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Interfacial tension of bilayer lipid membranes

Review Article

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Abstract: Interfacial tension is an important characteristic of a biological membrane because it determines its rigidity, thus affecting its stability. It is affected by factors such as medium pH and by the presence of certain substances, for example cholesterol, other lipids, fatty acids, amines, amino acids, or proteins, incorporated in the lipid bilayer. Here, the effects of various parameters to on interfacial tension values of bilayer lipid membranes are discussed.

The mathematically derived and experimentally confirmed results presented in this paper are of importance to the interpretation of phenomena occurring in lipid bilayers. These results can lead to a better understanding of the physical properties of biological membranes. The simple interfacial tension method proposed herein may be successfully used to determine the interfacial tension values of 1:1 lipid-lipid, lipid-cholesterol, lipid-fatty acid, lipid-amine, and lipid-amino acid systems.

Keywords: *Interfacial tension • Bilayer lipid membrane • pH effect • 1:1 complex • Adsorption equilibria*

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. Cell membranes are composed mostly of lipids and proteins, and they are very complex systems. For this reason, physicochemical experiments are usually done with simple membrane models, *e.g.* with artificial phospholipid membranes. Numerous functions of biological membranes have been reproduced and explained using these models. Many studies have demonstrated that the properties of the artificially derived lipid membranes are very similar to those of natural cell membranes [1-3].

Natural cell membranes have been studied by numerous techniques, including physicochemical ones. 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 the artificial phospholipid bilayer model membranes [3].

Various parameters of lipid bilayers were determined, *i.e.*, thickness, potential difference, electric capacitance, resistance, and others. An important characteristic of a biological membrane is its interfacial tension, which determines its rigidity and as a result affects its stability [4-5].

The interfacial tension of lipid bilayers was determined by measuring the energy of formation of the membrane [6], based on the dependence of the pressure difference on both sides of the lipid membrane and on its curvature (Young and Laplace's equation) [7]. To measure the pressure difference a glass rod with a micromanipulator was immersed in a solution placed in the chamber of the measuring vessel. Determination of the radius of curvature of a black membrane was based on the membrane surface area, its capacitance, and membrane curvature measurements. Depending on the electrolyte composition, the interfacial tension value for the phosphatidylcholine bilayer is 3.4±0.6 mN m^{-1} [6]. The membrane radius was assumed to be almost equal to the orifice radius in which it was formed. The energy of formation of the membrane was calculated from the radius of curvature and the pressure difference on both sides using suitable mathematical expressions [6]. For a typical BLM (bilayer lipid membrane) created in an orifice of 0.2 cm radius, the interfacial tension is equal to 2 mN m^{-1} (the hydrostatic pressure is equal about 0.04 cm H_2O) [2,4].

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The interfacial tension method presented in this study was previously described by Petelska and co-workers; the interfacial tension of a lipid bilayer sample was determined by measuring the radius of curvature of the convex surface formed when a pressure difference was applied across the bilayer. The measurement vessel consisted of two glass chambers separated by a mount holding a 1.5 mm diameter circular Teflon element axially pierced by a small orifice. Spherical membranes were formed by the Mueller-Rudin method [8] on the flat end of the Teflon element. Both chambers were filled with an electrolyte solution. The membrane-forming solution was introduced to the flat wall of the Teflon element using a micropipette, and pressure was applied to the left chamber using a manometer (VEB) [9-11].

The convexity of the spherical cap was measured using 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 diameter of the Teflon element diameter corresponding to the lipid spherical cap diameter, yielded the radius of curvature. The measurement of the spherical cap was difficult to do because the spherical cap was hardly visible, although using yellow light enabled for greater visibility [9-11].

The radius of curvature was determined using this value and the diameter of the Teflon element, corresponding to the diameter of the lipid cap and the convexity of the lipid membrane. The radius of curvature of the bilayer lipid membrane and the overpressure provoking the membrane convexity were measured. Then, the interfacial tension values were calculated from the radius of curvature and the pressure difference values according to Young and Laplace's equation. This method, although very difficult due to the small pressure difference on both sides of the lipid membrane, was precise [4,9-11].

The interfacial tension of lipid bilayers has been determined using computer simulation methods and theoretical discussion [12-15]. The interfacial tension value for bilayers, however, is controversial. Analogy to a shielded oil/water interface suggests a value around 20 mN m^{-1} [16], although it is easy to argue that interfacial tensions should be closer to fatty acid/water values of 5-10 mN m^{-1}. Estimates using monolayer data must proceed with numerous assumptions [17-18]: the monolayer tension is divided into independent headgroup/water and chain/air parts, with the latter approximated by alkane/air values and the former associated with the bilayer at the same surface area. McDonald and Simon [19] derived an interfacial tension near zero for dimyristoyl phosphatidylcholine bilayers using this method. In contrast, Feller and Pastor [15] obtained interfacial tension values of 15 mN m^{-1} (per interface) for dipalmitoyl phosphatidylcholine (DPPC) bilayers at 48°C based on 40 mN m^{-1} at 68 A^2/lipid for the monolayer [20] and 25 mN m^{-1} for hexadecane [21]. Experimental measurements, done on black lipid membranes, typically yield surface tensions in the range of 1-5 mN m^{-1} [9,11,22-34].

Interfacial tension is affected by such factors as medium pH and the presence of certain substances, such as cholesterol, other lipids, fatty acids, amines, amino acids, or proteins, incorporated in the lipid bilayer [9-11,23-34]. In this study, the effects of various parameters on interfacial tension values of bilayer lipid membrane are discussed.

2. Interfacial tension of lipid membrane

2.1. Interfacial tension of pure component

An important property of a bilayer lipid membrane is its interfacial tension. The interfacial tension method described in this paper is based on Young and Laplace's equation [7]. The tension in a lipid bilayer sample was determined by measuring the radius of curvature of the convex surface formed when a pressure difference was applied across the bilayer. The apparatus and measurement method were described in previous papers [9-10].

Table 1 presents the interfacial tension values of pure membrane formed from various components. The interfacial tension value for phosphatidylcholine (PC) is equal to 1.62 mN m^{-1} [9], in good agreement with previously reported values [6]. A large value of interfacial tension is characteristic for cholesterol (Ch) [9]. This molecule has a different structure and occupies more places in the hydrophobic layer of the membrane than in the polar group areas, unlike phospholipid molecules. Membranes formed from pure cholesterol are more stable and more reproducible than membranes created from pure phospholipids.

There is no accurate literature data on the interfacial tension values of pure fatty acids, amines, and amino acids because these components do not form bilayer membranes. Therefore, the hypothetical interfacial tension values for membranes built from fatty acids, amines, or amino acid were determined by adjusting the experimental curve with the polynomial of the other mark extrapolating the $x_2 = 1$ value (Fig. 1) [33]. A small or a

Table 1. Interfacial tension values for single-component bilayer lipid membranes.

Examined component	Interfacial tension [N m^{-1}]
PC	1.62×10^{-3} [9]
PE	3.34×10^{-3} [16]
Ch	4.72×10^{-3} [9]
SM	1.72×10^{-3} [24]
Cer	1.29×10^{-3} [24]
SA	-1.54×10^{-3} [17]
ST	4.40×10^{-3} [17]
DA	-4.20×10^{-3} [26]
DE	-7.50×10^{-3} [26]
Ile	-2.70×10^{-3} [27]
Phe	5.30×10^{-3} [27]
Tyr	-3.50×10^{-3} [27]
Val	7.00×10^{-4} [27]

where: PC-phosphatidylcholine, PE-phosphatidylethanolamine, Ch-cholesterol, SM-sphingomyelin, Cer-ceramide, SA-stearic acid, ST-stearylamine, DA-decanoic acid, DE-decylamine, Ile-isoleucine, Phe-phenylalanine, Tyr-tyrosine, Val – valine.

negative value of interfacial tension for the membrane built from pure fatty acids, amines, or amino acids points to the fact that it is not possible to create the bilayer membrane from SA, ST, DA, DE, Ile, Phe, Tyr, or Val. The thermodynamic potential for this bilayer would have a negative value, *i.e.* the bilayer does not form.

2.2. Two-component complex interfacial tension

2.2.1. Lipid-cholesterol and lipid-lipid complexes

Lipid-cholesterol interactions have been widely studied due to their importance in biological membranes [35-36]. Cholesterol is an important factor in controlling the physical properties of cell membranes and their functions [37-38]. The biological roles of cholesterol involve maintaining proper fluidity [39], reducing passive permeability [40], and increasing the mechanical strength [41] of the membrane. Because of these important roles, lipid-cholesterol interactions in the membrane have been studied extensively [42], revealing that cholesterol increases both the order of the hydrocarbon chains (an ordering effect) [43-44] and the surface density of the membrane (condensing effect) [45-46]. Ordering and condensing effects of cholesterol have been observed both in a model and in biological membranes [47-49]. There have also been a number of proposals suggesting that the lipid-cholesterol complexes exist in different stoichiometric ratios. However, there is no agreement concerning the stoichiometries of the formed complexes. The 1:1, 1:2, 2:1, 1:3, and 3:1 complexes are claimed to be present mostly in monolayers or bilayers [50-51]. On the other hand, subsequent investigators have suggested that specific associates [52], phase separation [53], domains [54], or a lattice-based structure [55-56] could be formed in the lipid-cholesterol membranes.

The interfacial tension values of a membrane formed from 1:1 lipid-cholesterol complexes are presented in Table 2. The interfacial tension values for PC-Ch, PE-Ch, and Cer-Ch complexes are greater than or equal to 2 mN m^{-1} [23,31]. Only the SM-Ch complex had an interfacial tension value that was two times greater.

The dependence of interfacial tension of the lipid membrane on composition was studied throughout the range of concentrations. For example, the dependence of the interfacial tension of the PC-Ch membrane on the molar fraction of cholesterol is presented in Fig. 2 [23].

Membranes may be assembled from two components capable of forming a complex. The stoichiometry of the complex may vary, but because the first stability constant of these complexes is usually the largest [57-58], we assumed that the complexes have primarily a 1:1 stoichiometry.

The equilibrium between the individual components and the complex is represented by:

$$\underset{\text{(Component 1)}}{A} + \underset{\text{(Component 2)}}{B} \Leftrightarrow \underset{\text{(Complex)}}{AB}$$

In cases where the membrane components form a 1:1 complex, interactions in the membrane may be described by a previously published set of equations [11,23].

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

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

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

$$x_1 + x_2 = 1$$

where: A_1, A_2, A_3 [m^2] - area occupied by compound 1, 2 and the complex, respectively; a_1, a_2, a_3 [mol m^{-2}] - surface concentration of 1, 2 and the complex, respectively; $\gamma_1, \gamma_2, \gamma_3$ [N m^{-1}] - interfacial tension of the membrane built of component 1, 2 and the complex, respectively; γ [N m^{-1}] – measured interfacial tension of the membrane; x_1, x_2 - molar fraction of component 1 and 2 in the solution forming membrane, respectively; K - stability constant of a 1:1 complex.

Elimination of a_1, a_2, a_3 yields the basic equation describing the interaction between components 1 and 2 and can be written as [11,23]:

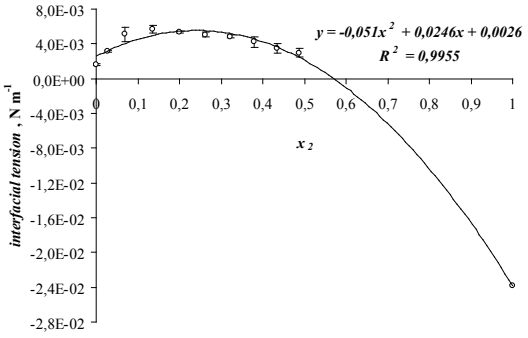

Figure 1. The interfacial tension γ of the phosphatidylcholine-decanoic acid membrane as a molar fraction of decanoic acid x_2 [33].

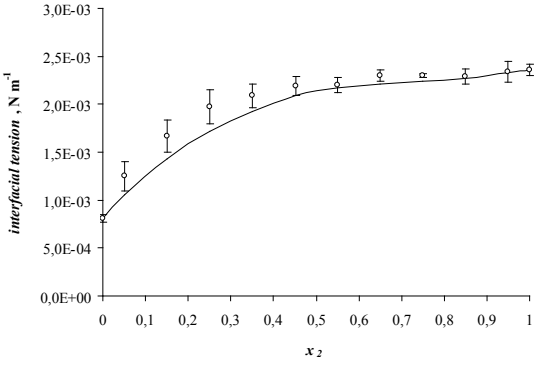

Figure 2. The interfacial tension, γ, of the phosphatidylcholine-cholesterol membrane as a molar fraction of cholesterol, x_2 (the experimental values are marked by points and the theoretical ones by curve) [23].

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

where: $B_1=\frac{A_3}{A_1}$ and $B_2=\frac{A_3}{A_2}$.

The resulting dependence deviates from linearity, indicating that some bonds are formed in the membrane. The experimental values in Fig. 2 are marked by points and the theoretical ones obtained from Eq. 1 by lines.

For systems containing two lipid components, formation of a 1:1 complex was assumed to be the explanation for deviation from the additivity rule. Model curves were constructed using calculated parameters such as equilibrium constants, molecular areas of the complexes, and interfacial tension of molecules and complexes. The accuracy of the models was verified by comparison to the experimental results.

Addition of cholesterol to the membrane constructed from phospholipids resulted in increased stability and reproducibility of the membranes. Cholesterol condenses some membrane components (the so-called condensation effect), making the membrane structures more rigid [3]. It also improves the packing of membrane lipids as they occupy more places in the hydrophobic layer of the membrane and fewer places in the polar groups range, unlike the phospholipid molecules.

The interfacial tension values for the 1:1 lipid-lipid complexes are presented in Table 3. The interfacial tension values for PC-PE and SM-Cer complexes were approximately equal to 1.60 mN m^{-1} [23,32].

2.2.2. Lipid-fatty acid, lipid-amine and lipid-amino acid complexes

The physicochemical studies of the phospholipids-fatty acid mixture may have significance other than the interest in relation to the membrane function alteration caused by fatty acids. Phospholipids, the major building blocks of most biomembranes, contain two fatty acids, which are esterified to glycerol. The interaction between different acyl chains within a phospholipid molecule or among different phospholipid molecules in the bilayer should determine the physical properties of biomembranes. Studying the phase behavior of the hydrated bilayer, composed of a phospholipid-fatty acid mixture, would be useful in understanding the acyl-acyl interactions which play such an important role in phospholipid bilayers [59].

Bilayer membranes can be very useful models in studying the organization of biological membranes and the interactions between fatty acids or amino acids and lipids. The study of bilayer lipid membranes is a fascinating branch of physical chemistry, with considerable implications in other fields, including chemistry, physics, material science, and biology. The examination of bilayer lipid membranes is of crucial importance to a great number of processes, including cell membrane modeling. Bilayer systems are often characterized by their interfacial tension values, which provide useful information about molecular-level interactions between the components.

The interfacial tension values of a membrane formed from 1:1 phosphatidylcholine-fatty acid and phosphatidylcholine-amine are presented in Table 4. The interfacial tension values for PC-fatty acid complexes were approximately 7.20 mN m^{-1}. The interfacial tension values determined for PC-amine complexes are smaller than interfacial tension values for PC- fatty acid complexes.

Amino acids are ubiquitously found in all living cells [60-61]. Although they are usually present in cells in only low concentrations, amino acids are interesting model substances being used to examine the interactions between amino acids and bilayer lipid membranes.

Table 2. Interfacial tension values for lipid-cholesterol complex (1:1) in bilayer lipid membranes.

Examined complex	Interfacial tension [N m^{-1}]
PC-Ch	2.17×10^{-3} [16]
PE-Ch	2.30×10^{-3} [16]
SM-Ch	4.48×10^{-3} [24]
Cer-Ch	2.33×10^{-3} [24]

where: PC-phosphatidylcholine, PE-phosphatidylethanolamine, Ch-cholesterol, SM-sphingomyelin, Cer-ceramide.

Table 3. Interfacial tension values for lipid-lipid complex (1:1) in bilayer lipid membranes.

Examined complex	Interfacial tension [N m^{-1}]
PC-PE	1.58×10^{-3} [16]
SM-Cer	1.62×10^{-3} [24]

where: PC-phosphatidylcholine, PE-phosphatidylethanolamine, SM-sphingomyelin, Cer-ceramide.

These interactions have been examined in numerous experimental studies [62-64]. MacCallum *et al*. [65-66] have calculated the distribution of small molecules mimicking 17 natural amino acids in a lipid bilayer in atomistic detail by molecular dynamics simulations. The results provide detailed insight into the molecular basis of the preferred location and orientation of each side chain as well as the preferred charge of ionizable residues.

The interfacial tension values of a membrane formed from the 1:1 phosphatidylcholine-amino acid complexes are presented in Table 5. The interfacial tension values for PC-Val, PC-Ile, and PC-Tyr complexes were greater than or equal to 2 mN m^{-1}, while those determined for the PC-Phe complex were larger [34].

In order to understand complex biological systems, it is valuable to analyze simple model systems first. It would be useful, for example, to be able to determine the influence of individual amino acids on the interactions of peptides with a cell membrane. Jacobs and White used a variety of techniques to examine the thermodynamics and binding of a general class of tripeptides to small phosphatidylcholine vesicles [67]. These experiments revealed that peptides induce alterations of the lipid order and modulate the lipid acyl chain motion. Later work related these thermodynamic parameters to the structural information from the neutron diffraction experiments [68]. These experiments were used to determine the general location of the peptide in the bilayer; for example, whether the peptide was inserted into the hydrocarbon region or was confined to the water-hydrocarbon interface. Specific information related to the structure of the lipid-peptide complex would be extremely useful in further understanding of the role of individual amino acids in peptide-lipid interactions.

The importance of the interactions between proteins and biological membranes is well known. Petelska *et al.* determined the interactions between the main structural elements of proteins and membranes, amino acid molecules and phospholipid bilayers, by using the interfacial tension method. By using this method, a remarkable progress has been made in understanding of lipid bilayer behavior influencing the peptide-lipid interactions. The effects of membrane composition on interfacial tension have been described in lecithin-other lipid [11], lecithin-fatty acid, lecithin-amine [24,33], and lecithin-amino acid (valine, isoleucine, tyrosine, and phenylalanine) systems [34]. The results showed that fatty acids interact more strongly with lecithin than do amines or amino acids [24,33,34].

The interfacial tension method has been used to characterize the physicochemical properties of lipid bilayers and provide a quantitative description of equilibria in a two-component membrane. Based on mathematical-modeling results, the existence of a very stable 1:1 complex between phosphatidylcholine and either fatty acid, amine, or amino acid is very likely.

2.3. Effect of pH of electrolyte solution

The effect of pH of an electrolyte solution on monolayer and bilayer lipid membranes built from different lipids was determined. The dependence of interfacial tension on the effect of pH of the electrolyte solution for phosphatidylcholine [10,25,26], phosphatidylserine [26,28], phosphatidylethanolamine [27] and sphingomyelin [32] was described by Petelska and Figaszewski. The curves obtained by Petelska and Figaszewski (Fig. 3) demonstrate that the maximal interfacial tension values occur at the isoelectric point [10]. Trends of these curves were well characterized by the simplified description based on the Gibbs isotherm, but only in the proximity of the isoelectric point [10]. Using precisely the definition of surface excess in the Gibbs equation allows one to explain the run of experimental curves throughout the whole pH range [25-28,32]. In earlier studies, Petelska and Figaszewski [10,25-28,32] proposed the models developed to describe adsorption of H^+ and OH^- ions on the phospholipid surfaces. These models would more correctly reproduce changes in interfacial tension, particularly in the ranges far from the isoelectric point. The models assume that the contribution of the individual lipid molecules to form interfacial tension of the bilayer is additive. Based on the studies described above it is very difficult to determine in which direction the further studies should go, but they

should focuse on the phospholipids phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and sphingomielin because they are the major lipids found in biological membranes.

The dependence of interfacial tension of lipid membranes on the pH solution can be described in terms of the acid-base equilibria, presented below [27]:

$$-PO^{(-)} + H^{(+)} \Leftrightarrow -PO^{(-)} - H^{(+)} \quad (2)$$

(A⁻) **(AH)**

$$-\overset{(+)}{N}H_3 + OH^{(-)} \Leftrightarrow -\overset{(+)}{N}H_3 - OH^{(-)} \quad (3)$$

(B⁺) **(BOH)**

$$\gamma = \gamma^0_{A^-}\left(\frac{1}{1+K_A a_{H^+}}\right) + \gamma^0_{AH}\left(\frac{K_A a_{H^+}}{1+K_A a_{H^+}}\right) + \\ + \gamma^0_{B^+}\left(\frac{1}{1+K_B a_{OH^-}}\right) + \gamma^0_{BOH}\left(\frac{K_B a_{OH^-}}{1+K_B a_{OH^-}}\right) \quad (4)$$

where: K_A, K_B - acid and base equilibrium constant, respectively; γ [N m^{-1}] – measured interfacial tension of the lipid membrane; $\gamma^0_{A^-}, \gamma^0_{AH}, \gamma^0_{B^+}, \gamma^0_{BOH}$ [N m^{-1}] - interfacial tension of the membrane components, respectively; a_{H^+}, a_{OH^-} - H^+ and OH^- ions activities, respectively.

The theoretical dependence of the interfacial tension of the lipid membrane on the electrolyte solution pH was derived from Eq. 4 and is shown by lines in Fig. 3. The experimental values are marked by points and the theoretical ones, obtained from Eq. 4, are marked by lines.

Table 6 presents the association constant values, bond energies (calculated from association constant values), interfacial tension values at the isoelectric point, and isoelectric point values for bilayer lipid membranes formed from PC, PS, PE, and SM.

The acid-base equilibria and isoelectric points of the lipid membranes can be determined by titration. However, it was difficult to determine their magnitudes because the phospholipid was insoluble in water. For this reason, the required values were determined using liposomes. It was assumed in the calculations that only the lipid molecules present in the outer layer of the liposome took part in the acid-base equilibria. Thus, the lipid concentrations used in the equations were half of those introduced into the solution. The dissociation constants of the membrane formed from the lipid were determined by titration of the previously obtained liposomes with hydrochloric acid and with sodium hydroxide. Having determined the dissociation constants of the membrane, the isoelectric point could be calculated from the equations presented in the paper [10]. The acid-base equilibria and isoelectric point values for the bilayer lipid membrane formed from PC, PE, PS, and SM were all obtained this way (Table 6). The acid equilibrium values for PC, PE, and SM were all similar ($K_a = 10^{2.58}$).

Table 4. Interfacial tension values for lipid-fatty acid and lipid-amine complexes (1:1) in bilayer lipid membranes.

Examined complex	Interfacial tension [N m^{-1}]
PC-SA	7.16×10^{-3} [17]
PC-ST	6.04×10^{-3} [17]
PC-DA	7.25×10^{-3} [26]
PC-DE	3.63×10^{-3} [26]

where: PC-phosphatidylcholine, SA-stearic acid, ST-stearylamine, DA-decanoic acid, DE-decylamine.

Table 5. Interfacial tension values for lipid-amino acid complex (1:1) in bilayer lipid membranes.

Examined complex	Interfacial tension [N m^{-1}]
PC-Val	2.04×10^{-3} [27]
PC-Ile	1.91×10^{-3} [27]
PC- Tyr	1.75×10^{-3} [27]
PC- Phe	3.69×10^{-3} [27]

where: PC-phosphatidylcholine, Ile-isoleucine, Phe-phenylalanine, Tyr-tyrosine, Val – valine.

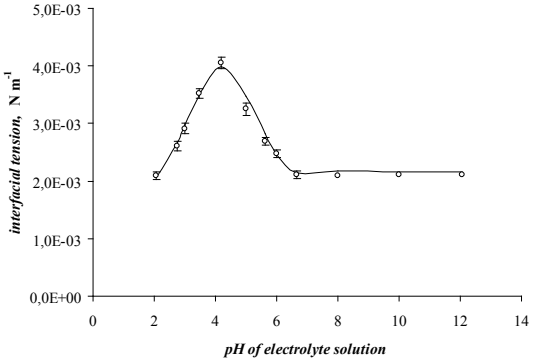

Figure 3. The dependence of the interfacial tension of a lipid membrane made from PE on the pH of the electrolyte solution the experimental values are marked by points and the theoretical ones by curve) [27].

The effect of pH on the interfacial tension of the bilayer lipid membrane formed from PC, PS, and PE was used to determine the effect of the hydrophilic head of lipids on interfacial tension values of the bilayer lipid membranes. The hydrophilic heads have been demonstrated to affect the interfacial tension values and, consequently, cell membrane properties. Unlike other phospholipids, *e.g.* lecithin or PS, the PE molecule is characterized by a small hydrophilic head. A previously found trend indicates that a larger hydrophilic head of a lipid brings about lower interfacial tension values.

Table 6. Physicochemical parameters of single-component bilayer lipid membrane (effect of pH).

Examined component	Association constants pK_a	pK_b	Energy of bond [kJ mol^{-1}]		Interfacial tension in isoelectric point [N m^{-1}]	Isoelectric point (pI)
PC [10]	2.58	5.69	-14.61	-32.19	3.53	4.12
PE [20]	2.42	5.98	-13.70	-33.85	4.06	4.18
PS [19]	2.58 4.14	9.55	-14.61 -23.43	-54.05	2.94	3.80
SM [25]	2.59	5.31	-14.66	-30.04	4.42	4.01

where: PC-phosphatidylcholine, PS-phosphatidylserine, PE-phosphatidylethanolamine, SM-sphingomyelin.

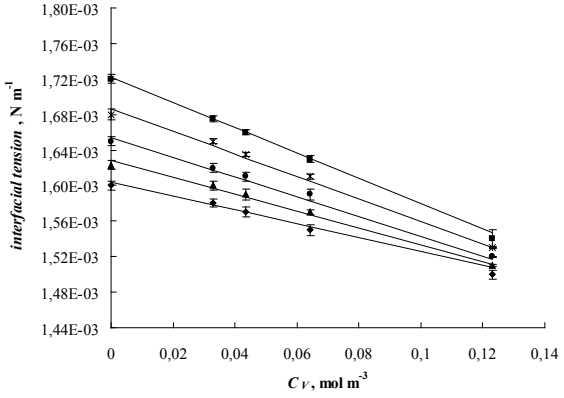

Figure 4. The dependence of the interfacial tension, γ of lecithin membrane in K^+ ion medium on valinomycin concentration, C_V for different electrolyte concentration where: (◊) – 0.0001 M KCl; (Δ) – 0.001 M KCl; (○) - 0.01 M KCl; (+) - 0.1 M KCl; (□) – 1 M KCl (the experimental values are marked by points and the theoretical ones by curves) [30].

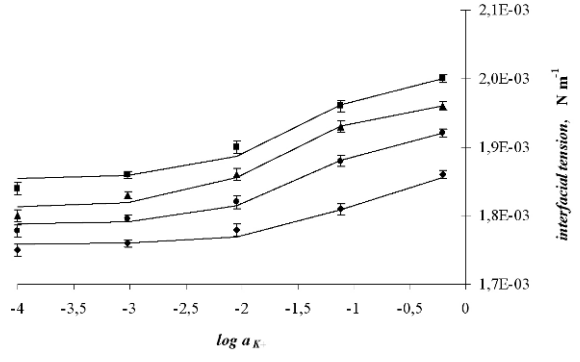

Figure 5. The dependence of the interfacial tension of a lipid membrane built from phosphatidylcholine modified with gramicidin D on the electrolyte solution concentration for different PC/G ratio where: (□) – 1:40; (Δ) – 1:30; (○) – 1:20; (◊) –1:10; the experimental values are marked by points and the theoretical ones by curves [29].

The calculated interfacial tension values were found to be 4.06 mN m^{-1} for PE, 3.53 mN m^{-1} for PC (large hydrophilic head), and 2.94 mN m^{-1} for PS.

In addition, a relationship between the size of the hydrophilic head of the lipid and the isoelectric point pH value has been found. For a lipid with a larger hydrophilic head, the isoelectric point appears at a lower pH. Interestingly enough, the interfacial tension value of the isoelectric point increases with a decrease in a diameter of the hydrophilic head of the lipid [11].

The maximal interfacial tension of the PC membrane was found to be 3.53 at pH 4.15, in good agreement with the value given by Coster and Simons [6]. The interfacial tension of lipid bilayers has been determined before [12-22]; the reported values ranged from 0.2 to 6.0 mN m^{-1} [4-5,22].

2.4. The effect of the presence of ionophores and substances able to form hydrophilic pores in the membrane

In biological membranes, among many different transport phenomena, ion transport is one of the major processes that are vital to all kinds of cell functions. The ion transport is all protein-mediated, and a wide variety of mechanisms by which a solute flux across membranes have been proposed. Over the past few decades extensive studies describing the mechanisms of ion transport across artificial membranes have revealed a great deal of information about ionophores themselves and about the properties which the lipid membranes must have for the ionophores to function efficiently. For example in the case of a carrier molecule it is of little value for it to have high ion selectivity if it does not combine with the membrane; or, if it does combine, if it does not move or bind ions in the membrane. In the case of pore-forming molecules, binding to different membranes may be very similar; however, the efficiency of the transport process may depend critically on the state of ionization of the lipid or on the membrane thickness [1,69].

Lipid bilayers have been most extensively applied to the modeling of transport phenomena (mainly in the case of electrolytes) across biological membranes. Antibiotics such as valinomycin, nonactin, eniatin, and their derivatives are important carriers across lipid membranes [70-72]. Valinomycin is a macrocyclic antibiotic. It has a markedly better solubility in lipids than in water. The valinomycin molecule in the membrane can form complexes with a cation, which is then situated inside the ring-shaped valinomycin molecule. In addition,

valinomycin binds water molecules of the first hydration layer of the cation and its hydrocarbon groups oriented outside the molecule facilitate complex dissolution in the hydrophobic membrane phase. The complex of valinomycin with the cation is electrically charged;, thus, it diffuses across the membrane. The reverse process occurs at the opposite surface of the membrane: the ion passes to the aqueous phase. Therefore, valinomycin group antibiotics are highly selective towards various cations [73-75].

A class of transport promoting substances consists of modifiers that form ionic channels in lipid membranes; gramicidin, alamecithin, monamosicin, nystatin, and hemocyanin belong to this group. Molecules of these modifiers are incorporated into the membrane and form water-saturated pores (ionic channels), which constitute ion passage paths [76,77]. The gramicidin peptide forms the smallest known protein ion channel, which is formed by a dimer consisting of two gramicidin molecules, each containing 15 amino acid residues. Because of its small size, it relatively easily partitions into membranes to form channels and is relatively easily synthesized [78-80]. Gramicidin has been used as a useful model system for the study of membrane permeability, the principles of membrane protein structure, and the mechanisms of protein-lipid interactions [78-83].

The functional roles played by channels and carriers are quite diverse. The complex formation equilibria between ionophores and monovalent cations for valinomycin-K^+ and gramicidin-K^+ systems have been reported previously [29,30]. The stability constant of the valinomycin-K^+ complex was determined using theoretical equations presented in the previous paper [30]. From these equations, a partition coefficient equal to 6.0 was obtained. This indicates that the valinomycin concentration in a lecithin membrane is six times higher than the valinomycin concentration in an electrolyte solution.

The dependence of interfacial tension of valinomycin-modified lipid membrane in the K^+ ion medium on valinomycin concentration in the membrane is given by Eq. 5 [30].

$$\gamma = \gamma_0 + \delta RTa_{K^+} - \delta BRTC_V \ln(1 + Ka_{K^+}) \quad (5)$$

where: C_V [mol m^{-3}] – total concentration of the valinomycin, R [J mol^{-1} K^{-1}] - gas constant, T [K] - temperature, δ [m] - lipid bilayer thickness; a_{K^+} [mol m^{-3}] - K^+ ion activity in the electrolyte solution, K - stability constant of the valinomycin-K^+ ion complex, γ_0 [N m^{-1}] - interfacial tension of bilayer lipid membrane.

The dependence of valinomycin-modified lecithin membrane interfacial tension values in K^+ medium on valinomycin concentration is presented in Fig. 4 [30]. The experimental values fit well with the theoretical ones obtained from Eq. 5.

The effect of the presence of gramicidin in the phospholipid bilayer on interfacial tension can be described as similar to that of cholesterol (the interfacial tension of such a membrane should be the sum of the lecithin and gramicidin interfacial tensions values) [9], because gramicidin can be integrally built into the membrane. The complex between gramicidin and K^+ was formed and the parameters describing the complex were determined using the following theoretical equations: surface area occupied by the GK^+ complex, the interfacial tension of the membrane formed from the GK^+ complex, and the stability constant of the gramicidin-K^+ complex [29]. The interfacial tension value of the gramicidin-K^+ complex was found to be 1.89 mN m^{-1}, whereas that for pure gramicidin is 1.76 mN m^{-1}.

Eq. 6 presents the theoretical dependence of the interfacial tension values of modified lipid membrane on gramicidin D (G) as a function of the K^+ concentration and the results are compared with experimental values, as shown in Fig. 5 [29].

$$\gamma = -\frac{(A_L n_G^{-1} + A_{GK^+})K}{A_L n_G^{-1} + A_G} a_{K^+}\gamma + \\ + \frac{(\gamma_L A_L n_G^{-1} + \gamma_{GK^+} A_{GK^+})K}{A_L n_G^{-1} + A_G} a_{K^+} + \frac{\gamma_L A_L n_G^{-1} + \gamma_G A_G}{A_L n_G^{-1} + A_G} \quad (6)$$

They are denoted by $\gamma_L, \gamma_G, \gamma_{GK^+}$ [mN m^{-1}] and are the interfacial tensions of the membrane formed from lecithin, gramicidin, and the GK^+ complex, respectively; S_L, S_G, S_{GK^+} are the surface fractions occupied by lecithin, gramicidin, and the GK^+ complex, respectively; A_L, A_G, A_{GK^+} [m^2 mol^{-1}] are the surface areas occupied by lecithin, gramicidin, and the GK^+ complex, respectively and a_L, a_G, a_{GK^+} [mol m^{-2}] are the activities of lecithin, gramicidin, and the GK^+ complex, respectively, n_G is the lecithin and the analytical gramicidin concentration ratio in the membrane, and a_{K^+} [mol m^{-3}] is K^+ ions activity in the solution.

Thus, n_G is described by the equation:

$$\frac{a_L}{a_G + a_{GK^+}} = \frac{m_L \cdot M_G}{m_G \cdot M_L} = n_G$$

where: m_L, m_G are the masses of lecithin and gramicidin in the forming solution, respectively, M_L, M_G are the molar masses of lecithin and gramicidin, respectively.

The surface energy value obtained for a gramicidin membrane was equal to 1995.7 J mol^{-1}, and for the gramicidin-K^+ complex membrane,

it was 1782.5 J mol^{-1}. The difference in these values (-213.2 J mol^{-1}) provides the energy required for K^+ ions to enter the gramicidin channel pore. It is very difficult to interpret these values because the energy required for K^+ entry into the gramicidin channel pore has a small value. In addition, it is not easy to compare this value with different bond energy values. The gramicidin channel is about 0.4 nm in diameter and is full of water. It permits water and partially hydrated K^+ ions to pass through the membrane. The energy necessary for K^+ ions to enter the gramicidin channel pore (-213.2 J mol^{-1}), determined by Petelska *et al.*, is the energy necessary to push out the water from the gramicidin channel and insert the K^+ ion inside the channel [29].

3. Conclusion

The interactions between the membrane lipids have been intensively studied by researching ~~of~~ the phenomena occuring in cellular membranes. However, quantitative descriptions of the systems are still unavailable. These are required for a better understanding of the processes that take place in biological membranes. Thus, it is necessary to form the artificial membranes that very closely resemble the properties of natural membranes in order to study the molecular structure and organization of phospholipids. Data presented in this work, determined from mathematical derivation and confirmed experimentally, are of great importance to the interpretation of all phenomena occurring in lipid monolayers and bilayers.

These results can lead to a better understanding of biological membranes and their biophysical properties. The simple interfacial tension method proposed in this work can be used successfully in determination of the interfacial tension values of any 1:1 lipid-lipid, lipid-cholesterol, lipid-fatty acid, lipid-amine, and lipid-amino acid complex; any 1:1 ionophore-monovalent ion complex; and acid-base equilibria established between any phospholipids and the ions from electrolyte solution (H^+ and OH^-).

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