$, $\gamma^0_{B_2OHA_2H}$ [N m$^{-1}$] specific interfacial tension of the membrane component, respectively.

For the calculations the Eq. (45) had a form:

$$\gamma = \frac{m_5a^2_{H^+} + m_6a_{H^+} + m_7a^2_{OH^-} + m_8a_{OH^-} + b}{1 - m_1a^2_{H^+} - m_2a_{H^+} - m_3a^2_{OH^-} - m_4a_{OH^-}} \quad (46)$$

### 2.3. Model III

In this model, the $H^+$ and $OH^-$ ions are adsorbed at the surface membrane formed from the phosphatidylcholine–phosphatidylserine system.

The adsorption equilibria are presented in the previous Section 2.1 with the aid of Eqs. (1)–(8). Acid–base equilibria can then be written as Eqs. (9)–(11) also presented in the previous Section 2.1. when surface concentration of the lipid was denoted by $s$.

The Gibbs equation assumes the form:

$$d\gamma = -\sum \Gamma_i d\overline{\mu}_i. \quad (47)$$

If the $H^+$ and $OH^-$ ions are adsorbed at the lipid surface then the Gibbs equation assumes the form:

$$d\gamma = -\Gamma_{A_1H}d\overline{\mu}_{H^+} - \Gamma_{A_2H}d\overline{\mu}_{H^+} - \Gamma_{B_1OH}d\overline{\mu}_{OH^-} - \Gamma_{B_2OH}d\overline{\mu}_{OH^-} \quad (48)$$

It was assumed in earlier work [5,6] that a surface excess of the $H^+$ and $OH^-$ ions is equal to their surface concentration. This assumption is common in describing adsorption phenomena [27,28] but it is correct in the case only where the adsorption is strong and the concentration of the adsorbed ion in the solution is low. In the present case, the surface excess definition resulting from deduction of the Gibbs equation [29] should be strictly respected.

The equations describing surface excess of the $H^+$ and $OH^-$ ions in terms of the definition resulting from the Gibbs equation can be presented in the form:

$$\Gamma_{OH^-} = a_{B_1OH} + a_{B_2OH} - V_{H^+}(a_{A_1H} + a_{A_2H})a_{OH^-} - V_{OH^-}(a_{B_1OH} + a_{B_2OH})a_{OH^-} \quad (49)$$

$$\Gamma_{H^+} = a_{A_1H} + a_{A_2H} - V_{H^+}(a_{A_1H} + a_{A_2H})a_{H^+} - V_{OH^-}(a_{B_1OH} + a_{B_2OH})a_{OH^+} \quad (50)$$

$V_{H^+}$ [m$^3$ mol$^{-1}$] – $H^+$ ion volume in the adsorption layer; $V_{OH^-}$ [m$^3$ mol$^{-1}$] – $OH^-$ ion volume in the adsorption layer.

Substitution of Eqs. (49) and (50) to the Gibbs equation yields:

$$\begin{aligned} d\gamma = {} & -RTa_{A_1H}\frac{da_{H^+}}{a_{H^+}} - RTa_{A_2H}\frac{da_{H^+}}{a_{H^+}} + RTV_{H^+}a_{A_1H}da_{H^+} \\ & + RTV_{H^+}a_{A_2H}da_{H^+} + RTV_{OH^-}a_{B_1OH}da_{H^+} \\ & + RTV_{OH^-}a_{B_2OH}da_{H^+} - RTa_{B_1OH}\frac{da_{OH^-}}{a_{OH^-}} - RTa_{B_2OH}\frac{da_{OH^-}}{a_{OH^-}} \\ & + RTV_{H^+}a_{A_1H}da_{OH^-} + RTV_{H^+}a_{A_2H}da_{OH^-} \\ & + RTV_{OH^-}a_{B^1OH}da_{OH^-} + RTV_{OH^-}a_{B_2OH}da_{OH^-}. \end{aligned} \quad (51)$$

Eqs. (5)–(12) are then used to determine $a_{A_1H}$, $a_{A_2H}$ and $a_{B_1OH}$, $a_{B_2OH}$ which are expressed by equations:

$$a_{A_1H} = \frac{K_{A_1}sa_{H^+}}{1 + K_{A_1}a_{H^+}} \quad (52)$$

$$a_{B_1OH} = \frac{K_{B1}sa_{OH^-}}{1 + K_{B_1}a_{OH^-}} \quad (53)$$

$$a_{A_2H} = \frac{K_{A_2}sa_{H^+}}{1 + K_{A_2}a_{H^+}} \quad (54)$$

$$a_{B_2OH} = \frac{K_{B_2}sa_{OH^-}}{1 + K_{B_2}a_{OH^-}} \quad (55)$$

Eqs. (52)–(55) are substituted for Eq. (51); $a_{A_1H}$, $a_{A_2H}$ and $a_{B_1OH}$, $a_{B_2OH}$ being hereby eliminated. The equation is then

integrated taking the conditions $\gamma=\gamma_{\max}$, $a_{\mathrm{H}}^{+}=a_{\mathrm{H}^+}^{\max}$, and $a_{\mathrm{OH}^-}=a_{\mathrm{OH}^-}^{\max}$ resulting in the equation:

$$\begin{aligned}\gamma = \gamma_{\max} &- sRT\left(1+\frac{V_{\mathrm{H}^+}}{K_{\mathrm{A}_1}}-V_{\mathrm{H}^+}K_{\mathrm{w}}K_{\mathrm{A}_1}\right)\ln\frac{1+K_{\mathrm{A}_1}a_{\mathrm{H}^+}}{1+K_{\mathrm{A}_1}a_{\mathrm{H}^+}^{\max}}\\ &- sRT\left(1+\frac{V_{\mathrm{H}^+}}{K_{\mathrm{A}_2}}-V_{\mathrm{H}^+}K_{\mathrm{w}}K_{\mathrm{A}_2}\right)\ln\frac{1+K_{\mathrm{A}_2}a_{\mathrm{H}^+}}{1+K_{\mathrm{A}_2}a_{\mathrm{H}^+}^{\max}}\\ &- sRT\left(1+\frac{V_{\mathrm{OH}^-}}{K_{\mathrm{B}_1}}-V_{\mathrm{OH}^-}K_{\mathrm{w}}K_{\mathrm{B}_1}\right)\ln\frac{1+K_{\mathrm{B}_1}a_{\mathrm{OH}^-}}{1+K_{\mathrm{B}_1}a_{\mathrm{OH}^-}^{\max}}\\ &- sRT\left(1+\frac{V_{\mathrm{OH}^-}}{K_{\mathrm{B}_2}}-V_{\mathrm{OH}^-}K_{\mathrm{w}}K_{\mathrm{B}_2}\right)\ln\frac{1+K_{\mathrm{B}_2}a_{\mathrm{OH}^-}}{1+K_{\mathrm{B}_2}a_{\mathrm{OH}^-}^{\max}}\\ &- sRTV_{\mathrm{H}^+}K_{\mathrm{w}}K_{\mathrm{A}_1}\ln\frac{a_{\mathrm{H}^+}}{a_{\mathrm{H}^+}^{\max}}-sRTV_{\mathrm{H}^+}K_{\mathrm{w}}K_{\mathrm{A}_2}\ln\frac{a_{\mathrm{H}^+}}{\mathrm{A}_{\mathrm{H}^+}^{\max}}\\ &- sRTV_{\mathrm{OH}^-}K_{\mathrm{w}}K_{\mathrm{B}_1}\ln\frac{a_{\mathrm{OH}^-}}{a_{\mathrm{OH}^-}^{\max}}-sRTV_{\mathrm{OH}^-}K_{\mathrm{w}}K_{\mathrm{B}_2}\ln\frac{a_{\mathrm{OH}^-}}{a_{\mathrm{OH}^-}^{\max}}\\ &+ sRTV_{\mathrm{H}^+}\left(a_{\mathrm{H}^+}-a_{\mathrm{H}^+}^{\max}\right)+sRTV_{\mathrm{OH}^-}\left(a_{\mathrm{OH}^-}-a_{\mathrm{OH}^-}^{\max}\right).\end{aligned} \tag{56}$$

## 3. Experimental

### 3.1. 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 and Laplace's equation [30].

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

### 3.2. Measurements

The apparatus and the measurement method were described in previous papers [5,6]. The lipid membranes were formed by the Mueller–Rudin method [31]. They were formed in a Teflon diaphragm of 1.5 mm outer diameter containing an orifice along its axis. Some electrolyte solution was present on both sides of the orifice.

The convexity of the lipid membrane cap was measured with a 0.05 mm precision instrument reading. This value together with the Teflon element diameter corresponding to the lipid cap diameter yielded the radius of the curvature.

The interfacial tension was measured on a freshly created lipid bilayer membrane 12–15 times for each pH electrolyte solution. For each membrane about 10 instrument readings were taken of the lipid spherical cap diameter, formed by a pressure difference applied on both sides. These measurements were made within 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 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 PS and PC mixture (1:1) in an *n*-decane:butanol mixture (3:1). The PC and PS were dissolved in chloroform to prevent oxidation; the solvent was evaporated in an atmosphere of argon and the residue was dissolved in *n*-decane:butanol mixture. The pH electrolyte was carefully controlled during the measurements.

### 3.3. Materials

Egg PC (99%) from Fluka (Neu-Ulm, Germany) 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%.

3-*sn*-phosphatidyl-L-serine from bovine brain (99%) from Fluka (Neu-Ulm, Germany) was used in the experiment.

Buffers of 2–12 pH ranges were prepared according to Britton and Robinson [32] 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%), 0.04 M phosphoric acid and 0.04 M boric acid from POCh (Gliwice, Poland). A suitable pH of the buffer was established depending on the amount of added sodium hydroxide.

## 4. Results and discussion

Effect of pH on interfacial tension of the lipid bilayer formed from a phosphatidylcholine–phosphatidylserine system has been studied. The measurements were carried out at room temperature in the whole pH range. The dependence of interfacial tension a membrane formed from PC–PS system on pH of electrolyte solution is presented in Fig. 2. The maximal interfacial tension value is 2.98 mN $m^{-1}$ at a pH equal to 4.12. The maximal interfacial tension values for pure phosphatidylcholine and phosphatidylserine were determined earlier and these values are equal 3.53 mN $m^{-1}$ at pH equal to 4.12 [6] and 2.94 mN $m^{-1}$ at pH equal to 3.80 [14], respectively.

Interfacial tension of the membrane formed from phosphatidylcholine–phosphatidylserine is plotted in Fig. 2 vs. pH of the electrolyte solution. In this figure the experimental values and the theoretical values are marked as points, calculated from Eq. (22), and are presented by continuous line. Fig. 2 refers to the above presented Model I. As it is seen in Eq. (22), the total interfacial tension value of the PC–PS membrane is a sum of interfacial tension values of it components, i.e. $A_1^-$, $A_1H$, $B_1^+$, $B_1OH$, $A_2^-$, $A_2H$, $B_2^+$ and $B_2OH$.

The acid–base equilibrium constants for PC membrane are $K_{\mathrm{A}_1}=10^{2.581}$ and $K_{\mathrm{B}_1}=10^{5.687}$ determined in the work [6] was assigned to the $-PO^{(-)}$ and $-N^{(+)}(CH_3)_3$ groups. The acid–base equilibrium constants for a PS membrane were presented in an earlier article [14], thus: $K_{\mathrm{A}_1}=10^{2.581}$, $K_{\mathrm{A}_2}=10^{4.139}$ and $K_{\mathrm{B}_2}=10^{9.55}$ for $-PO^{(-)}$, $-COO^{(-)}$ and $-N^{(+)}H_3$ groups, respectively.

Introducing the $K_{\mathrm{A}_1}$, $K_{\mathrm{A}_2}$, $K_{\mathrm{B}_1}$, $K_{\mathrm{B}_2}$ values obtained in this way to Eq. (22) yielded specific interfacial tension values of the

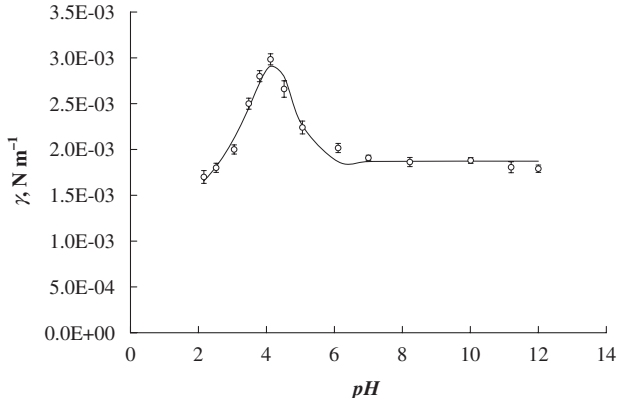


Fig. 2. The dependence of the interfacial tension $\gamma$ of a bilayer lipid membrane formed from PC–PS system on the pH of the electrolyte solution, calculated from Model I (the experimental values are marked by points and the theoretical ones by a curve).

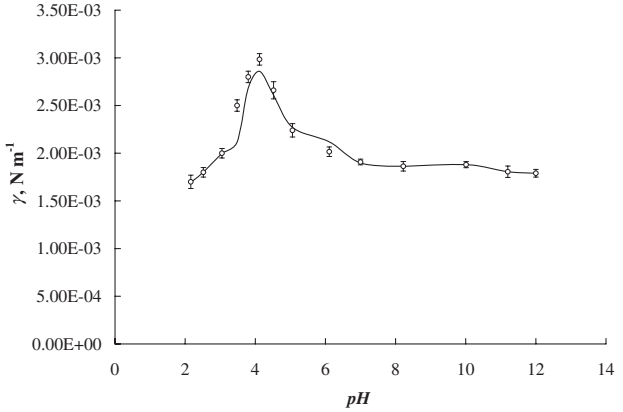


Fig. 4. The dependence of the interfacial tension $\gamma$ of a bilayer lipid membrane formed from a PC–PS system on the pH of the electrolyte solution, calculated from Model II, the experimental values are marked by points and the theoretical ones by a line.

individual forms of the PC–PS membrane. The results were obtained using the linear regression method of the Excel 2003 program. The $\gamma^0_{A_1^-}$, $\gamma^0_{A_1H}$, $\gamma^0_{B_1^+}$, $\gamma^0_{B_1OH}$, $\gamma^0_{A_2^-}$, $\gamma^0_{A_2H}$, $\gamma^0_{B_2^+}$, $\gamma^0_{B_2OH}$ values determined in this way were $2.84\times10^{-3}$, $1.51\times10^{-3}$, $-4.20\times10^{-4}$, $-3.36\times10^{-3}$, $-5.70\times10^{-4}$, $-6.61\times10^{-4}$, $3.78\times10^{-3}$ and $5.63\times10^{-5}$ $Nm^{-1}$, respectively. When the interfacial tension of bilayer lipid membranes formed from individuals only has negative values, it is possible to suppose, then the bilayer membrane formed from this form does not exist.

Coverage of the lipid membrane surface by the $H^+$ and $OH^-$ ions vs. pH of the electrolyte solution is presented in Fig. 3. As can be seen, the membrane surface is not covered by the $H^+$ and $OH^-$ ions in proximity of its isoelectric point, i.e. at a pH equal to about 4.

Interfacial tension of the membrane formed from phosphatidylcholine– phosphatidylserine is also plotted in Fig. 4 vs. pH of electrolyte solution; the experimental values are presented by points and the theoretical ones calculated from Eq. (45), which has been derived according to Model II, and may be represented by a continuous line. It is seen in Eq. (45) that the total interfacial tension values of PC–PS membrane is the sum of its individual components, i.e. $A_1^-$, $A_1H$, $B_1^+$, $B_1OH$, $B_2^+A_2^-$, $B_2OHA_2^-$, $B_2^+A_2H$, $B_2OHA_2H$. However, determination of interfacial tension of the individual components was difficult because the association constant values $K_3$, $K_4$, $K_5$, were unknown (the association constant $K_1=K_{A_1}$ and $K_2=K_{B_1}$). For this reason, the individual equation coefficients, $m_1$, $m_2$, $m_3$, $m_4$, $m_5$, $m_6$, $m_7$, $m_8$ and $b$ were obtained using the linear regression method of the Excel 2003 program.

As it is seen in Fig. 2, Model I yields a much better agreement of calculated and experimental data in all the pH range. It indicates that it is closer to reality.

In paper [14] models were presented which describe the surface of the membrane built from pure phosphatidylcholine and pure phosphatidylserine. For a membrane built from pure lecithin, a model which was closer to reality would suppose an equilibria between functional groups $-PO^{(-)}$ and $-N^{(+)}(CH_3)_3$ distributed on its surface and $H^+$ and $OH^-$ (experimental points are in best agreement with theoretical ones calculated in an earlier study [14]). While, for the phosphatidylserine membrane the best was the model which supposes equilibria between functional groups ($-PO^{(-)}$, $-COO^{(-)}$ and $-N^{(+)}H_3$) distributed on its surface and $H^+$ and $OH^-$ (experimental points are in best agreement with theoretical

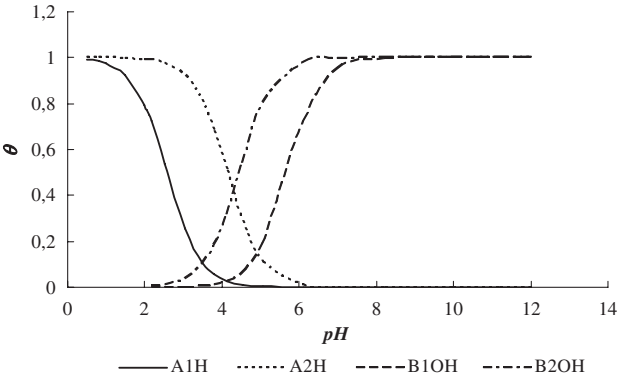


Fig. 3. The calculated from the Model I, $\theta$ in associated forms $A_1H$, $B_1OH$, $A_2H$ and $B_2OH$ groups in the interfacial tension of the bilayer formed from a PC–PS mixture, as a function of pH of the electrolyte solution.

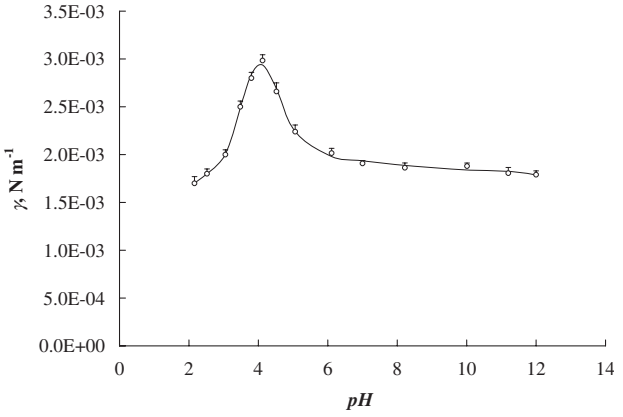


Fig. 5. The dependence of the interfacial tension $\gamma$ of a bilayer lipid membrane formed from PC–PS on the pH of the electrolyte solution, calculated from Model III (the experimental values are marked by points and the theoretical ones by a line).

ones calculated in an earlier study [14]). Therefore a description of PC–PS system was made using the model described above and presented in an earlier study [14].

Another structural model (Model III) of PC–PS bilayer surface was considered. In Model III in the bilayer surface to explain acid–base equilibria between the functional groups of PC–PS molecule and the $H^+$ and $OH^-$ ions in solution was described in terms of the Gibbs isotherm. As is observed in Fig 5, the experimental data agree with the calculation results. Eq. (56), used theoretically, describes the experimental results in the whole pH range.

## 5. Conclusion

The interactions between membrane lipids are nowadays intensively developed. This results from intensely studied areas for the understanding of phenomena occurring in cellular membranes. However, there is still a lack of a quantitative description of the systems involved. Thus a better understanding is required for the processes that take place in biological membranes with the aim of forming the artificial membrane that would very closely resemble the properties of the natural membrane. Therefore, the knowledge of molecular structure and organisation of phospholipids is necessary. Data presented in this work, received from mathematical derivation and confirmed experimentally are of great importance for the interpretation of phenomena occurring in lipid bilayers. These results can help in a better understanding of biological membranes and in their biophysical studies. Simple and very interesting methods proposed in earlier studies [6,14,16,18,19] and in this paper can be used with success for the determination of the equilibrium constant value of any 1 : 1 lipid–other lipid system and for acid–base equilibria between any phospholipids and ions from electrolyte solution ($H^+$ and $OH^-$).

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