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Impedance Analysis of Phosphatidylcholine/α-Tocopherol System in Bilayer Lipid Membranes

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Abstract. The effect of α-tocopherol on the electrochemical features of the phosphatidylcholine membrane was investigated by impedance spectroscopy. Phosphatidylcholine and α-tocopherol were chosen for the study because they are present in biological membranes and they fulfill essential functions in living organisms. The experimental impedance values obtained in the presence of different amounts of α-tocopherol showed evidence of domain structures within the bilayer containing less than 0.048 molar fraction of α-tocopherol. Based on derived mathematical equations, the surface area of phospholipid/α-tocopherol domain was calculated; it amounts to 832 $Å^2$. This value is consistent, taking into consideration ordering and condensation effects of α-tocopherol, with the acknowledged, well documented, stoichiometry of such a domain of 10:1. The result of the investigation is the proposal of a new method for the determination of the surface area and description of the stoichiometry of domains formed in any two-component system.

Key words: Impedance spectroscopy — Bilayer lipid membrane — Surface area — Phosphatidylcholine α-tocopherol domain

Introduction

Most biological membranes are extremely complex structures consisting of hundreds or thousands of different molecules. The present view of the organization of these membranes is based on the Singer-Nicolson fluid mosaic model [33]. According to this model, phospholipids together with some other lipids, such as cholesterol, form a fluid bilayer in which the incorporated proteins as well as lipids themselves are free to diffuse laterally. The idea of a free lateral diffusion implies that, generally, both lipids and proteins would be more or less randomly distributed. However, as has been repeatedly emphasized [8, 11, 16, 24, 35], a considerable amount of experimental and theoretical data have accumulated since, indicating that membranes are not homogeneous but domains with distinct lipid and protein composition exist. Domains have been shown to exist as macrodomains that are protein-driven and microdomains that are to a large extent lipid-driven. Protein-based macrodomains are known to be stable and are often well-characterized entities, while little is known about lipid microdomain composition, stability and size. It has been suggested that lipid microdomains may range from as little as 20 molecules up to tens of square microns in size and range in stability from nanoseconds up to the lifetime of a cell [3, 42]. The effect of lipids on microdomain size and stability must reflect both phospholipid head-group structure, size and charge as well as the composition (chain length and degree of unsaturation) of the acyl chains. Lipid microdomains are therefore largely the result of lipid-lipid interactions [32].

Most of the techniques used to characterize domains are based on fluorescence spectroscopy. A limitation of fluorescence-based measurements is the inability to provide information at the level of molecular structure and interactions of domain constituents. Furthermore, the area of the domains detected is limited to greater than $\sim 10^6$ $Å^2$. IR and Raman spectroscopies provide a means to overcome these limitations. Direct molecular structure information is inherent in vibrational spectroscopy. Certain IR spectral parameters are sensitive to domain formation in a size regime much smaller than fluorescence-determined domains [23].

It is possible, perhaps likely, that biological membranes are largely composed of a patchwork of lipid microdomains formed as a result of fluid-fluid phase separations. In the present work we model biomembranes using a two-component bilayer system.

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The lipids chosen are phosphatidylcholine from egg yolk (PC) and α-tocopherol (α-T). Egg PC was selected mainly due to its acyl chain composition, which resembles many biological membranes. α-Tocopherol was used because the creation of domains within the phospholipid bilayer that are enriched with respect to α-tocopherol is very well documented [21, 30, 41]. We therefore predicted that our two-component model membrane would contain the PC/α-T domains. We have utilized electrochemical impedance spectroscopy (EIS) to study the formation of domains in lipid bilayers. The determination of the area occupied by one PC/α-T domain is the final research result. Equations presented in this paper can be used, e.g., for determination of area and to describe the stoichiometry of domains formed in any two-component system.

Theory

A two-component forming solution can be used to obtain a lipid membrane. The components may or may not form another compound.

In the case where the membrane components do not form chemical compounds, any two-component system, regardless if it is a monolayer or a bilayer, can be described in terms of the presented equations here, describing additivity of electric capacity and electric conductance:

$$C_m = C_1 S_1 + C_2 S_2 \tag{1a}$$

and

$$R_m^{-1} = R_1^{-1} S_1 + R_2^{-1} S_2 \tag{1b}$$

here:

$$S_1 + S_2 = 1 \tag{2}$$

$$S_1 = a_1 A_1 \tag{3}$$

$$S_2 = a_2 A_2 \tag{4}$$

$$x_1 = \frac{a_1}{a_1 + a_2} \tag{5}$$

$$x_1 + x_2 = 1 \tag{6}$$

where C_m [μF cm^{-2}] is capacitance of the membrane; C_1, C_2 [μF cm^{-2}] are capacitances of the membrane built of components 1 and 2, respectively; R_m^{-1} [Ω^{-1} cm^{-2}] is conductance of the membrane; R_1^{-1}, R_2^{-1} [Ω^{-1} cm^{-2}] are conductances of the membrane built of components 1 and 2, respectively; S_1, S_2 are surface fractions of the membrane covered by components 1 and 2, respectively; a_1, a_2 [mol m^{-2}] are surface concentrations of components 1 and 2, respectively, in the membrane; A_1, A_2 [m^2 mol^{-1}] are surface areas of one mole of the membrane formed from components 1 and 2, respectively; x_1, x_2 are molar fractions of components 1 and 2, respectively.

After solution of equations 1–6 dependencies of linear type are derived:

$$(C_m - C_1)A_2^{-1}x_1 + (C_m - C_2)A_1^{-1}x_2 = 0 \tag{7a}$$

$$(R_m^{-1} - R_1^{-1})A_2^{-1}x_1 + (R_m^{-1} - R_2^{-1})A_1^{-1}x_2 = 0 \tag{7b}$$

Spatial regionalization of components occurs in biological membranes. It is due to specific interactions between membrane components resulting in the appearance of membrane regions of diverse chemical character, structure, and functions. Such specialized structures of various sizes have been called domains [6]. The equilibrium of domain formation can be described in terms of such physicochemical parameters like electric capacity and electric conductance. Let us assume that in the domain (compound 3) formation process in a two-component lipid membrane every molecule of component 2 is surrounded by a certain, possibly from determination, quantity of component 1. The equilibrium state of the discussed system is described by the following equations:

$$C_m = C_1 S_1 + C_3 S_3 \tag{8a}$$

and

$$R_m^{-1} = R_1^{-1} S_1 + R_3^{-1} S_3 \tag{8b}$$

in which:

$$S_1 + S_3 = 1 \tag{9}$$

$$S_3 = a_3 A_3 \tag{10}$$

$$x_1 = \frac{a_1}{a_1 + a_3} \tag{11}$$

$$x_1 + x_3 = 1 \tag{12}$$

where C_3 [μF cm^{-2}] is the capacitance of the membrane built of compound 3; R_3^{-1} [Ω^{-1} cm^{-2}] is the conductance of the membrane built of compound 3; S_3 is the surface fraction of the membrane covered by compound 3; a_3 [mol m^{-2}] is the surface concentration of compound 3 in the membrane; A_3 [m^2 mol^{-1}] is the surface area of one mole of the membrane formed from compound 3; x_3 is the molar fraction of compound 3. Elimination of S_1 S_3, a_1, and a_3 yields the following equations:

$$C_m = \frac{C_1 A_1 + (C_3 A_3 - C_1 A_1)x_3}{A_1 + (A_3 - A_1)x_3} \tag{13a}$$

$$R_m^{-1} = \frac{R_1^{-1} A_1 + (R_3^{-1} A_3 - R_1^{-1} A_1)x_3}{A_1 + (A_3 - A_1)x_3} \tag{13b}$$

Eqs. (13a) and (13b) are quotients of polynomials. Dividing the numerator of each quotient by its denominator yields a series of increasing exponents of the power of mole fraction, x_3. Further, taking into account two first terms of each series results in linear expressions, which are correct at low mole fractions (for $x_3 \rightarrow 0$):

$$C_m x_3^{-1} = C_1 x_3^{-1} + (C_3 - C_1) A_1^{-1} A_3 \quad (14a)$$

$$R_m^{-1} x_3^{-1} = R_1^{-1} x_3^{-1} + (R_3^{-1} - R_1^{-1}) A_1^{-1} A_3 \quad (14b)$$

Materials and Methods

Reagents and Preparation of the Bilayer-Forming Solutions

99% egg lecithin was purchased from Fluka (Neu-Ulm, Germany) and it had the following fatty-acid composition: 16:0 ~ 33%, 18:0 ~ 4%, 18:1 ~ 30%, 18:2 ~ 14%, 20:4 ~ 4%. 99 % α-tocopherol was obtained from Sigma (St. Louis, MO). The lipids were dissolved in chloroform and mixed in appropriate proportions to achieve the desired molar fractions. The solvent was evaporated under a stream of argon. Dried residues were dissolved in a hexadecane-butanol mixture (10:1 by volume). The samples were stored for at least 5 days at 4°C before examination.

The solvents were of chromatographic standard grade: chloroform and butanol were from Aldrich (Milwaukee, WI), hexadecane was from Fluka (Neu-Ulm, Germany).

Potassium chloride solution of 0.1 mol dm^{-3} was used as the electrolyte for experiments. KCl was analytical grade and was roasted prior to use at 400°C for 4 h to remove traces of organic material. Water purified by Milli-Qll (18.2 M, Millipore, USA) was used to make the electrolyte and in all cleaning procedures.

Preparation of the Bilayer Membranes

Bilayer membranes were obtained as bubbles at the teflon cap constituting a measuring-vessel component. With hexadecane as the solvent one can obtain membranes of thickness and capacity values similar to those of membranes formed of monolayers [2]. Butanol is of little use, as it does not change the values of the impedance parameters of the bilayers created, but considerably accelerates membrane formation. The formation of the bilayers was monitored visually and electrically by measuring the membrane capacitance. Capacity of membranes increased with time after bilayer formation until a steady-state value was reached some 10–20 min later. The measurements were started 20–30 min after the membranes turned completely black. The bilayer area was determined with a microscope with a micrometer scale built into the lens and was between 4×10^{-2}–8×10^{-2} cm^2 (margin-subtracted values are given for the bilayer area).

Impedance Analysis

Electrochemical impedance spectroscopy was performed with an a.c. impedance system (EG&G, Princeton Applied Research, Model 388) that included a personal computer, a two-phase lock-in amplifier (Model 5208) and a potentiostat/galvanostat (Model 273), in which a four-electrode input was applied within the pre-amplifier. The electrochemical cell contained two identical reversible silver-silver chloride electrodes and two identical current platinum electrodes, and was described in detail in [25, 26]. The use of the four-electrode system in the studies of electric phenomena occurring in membranes makes it possible to considerably reduce the errors caused by electrode and electrolyte impedance. A 4-mV amplitude sine-wave signal perturbation was applied in the 0.1–10000 Hz frequency range. Impedance data were analyzed by using the nonlinear least squares (NLLQ) fitting to a model represented by an equivalent electrical circuit. The NLLQ program used in this work was EQUIVCTR.PAS B.A. Boukamp [5]. All experiments were carried out at room temperature of 20 ± 1°C.

Results and Discussion

The effect of α-tocopherol on the phosphatidylcholine capacitance and resistance was examined in the presence of different amounts of α-T using impedance spectroscopy. The capacity of a black bilayer membrane (BLM) is well defined when it is in the black state. The resistance may vary by at least one order of magnitude, possibly because of impurities of the BLM, border leakage at the membrane support, the appearance of lipid "crystals" at the periphery of the BLM, way of introducing the lipid solution (if the forming solution is introduced with a micro-syringe, instead of with a brush, the irreproducibility of bilayer can be minimized). The resistance of a single membrane, however, is usually constant until a short time before the membrane ruptures. Therefore, any changes in resistance due to addition of ions, proteins, drugs, etc., can be determined with a relatively high degree of accuracy [36]. The EIS technique was used in our study to characterize the membrane features, as this method has been shown to be able to measure the membrane capacitance and resistance on bilayer lipid membranes accurately. The mean values of the measured parameters were obtained from ten independent measurements of the lipid bilayer. Based on numerous results given in the literature and our own experimental results, we assume that the membranes created by us do not contain solvent. If some of these quantities, which are not large in number, are contained in the membranes, then one should treat them as traces of impurities. As it is impossible to determine the quantity of these impurities, it is impossible to make a thorough qualitative determination of their nature and so one cannot take them into account in quantitative considerations (except as a possible qualitative indication). If quantitative analysis were possible, we would take into account the possibility of solvent's presence in the derived equations.

The experimental impedance values presented here refer to the bilayer surface area unit. The α-T content was varied up to a 0.33 mole fraction (46 mol %), which is close to the maximum of 40 mol %, which can be accommodated in such bilayers [4, 19]. At 50 mol % α-T, the lipid mixture was difficult to

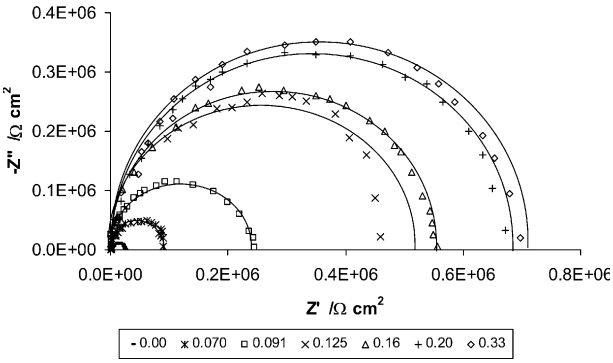

Fig. 1. Impedance spectra of the imaginary component of impedance $-Z''$ versus the real component of impedance Z' over a frequency range of 0.1 Hz – 10 kHz for a phosphatidylcholine bilayer modified with α-tocopherol. A different content of α-tocopherol (expressed as a molar fraction) is illustrated by the different point's shapes of the impedance spectra. (–) NLLQ fit to the equivalent circuit shown in Fig. 2.

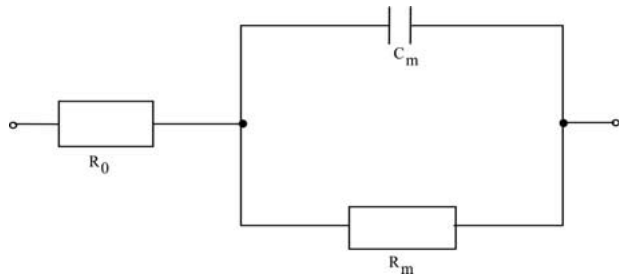

Fig. 2. An equivalent circuit representing electric properties of the phosphatidylcholine membrane modified with α-tocopherol: R_0, electrolyte resistance; C_m, capacitance of the membrane; R_m, resistance of the membrane.

disperse and macroscopic phase separation occurred. It has been reported before [38] that, at concentrations higher than 40 mol %, aggregates could be seen using a light microscope equipped with a Normarski interference-contrast accessory.

Any impedance Z is defined by an amplitude ratio and a phase angle. This enables one to treat impedances as complex numbers and leads to the following definition of impedance:

$$Z = Z' + jZ'' \tag{15}$$

where: Z' is the real component of impedance, Z'' is the imaginary component of impedance, and

$$j = \sqrt{-1}.$$

In the electrochemical literature the notation $Z = Z'\text{-}jZ''$ is frequently employed instead of the formal notation above. This is related to the fact that often in electrochemistry the value of the phase angle is negative, combined with the desire to have Z'' positive. In view of the widespread importance of the impedance method, also outside the field of electrochemistry, the notation as in Eq. (15) should be preferred [34].

Graphical representation of primary (''raw'') impedance data can be quite useful from a diagnostic point of view. The most common form of representation is the complex-plane diagram, i.e., the plot of Z'' against Z' at varied frequency ω. Such a plot is known under different names: ''Argand diagram'' (general for representation of complex numbers), ''Nyquist diagram'' (more particular in use for representation of complex transfer functions of electronic control devices), ''Sluyters diagram'' (more particular in use in electrochemistry, especially corrosion literature) or ''Cole-Cole plot'' (in analogy with the graphic representation of the dielectric constant [34]).

Fig. 1 shows the results of impedance measurements conducted on the phosphatidylcholine bilayers, pure and containing different amounts of α-T. The NLLQ fits (according to the equivalent circuit shown in Fig. 2) are represented by the curves and are in good agreement with the data obtained. For the sake of clarity, spectra for some mole fractions have been omitted (otherwise the figure would be illegible by superimposed spectra caused by too little differences in the impedance parameter values). Very simple impedance diagrams were obtained for all examined membranes; they had the form of impedance semicircles in the entire analyzed frequency range. The centers of the semicircles lie on the real axis, provided that the lipid bilayer is considered as a dielectric layer with leakage [25]. The spectra of PC/α-T bilayers were higher than those of unmodified membranes, confirming that α-T has successfully been incorporated into the lipid bilayer and has a contrast effect on the membrane capacitance and resistance; it caused C_m to decrease and R_m to increase.

An equivalent circuit presented in Fig. 2 describes the electric properties of the analyzed lipid membrane. The impedance of the PC/α-T bilayer is presented by the electrolyte solution resistance R_0 (it was assumed to be of ohmic nature and not to perturb the membrane properties), which is in series with a parallel circuit, composed of the resistance of the membrane R_m and of the membrane capacitance C_m. The possibility of misinterpretation of the recorded data is reduced by simplicity of the circuit. This electric circuit is characteristic for an artificial lipid membrane only when ionophore systems, specific channels, pores, and adsorption are absent [18]. However, in many cases, the centre of the semicircle does not lie on the real axis, and can be looked upon as a semicircle rotated in the clockwise sense around the origin by a certain angle α. An inclined semicircle on the complex plane might be caused by a distribution in membrane thicknesses [9]. In our studies, α was varied from 0.97 to 1.0 (α equal to 1.0 shows that the membrane thickness is totally uniform), and its value was not taken into

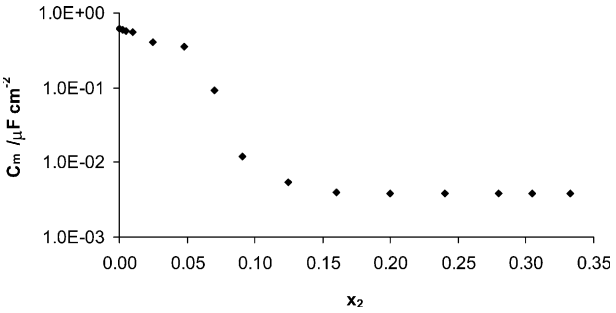

Fig. 3. Dependence of capacitance C_m of the phosphatidylcholine/α-tocopherol membrane on the mole fraction of α-tocopherol x_2. Experimental errors are smaller than (or comparable to) the symbols used to represent the data.

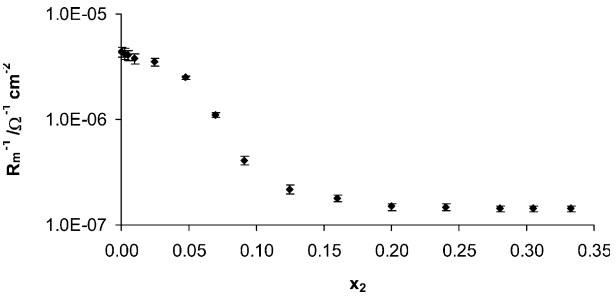

Fig. 4. Dependence of conductance R_m^{-1} of the phosphatidylcholine/α-tocopherol membrane on the mole fraction of α-tocopherol x_2. Error bars indicate the experimental scatter.

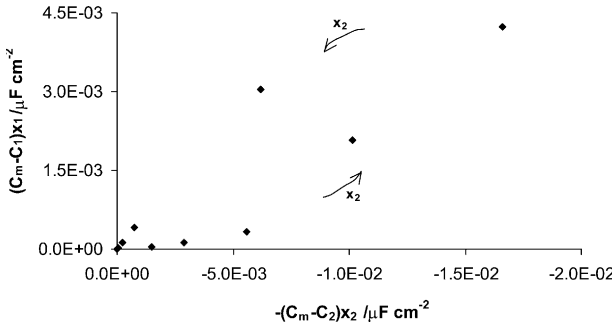

Fig. 5. The dependence of $(C_m\text{-}C_1)x_1$ vs $(C_m\text{-}C_2)x_2$. C_m, capacitance of the phosphatidylcholine/α-tocopherol membrane; C_1, capacitance of the phosphatidylcholine membrane; C_2, capacitance of the α-tocopherol membrane; x_1, molar fraction of the phosphatidylcholine and x_2, molar fraction of α-tocopherol. The arrows denote the direction of the increasing x_2 values.

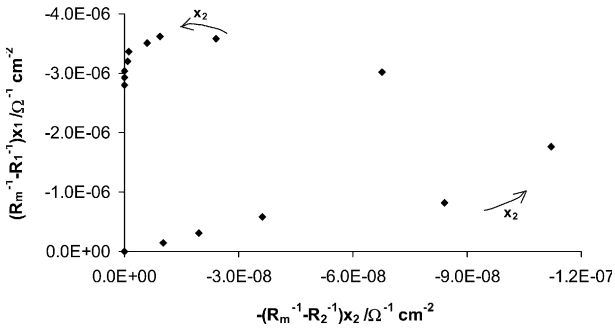

Fig. 6. The dependence of $(R_m^{-1}-R_1^{-1})x_1$ vs $(R_m^{-1}-R_2^{-1})x_2$. R_m^{-1}, conductance of the phosphatidylcholine/α-tocopherol membrane; R_1^{-1}, conductance of the phosphatidylcholine membrane; R_2^{-1}, conductance of the α-tocopherol membrane; x_1, molar fraction of the phosphatidylcholine and x_2, molar fraction of α-tocopherol. The arrows denote the direction of increasing x_2 values.

account during calculation of the membrane impedance parameters.

Based on this equivalent circuit (Fig. 2), the nonlinear least squares analysis was used to simulate the impedance plots; then the values of R_m and C_m were extracted from the fit. Dependencies of the capacitance and the conductance (reciprocal of resistance) of the PC/α-T membrane on the mole fraction of α-tocopherol are presented in Figs. 3 and 4, respectively. The resulting curves deviate from linearity, indicating that some bonds are formed in the membrane. The C_1 and R_1^{-1} values for a pure bilayer of lecithin are equal to 0.62 μF cm^{-2} and 4.35×10^{-6} Ω^{-1} cm^{-2}, respectively. α-T had a significant effect on the capacitance and the conductance membranes up to 0.24 mole fraction, C_m and R_m^{-1} reached a plateau value when the α-T mole fraction was over 0.24. The C_2 and R_2^{-1} values for a pure bilayer of α-tocopherol (evaluated from plateau values) are equal to 0.0038 μF cm^{-2} and 1.43×10^{-7} Ω^{-1} cm^{-2}, respectively.

These results are in contrast to the data reported by Koronkiewicz et al. [17]. The capacity of the lipid bilayers made by them decreased only by about 15% when the mole fraction of α-tocopherol was about 0.2. The main reason for the divergence of our results is lack of an external electric field applied to the membrane (mimicking the electric environment of membranes of the living cell); besides, these authors were using another kind of solvent plus phosphatidylcholine and so they did not take into account influence of the Plateau-Gibss border on the electrical properties of lipid bilayers. The effect of α-tocopherol on the lipid membrane is strongly dependent on the external electric conditions of the membrane. Koronkiewicz demonstrated that α-tocopherol in high concentrations can act destructively in the membrane: under an external electric field, α-tocopherol led to the destabilization of model bilayer lipid membranes. On the contrary, our results confirm the suggestion by several authors [7, 14, 37] that α-tocopherol acts as a structural component which stabilizes biomembranes.

Figs . 5 and 6 present the dependencies described by Eqs. 7a and 7b, respectively.

According to Eq. (7), as in the case when the membrane components do not form chemical compounds, the values of these functions should form straight lines. As one can see, this is not the case, which suggests that there is a chemical compound

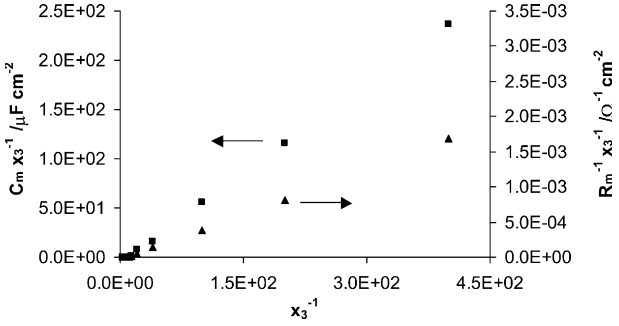

Fig. 7. A plot illustrating Eqs. (14a) and (14b), from which the surface area of phosphatidylcholine/α-tocopherol domain can be determined. C_m represents capacitance of the membrane; R_m^{-1}, conductance of the membrane and x_3, molar fraction of the domain.

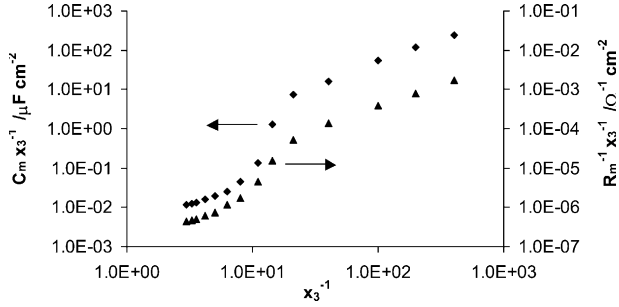

Fig. 8. Eqs. (14a) and (14b) are plotted on a logarithmic scale to emphasize the straight lines obtained for the initial five points.

formation in the PC/α-T bilayer. Since Eqs. 7a and 7b do not describe the system under study sufficiently, we assume, on basis of the literature [21, 30, 41], the creation of domains within the phospholipid bilayer that are enriched with α-tocopherol and that all α-tocopherol is present in the PC/α-T domains [40]. Consequently, Eqs. (8a) and (8b), describing a domain formed in the bilayer lipid membrane, complete the theoretical description. After simple modifications of Eq. (8), one can obtain information of great interest from our point of view, presented by Eqs. (14a) and (14b).

Fig. 7 presents the dependences illustrating Eq. (14) in the whole analyzed mole fraction of the α-T range. Provided that a domain is formed, the plots of Eq. (14) show straight lines. This is illustrated more distinctly when these equations are plotted in a logarithmic scale (Fig. 8). The first of the five points lie on ideal straight lines, as is precisely shown on the figure. These points correspond to a range from 0.0025 to 0.048 (8 mol %) molar fraction of α-tocopherol and confirm, that in this molar fraction range there are created domains with a defined stoichiometry and a relatively constant area. The freeze-fracture electron microscopic method combined with X-ray diffraction study [39] also indicate that α-tocopherol/phospholipid domains exist in mixtures containing less than 10 mol % α-T.

A linear regression analysis was carried out for five of the least molar fractions of α-tocopherol and gave a straight line of the forms: $y = 0.606x - 5.844$ with the correlation coefficient $R^2 = 0.9998$ (from capacitance measurements) and $y = 4.29 \times 10^{-6}x - 4.09 \times 10^{-5}$ with the correlation coefficient $R^2 = 1.0000$ (from conductance measurements). The slope values of the straight lines are equal to C_1 and R_1^{-1}, respectively, and are in agreement (in deviation limits) with experimental values obtained for a pure phosphatidylcholine membrane. The intercepts with the y-axis yield values that allow one to determine an area occupied by one PC/α-T domain (denoted by A_3). The surface area occupied by one PC molecule (A_1) is also necessary for calculation of the A_3 value. This surface depends on the way the phospholipid is prepared, because this affects the length, conformation and degree of unsaturation of the fatty acids chains. Therefore, the values of the literature range between 54 Å^2 and 99 Å^2 [10, 12]. We chose the surface area per phosphatidylcholine molecule determined by us [31], equaling 85 Å^2. Knowing the A_1, C_1 and C_3, as well as R_1^{-1} and R_3^{-1}, the area occupied by one PC/α-T domain could be determined. The resulting A_3 values were 825 Å^2 from capacitance measurements and 838 Å^2 from conductance measurements, which gave the mean value, which amounts to 832 Å^2.

Preferential interactions between α-tocopherol and phospholipids and indirect evidence for the formation of specific complexes have been obtained from a number of different experimental approaches. Recent X-ray diffraction and calorimetric studies of mixed aqueous dispersions of α-T and phosphatidylcholine [30] have provided evidence for the formation of complexes in both the gel and fluid phases of the phospholipid, which have an approximate stoichiometry of phospholipids: α-tocopherol of 10:1. These studies support other evidence that indicates α-T is not randomly distributed in the phospholipid bilayer membranes [1, 13] but tends to be localized in domains within membranes.

The effect of α-tocopherol on phosphatidylcholine is similar, but less significant, in many respects to that of cholesterol, i.e., α-T condenses the membrane of PC due to an interaction between α-T and the acyl chains of the phospholipid molecules. The limited freedom of acyl chains causes the membrane to condense, with a reduction in area, closer packing and decreased fluidity [4, 20, 27, 28]. However, a very important difference exists in interactions shown by both of these compounds. Since the phytanoyl chain of α-tocopherol only resembles cholesterol in an all-trans conformation, rotational mobility would eliminate any suggested static complex between α-T and a fatty acyl chain similar to that suggested for cholesterol [22]. This is

confirmed by calorimetric and infrared spectroscopic studies, which indicate that α-T tends to partition into fluid rather than rigid domains within the phospholipid bilayers [29, 38].

Taking into account the surface area occupied by one α-T molecule (equal to 60 $Å^2$ [7]), the surface area occupied by one PC (mentioned above) and the well-estimated stoichiometry of PC/α-T of 10:1, the theoretical surface area per one domain was obtained: it is equal to 910 $Å^2$. Deviation of the surface area calculated on the basis of experiments (the experimental surface value amounts to 832 $Å^2$) from the theoretical value shows a negative value, which means that a reduction in the surface area occurs as a result of component mixing. This is thus a condensation effect observed between the PC and α-T, which indicates that α-tocopherol can work as a reinforcer for PC bilayers. The condensation can be attributed to area changes within the PC when this lipid is mixed with α-T, since α-tocopherol is a rigid molecule whose area does not significantly change when a PC/α-T bilayer is formed. The surface area occupied by one PC molecule within the domain amounts to about 77 $Å^2$; while presence of cholesterol in the PC bilayer can reduce the molecular area of the phosphatidylcholine even to 56 $Å^2$ [10].

Conclusion

Application of impedance spectroscopy to the study of electrochemical behavior of lipid bilayers allows one to provide a quantitative description of equilibria in a two-component membrane. Based on derived mathematical equations, a new method for calculation of the surface area of the domain between phosphatidylcholine and α-tocopherol was proposed. The domain formation is the main reason for which deviation from rectilinearity of the parameters of the system described by the additivity rule is observed.

Data presented in this work, obtained from the mathematical derivation and confirmed experimentally are of great importance for the interpretation of phenomena occurring in lipid monolayers and bilayers. In our opinion, these results can help in a better understanding of biological membranes and with biophysical studies. A new, simple and very interesting method proposed by us can be used with success for the quantitative determination of area and to describe the stoichiometry of domains formed in any two-component system.

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