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# Impedance spectroscopy measurements of phosphatidylcholine bilayers containing ether dibenzo-18-crown-6

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The effect of ion carrier crown ether dibenzo-18-crown-6 on the electrochemical features of the phosphatidylcholine bilayer membrane was investigated by impedance spectroscopy. The experiments have been carried out with various forming solution compositions and at various potassium ion concentrations in the electrolyte solution. Potassium chloride was used as the electrolyte. A complex was formed between the dibenzo-18-crown-6 molecule and $K^+$ ion on the lipid bilayer/electrolyte solution interface. Based on derived mathematical equations, the heterogeneous equilibrium constant ($K_h$), association rate constant of the complex ($k_R$) and dissociation rate constant of the complex ($k_D$) were determined.

**Key words**: Bilayer lipid membrane, impedance spectroscopy, phosphatidylcholine, crown ether.

## INTRODUCTION

Biological membranes show selectivity to penetration of different ions even if their physico-chemical parameters are very similar; selectivity to sodium or potassium ion is a classical example. The carrier theory is an attempt to explain the selectivity of the membranes: the ion is stated to form a transition complex with a membrane component, which enables their transport across the membrane. Some compounds are able to form complexes with mono- or divalent cations. This property makes it possible to use these compounds as artificial ion carriers to the cell or through mitochondrial membranes. Detailed studies on ion transport are facilitated by simple structure of artificial membranes in contrast to that of complex lipid and protein mixtures present in natural membranes. Several classes of macrocyclic compounds are frequently used in the studies on potassium ion penetration through lipid bilayers. Among them, there are depsipeptides like enniatin B or valinomycin and its analogues, polyesters-polyethers like monactin-dinactin and pure polyethers, e.g. crown ethers [1].

Crown ethers have been studied extensively since their discovery nearly four decades ago [2, 3]. Literally, thousands of crown ether derivatives [4] have been prepared and their ability to complex cations [5–7] under equilibrium conditions [8] has been evaluated. In addition, there are numerous reports of cation transport through bulk liquid membranes mediated by crowns of widely varying structures [9]. Sodium and potassium are the two most common cations in solutions *in vivo* and agents that complex and alter their natural balance are expected to exhibit biological effects. Indeed, the toxicity of certain crown ethers was established shortly after their discovery [10–13].

The first one discovered and most versatile of the aromatic crown compounds is dibenzo-18-crown-6 (Fig. 1) yielding 1:1 complex with the potassium ion. The aim of the authors was to utilize electrochemical impedance spectroscopy to study the formation of this complex at the membrane/electrolyte solution interface. The heterogeneous reaction was described by mathematical equations and was further verified experimentally. The following parameters, describing the complex, were determined: association rate constant of the complex, dissociation rate constant of the complex and heterogeneous equilibrium constant.

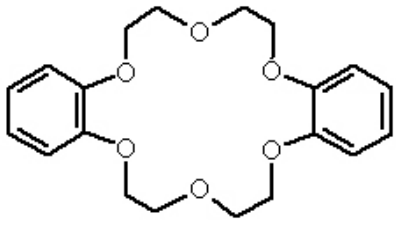

Fig. 1. The structure of 2,3,11,12-dibenzo-1,4,7,10,13,16-hexaoxacyclooctadeca-2,11-diene (dibenzo-18-crown-6).

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## THEORY

In the following we base the impedance analysis of the phosphatidylcholine membranes, modified with crown ether dibenzo-18-crown-6, on a model of carrier-mediated ion transport that has already been used for the treatment of phosphatidylcholine membranes containing valinomycin [14]. Specifically, this model assumes that a mobile, positively charged 1:1 complex MS+, is responsible for charge transport through the membrane. The formation of the complexes, which cross the membrane, preferentially occurs at the interfaces, where carrier molecules S from the membrane combine with cations M+ from the aqueous phases. This heterogeneous reaction can be described by rate constants $k_R$ (association, recombination) and $k_D$ (dissociation) and its mechanism can be formally written as:

$$M^+ + S \underset{k_D}{\overset{k_R}{\rightleftarrows}} MS^+ \tag{1}$$

This reaction is at equilibrium:

$$K_h = \frac{k_R}{k_D} \tag{2}$$

where Kh is the heterogeneous equilibrium constant ($cm^3 \cdot mol^{-1}$).

If the volume concentrations of the complex MS+ and the free carrier S are denoted by $c^b_{MS}$ and $c^b_S$ (expressed in $mol \cdot cm^{-3}$) and the ion activity by $a_M$ (expressed in $mol \cdot cm^{-3}$), the heterogeneous equilibrium constant has the form:

$$K_h = \frac{c^b_{MS}}{c^b_S \cdot a_M} \tag{3}$$

As membrane component concentrations can be related to its surface area by multiplying volume concentrations by the lipid bilayer thickness, the heterogeneous equilibrium constant is given also by the expression:

$$K_h = \frac{N_{MS}}{N_S \cdot a_M} \tag{4}$$

where: $N_{MS}$ - surface concentration of the complex ($mol \cdot cm^{-2}$), $N_S$ - surface concentration of the free carrier ($mol \cdot cm^{-2}$).

Introducing the total carrier surface concentration in the bilayer NT as the sum of complex and free carrier surface concentrations

$$N_T = N_{MS} + N_S \tag{5}$$

and combining Eqns. (4) and (5), the surface concentration of the complex is derived:

$$N_{MS} = \frac{K_h \cdot a_M \cdot N_T}{1 + K_h \cdot a_M} \tag{6}$$

The total quantity of the carrier, added to the solution forming the membrane, can be expressed as follows:

$$c_f V_f = c_m V_m + c_{aq} V_{aq} \tag{7}$$

here: $c_f$, $c_m$, $c_{aq}$ – concentrations of the carrier in the membrane-forming solution, the membrane and the electrolyte solution ($mol \cdot cm^{-3}$), respectively; $V_f$, $V_m$, $V_{aq}$ – volumes of the membrane-forming solution, the membrane and the electrolyte solution ($cm^3$), respectively.

The partition coefficient of the carrier $\gamma_S$ can be represented in the form:

$$\gamma_S = \frac{c_m}{c_{aq}} \tag{8}$$

Therefore, from Eqns. (7) and (8), the total carrier surface concentration can be expressed by the equation:

$$N_T = \frac{\gamma_S \cdot c_f \cdot V_f \cdot d}{\gamma_S \cdot V_m + V_{aq}} \tag{9}$$

in which $d$ is lipid bilayer thickness (cm).

Determination of membrane conductivity $R_m^{-1}$ in terms of Ohm's Second Law yields:

$$R_m^{-1} = \frac{S}{d} \cdot \mu_{MS} \cdot \frac{N_{MS}}{d} \cdot F \tag{10}$$

here: $S$ - membrane surface area ($cm^2$), $\mu_{MS}$ – mobility of the complex ($cm^2 \cdot V^{-1} \cdot s^{-1}$), $F$ – Faraday's constant ($C \cdot mol^{-1}$).

If Eqn. (6) is inserted into Eqn. (10), the following expression for the membrane conductivity as a function of total carrier and/or electrolyte concentration is derived:

$$R_m^{-1} = \frac{S}{d^2} \cdot \mu_{MS} \cdot F \cdot \frac{K_h \cdot a_M \cdot N_T}{1 + K_h \cdot a_M} \tag{11}$$

The $k_D$ value can be determined by the equations determining the real and imaginary parts of transfer across interface impedance [15]:

$$R_{it} = \frac{\nu^2 RT}{n^2 F^2} \cdot \frac{1}{N_{MS} \cdot k_D} \cdot \frac{1}{1 + (\omega / k_D)^2} \tag{12}$$

$$\frac{1}{\omega \cdot C_{\mathrm{it}}} = \frac{\nu^2 RT}{n^2 F^2} \cdot \frac{1}{N_{MS} \cdot k_{\mathrm{D}}} \cdot \frac{\omega / k_{\mathrm{D}}}{1 + (\omega / k_{\mathrm{D}})^2} \quad (13)$$

in which: $R_{\mathrm{it}}$ – resistance of the transfer across interface ($\Omega \cdot \mathrm{cm}^2$); $C_{\mathrm{it}}$ - capacity of the transfer across interface ($\mu\mathrm{F} \cdot \mathrm{cm}^{-2}$); $\nu$ – stoichiometric coefficient of the complex; $\omega$ – angular frequency ($\mathrm{s}^{-1}$); $R$, $T$, $n$, $F$ have their meaning.

At low frequencies, where $\omega$ is considerably smaller than $k_{\mathrm{D}}$, the above formulae are reduced to:

$$R_{\mathrm{it}} = \frac{\nu^2 RT}{n^2 F^2} \cdot \frac{1}{N_{MS} \cdot k_{\mathrm{D}}} \quad (14)$$

$$\frac{1}{\omega \cdot C_{\mathrm{it}}} = \frac{\nu^2 RT}{n^2 F^2} \cdot \frac{1}{N_{MS} \cdot k_{\mathrm{D}}} \cdot \frac{\omega}{k_{\mathrm{D}}} \quad (15)$$

It results from Eqns. (14) and (15) that the resistance of the transfer across the interface is frequency independent for the frequencies approaching zero, whereas $1/\omega \cdot C_{\mathrm{it}}$ increases proportionally to $\omega$ .

At high frequencies, where $\omega$ is considerably greater than $k_{\mathrm{D}}$, Eqns. (12) and (13) are simplified into:

$$R_{\mathrm{it}} = \frac{\nu^2 RT}{n^2 F^2} \cdot \frac{1}{N_{MS} \cdot k_{\mathrm{D}}} \cdot \left( \frac{k_{\mathrm{D}}}{\omega} \right)^2 \quad (16)$$

$$\frac{1}{\omega \cdot C_{\mathrm{it}}} = \frac{\nu^2 RT}{n^2 F} \cdot \frac{1}{N_{MS} \cdot k_{\mathrm{D}}} \cdot \frac{k_{\mathrm{D}}}{\omega} \quad (17)$$

It means that the resistance and the capacity of the transfer across the interface approach zero at high frequencies: both $1/\omega \cdot C_{\mathrm{it}}$ and $R_{\mathrm{it}}$ decrease with the increasing value of $\omega$.

## EXPERIMENTAL

### *Reagents and preparation of the forming solutions*

99% pure egg phosphatidylcholine 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%. The 98% dibenzo-18-crown-6 was obtained also from Fluka (Neu-Ulm, Germany). Phosphatidylcholine was dissolved in chloroform to prevent oxidation and the solvent was evaporated in an atmosphere of argon. Dibenzo-18-crown-6 was added as a solution in chloroform (20 $\mathrm{mg \cdot ml^{-1}}$) and the solvent was again removed by argon. Dried residues (phosphatidylcholine or phosphatidylcholine and dibenzo-18-crown-6 mixture) were dissolved in a hexadecane-butanol mixture (10:1 by volume). The forming solutions contained phosphatidylcholine (20 $\mathrm{mg \cdot ml^{-1}}$ of solvent system) or a phosphatidylcholine-dibenzo-18-crown-6 mixture (weight ratios: 100:1, 90:1, 80:1, 70:1, 60:1, 50:1 and 40:1) and were stored at 4°C for less than a week. The method of preparation and storage gave reproducible electrochemical features of the membranes when samples prepared at different times were examined by impedance spectroscopy.

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

1, 0.1, 0.01, 0.001 and 0.0001M potassium chloride solutions were used as electrolytes for the experiment. Potassium chloride produced by POCh Co. (Poland) was analytical grade of purity and was calcined 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 in all solutions and in all cleaning procedures.

All experiments were performed at room temperature 20 ± 1°C.

### *Preparation of the bilayer membranes*

Bilayer membranes were obtained as bubbles at the Teflon cap, constituting a measuring vessel component. The use of hexadecane as the solvent allows one to obtain membranes of thickness and capacity values similar to those of membranes formed of monolayers [16, 17]; there is almost no solvent retained in the bilayer. Small quantity of butanol has a negligible effect on the impedance parameters of the bilayers created, but however it considerably accelerates the membranes formation. The thinning of the membranes was monitored visually by means of the microscope, which was being reached by reflected white light. The reflected light beam showed the grey colour initially, then, along with decreasing of thickness of the membrane, interference colours were appearing, until the image attained the black colour finally. After obtaining the black colour, the process of forming was ended - no further changes were being observed. The formation of the bilayers was also monitored electrically by measuring the membrane capacitance at low frequency. The capacity of the membranes increased with time after bilayers formation until a steady-state value was reached some 10–20 min later. The measurements started only after the low frequency capacitance became stable; increasing by less than 1% per hour. When the capacitance had stabilized it was assumed that diffusion of solvent out of the bilayer was complete, although some hexadecane molecules might remain “dissolved” in the membrane interior. The bilayers area was determined by a microscope with a micrometer scale built into the

lens and was between $4\times10^{-2}$–$8\times10^{-2}$ cm$^2$ (the values are given for the bilayers area with subtracted margin).

*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 it was described in details in [18–20]. 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 [21, 22]. A 4-mV amplitude sine-wave signal perturbation was applied in the 0.1–10000 Hz frequency range. The PowerSuite 2.4 software package was used for acquisition of impedance data. These data were analyzed using complex nonlinear least squares (CNLS) fit to a model, represented by an equivalent electrical circuit. The CNLS program used in this work was ZSimpWin 3.21.

## RESULTS AND DISCUSSION

Dependence of crown ether-modified phosphatidylcholine membranes in a potassium ion medium was measured as function of dibenzo-18-crown-6 concentration using electrochemical impedance spectroscopy. Impedance measurements of the lipid membranes were carried out with unmodified membranes and with membranes modified by seven different carrier concentrations and at five different KCl concentrations. The total carrier surface concentration in the individual forming solution was calculated using Eqn. (9), taking into account the partition coefficient of the carrier to be equal to $1.26\times10^3$ [23]. The following values NT were obtained: $2.54\times10^{-14}$, $2.82\times10^{-14}$, $3.17\times10^{-14}$, $3.62\times10^{-14}$, $4.21\times10^{-14}$, $5.03\times10^{-14}$ and $6.26\times10^{-14}$ mol·cm$^{-2}$. The arithmetic mean values of the impedance parameters were determined based on six independent measurements of the lipid bilayer.

Fig. 2 depicts typical impedance spectra of the phosphatidylcholine bilayers, both pure and containing dibenzo-18-crown-6. Very simple impedance diagrams were obtained in the absence of crown ether; they had the form of impedance semicircles in the entire analyzed frequency range; it was the evidence that the lipid bilayer was a dielectric layer with leakage (Fig. 2a). The semicircles were distorted because the lipid bilayer itself was not a simple and uniform dielectric layer. The dielectric layer was composed of substructures, which are difficult to extract unless the phase angle can be determined separately at each frequency and very accurately. Karolis *et al*. [17] demonstrated the presence of seven separate elements of lipid bilayer/electrolyte systems on the basis of low frequency impedance measurements of pure phosphatidylcholine bilayers. Four of these can be attributed to the acyl chain, carbonyl, glycerol bridge and phosphatidylcholine regions of the lecithin molecule. The equivalent circuit used for data analysis (Fig. 3a), consists of a parallel arrangement of the capacitor $C_m$ and resistor $R_m$, attributed to the electrical properties of the bilayer, completed with a serial resistor $R_0$ for the conductivity of the bulk. The possibility of misinterpretation of the recorded data is reduced by the 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 [24]. Based on this equivalent circuit, 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. The CNLS fit is represented by the solid line in Fig. 2a and it is in good agreement with the data obtained.

The frequency response was drastically different, when ion carrier was added to the membrane (Fig. 2b). The impedance diagrams of the bilayers, modified with crown ether, exhibited capacitive contribution at high frequencies, with the indication of a second semicircle at low frequencies related to potassium ion transport in the area close to the membrane surface. The impedance experiments have been carried out with various forming solution compositions and at various potassium ion concentrations in electrolyte solution. Except for the $Z$ values, all recorded impedance spectra are characterized by common general features and the same dynamic behaviour. For this reason, the data for one KCl concentration and for one ion carrier concentration are shown in Fig. 2b. Fig. 3b represents the equivalent circuit, used to describe the transport of ions through the bilayer. This circuit takes into account the impedance components of the membrane and the impedance representing the situation at the membrane interface. The membrane impedance is composed of the electric capacity of the membrane $C_m$, and of the electric resistance of the charged complex transport inside the membrane $R_m$.

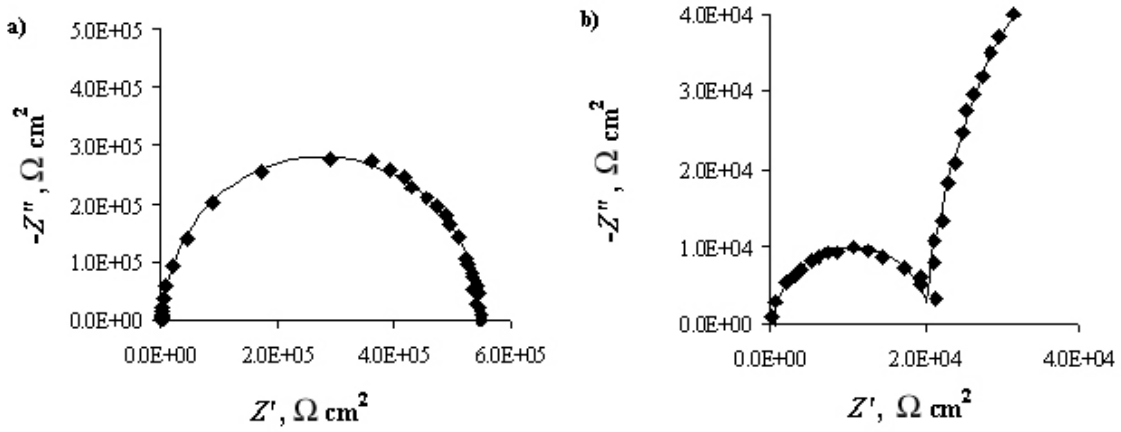


Fig. 2. Impedance diagrams obtained in a solution containing 0.0001 M KCl: a) a membrane made of phosphatidylcholine, b) a phosphatidylcholine bilayer, modified with dibenzo-18-crown-6 (total carrier surface concentration is equal $4.21\times10^{-14}$ mol·cm$^{-2}$). The solid lines represent the results of the fitting procedure.

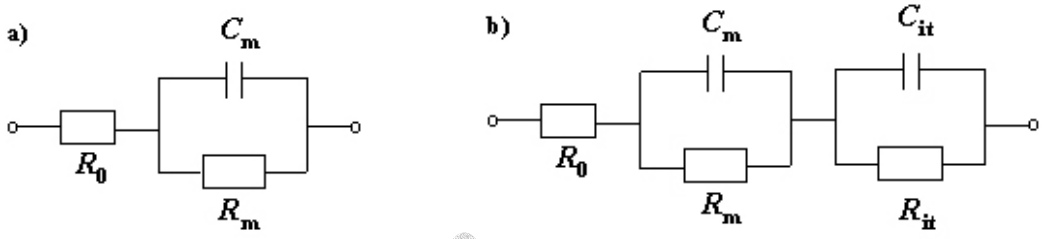


Fig. 3. Equivalent circuits representing the electrical properties of pure phosphatidylcholine membranes (a) and phosphatidylcholine membranes containing dibenzo-18-crown-6 (b). $R_0$ – electrolyte resistance; $C_m$ and $R_m$ – capacitance and resistance of the membrane, respectively; $C_{it}$ and $R_{it}$ capacitance and resistance of the transfer through interface membrane/electrolyte solution.

Capacity and resistance of the transfer through interface membrane/electrolyte solution are denoted by $C_{it}$ and $R_{it}$, respectively (subscript it stands for transfer across interface). Based on this equivalent circuit, the nonlinear least squares analysis was used to simulate the plots; then the values of the impedance parameters were extracted from the fit (the CNLS fit is represented by the solid lines in Fig. 2b).

The Figs. 4 and 5 illustrate the experimental values of the $R_m^{-1}$, $C_m$, $R_{it}$, and $C_{it}$ parameters as functions of potassium ion concentration in the solution and of total dibenzo-18-crown-6 surface concentration in the membrane. The presence of the crown ether in the membrane and of the $K^+$ ion in the solution has no significant effect on membrane capacity, which varies in the 0.6 μF·cm$^{-2}$ < $C_m$ < 0.8 μF·cm$^{-2}$ range (Fig. 4b). This can be explained by the higher water content in the bilayer, which enhanced electric permittivity, and thus, the capacity of the membrane. No clear variation of the membrane capacity values with electrolyte concentration was also observed by other authors who studied the effect of carrier on potassium ion transport through lipid bilayers [25]. The standard deviations are not shown in Figs. 4a, 5a and 5b for the sake of clarity (otherwise, figures would be illegible by close super-imposed data due to too little difference in the impedance parameters values). Deviations amounted up to 5% of the mean capacity of the transfer across interface values and up to 15% of the mean resistance values.

The scatter of the results increased with increasing crown ether concentration as the membrane stability became then poorer. The capacity and resistance of the transfer through interface membrane/electrolyte solution values were not determined for 1 M KCl because the formation of a second semicircle was observed to start at a KCl concentration as high as 0.1 M KCl.

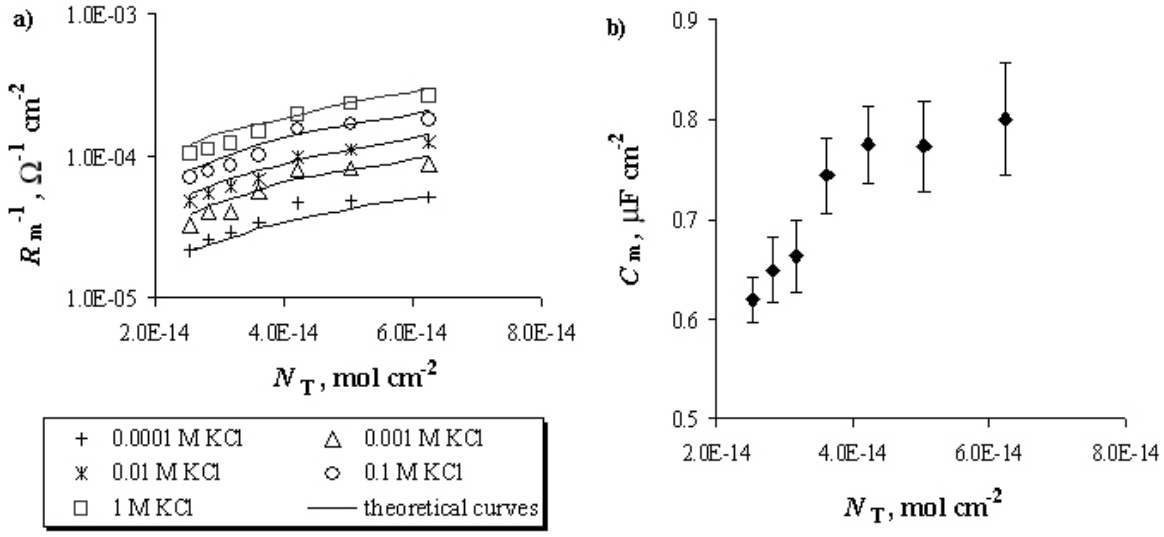


Fig. 4. The dependence of the conductance of the membrane (a) and the capacitance of the membrane (b) on the total dibenzo-18-crown-6 surface concentration at various electrolyte concentrations. The experimental values are marked by points and the theoretical values by solid lines.

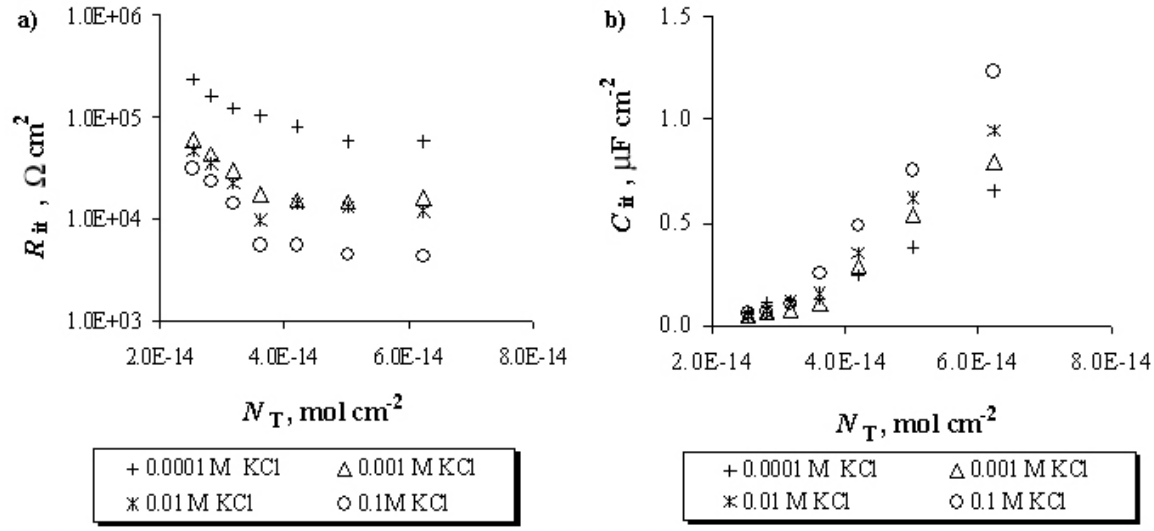


Fig. 5. The dependence of the resistance of the transfer across interface (a) and the capacitance of the transfer across interface (b) on the total dibenzo-18-crown-6 surface concentration at various electrolyte concentrations.

Analyzing the data represented in Figs. 4 and 5 it is possible to state, that an increase in potassium ion concentration at constant dibenzo-18-crown-6 surface concentration provokes a noticeable decrease in Rm (Fig. 4a) and in Rit (Fig. 5a) as well as a noticeable increase in Cit (Fig. 5b). Similarly, both resistances decrease and the capacity of transfer across interface increases with increasing carrier concentration at constant potassium ion concentration (increasing membrane conductivity). The increase in conductivity was due to the increasing amount of the dibenzo-18-crown-6-$K^+$ complex in the membrane, resulting from increasing potassium ion and crown ether concentration. The complex is more soluble in the hydrophobic phase than the hydrated potassium ion itself is. The conductivity increases in the presence of such a complex in the lipid phase, because the macrocyclic compound-potassium ion complex has a net positive charge. This point of view is supported by the results of the study of membrane non-isothermal potential and of kinetic studies on biphasic extraction [26 and literature cited there in].

There are several classes of macrocyclic compounds, which can yield observable changes in the selective $K^+$ permeability of the lipid membranes; the most important among them are depsipeptides and polyethers. The number of ring atoms in these active compounds varies from 18 in the case of enniatin B and dibenzo-18-crown-6 to 36 in valino-

mycin. Enniatin B and dibenzo-18-crown-6 affect the membrane resistance to much weaker extent than valinomycin and the polyene antibiotic, monactin-dinactin, with a 32-atom ring. Polyethers with less than 18 ring atoms cause a greater permeability increase through the lipid bilayer for the sodium ion than that for the potassium ion. Although the substituents on the rings vary in these different compounds, they are all aliphatic in character and lack functional groups. All these compounds, which are able to increase the permeability of the lipid bilayer to the potassium ion, are uncharged [1, 27].

According to Eqn. (11), the membrane conductivity can be expressed as a function of total carrier and/or electrolyte concentration. This Eqn. (11) is of the $y = ax$ type, where: $y = R_m^{-1}$, $x = N_T$ and $a = (S/d^2)\cdot\mu_{MS}\cdot F\cdot(K_h\cdot a_M)/(1 + K_h\cdot a_M)$. The $a$ coefficient was determined using linear regression and it was applied to present the agreement of the Eqn. (11) data (solid lines) with the experimental data (points) in Fig. 4a. It can be seen from this figure that the agreement between the experimental and theoretical points is good, which verifies the correctness of equations presented in this article. From the linear dependence of the membrane conductance on the total crown ether surface concentration one can conclude that a single dibenzo-18-crown-6 molecule is the smallest transporting unit and that this molecule participates as a carrier but not as a channel [28]. The linear increase in the transport of cations together with the growing concentration of crown ether is in agreement with the classical carrier model for ion transport [29]; such a behaviour being observed also in case of polymer [30] and liquid [31] membranes, modified with dibenzo-18-crown-6. The conductance is also proportional to the potassium ion concentration logarithm; this dependence is illustrated graphically in Fig. 6. This fact, together with the Fig. 4a data, suggests that the 1:1 dibenzo-18-crown-6-$K^+$ complex is the carrier of charge in the membrane.

The above equation can be presented in another way, more suitable for calculation:

$$\frac{p}{a_M} = \frac{S}{d^2}\cdot\mu_{MS}\cdot F\cdot K_h - K_h\cdot p \qquad (19)$$

This equation is of the $y = ax + b$ type, where: $y = p/a_M$, $x = p$, $a = K_h$ and $b = (S/d^2)\cdot\mu_{MS}\cdot F\cdot K_h$.

The heterogeneous equilibrium constant of the 1:1 dibenzo-18-crown-6-$K^+$ ion complex formation calculated based on the parameter $a$ amounts to about $3.43\times10^3$ $dm^3\cdot mol^{-1}$.

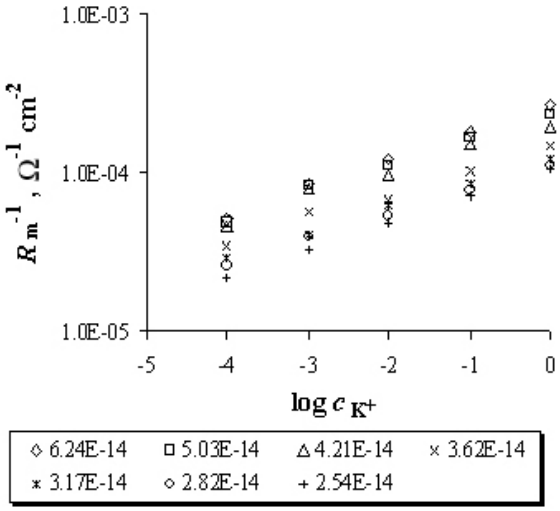


Fig. 6. The dependence of the conductance of the membrane on potassium ion concentration logarithm at various total dibenzo-18-crown-6 surface concentrations.

The values of the stability constants for the 1:1 complexes of the potassium ion with dibenzo-18-crown-6 in various solvents [32–40] are shown in Table 1. The nature of solvent plays the most important role in the complexation reaction because general and specific solvation effects differ in complexed and uncomplexed states [32]. The method of the measurement and the applied counter-ion has a smaller influence on the values of stability constant. The systems in which solvents are showing various solvating effects (from very solvating water to almost inert 1,2-dichloroethane) are represented in Table 1. The data collected in Table 1 reveal that the formation constants of the 1:1 dibenzo-18-crown-6-$K^+$ ion complexes in inert solvents (1,2-dichloroethane) are quite large compared with those in solvating solvents, for example, $10^8$ times larger than those in water and $10^4$ times larger than those in methanol. The desolvation process plays an important role in the complexation in solutions. Particularly, it was reported that the desolvation of metal ions has a large effect in solvating solvents [41].

**Table 1.** Selected values of stability constants of $K^+$ complexes with dibenzo-18-crown-6 in solvents differring in polarity at 25ºC.

| Solvent | log$K$ | References |
|---|---|---|
| water | 1.74 | [33, 34] |
| acetonitrile | 4.80 | [35] |
| metanol | 5.00 | [32, 36] |
| | 5.08 | [35] |
| nitromethane | 5.94 | [34] |
| | 5.39 | [37] |
| nitrobenzene | 6.90 | [38] |
| 1,2-dichloroethane | 9.36 | [39] |
| | 9.90 | [40] |

The value of heterogeneous equilibrium constant presented in this paper describing the chemical reaction at the interface between a carrier molecule from the membrane and a potassium ion from the aqueous phase ($\log K_h$ is equal to 3.54) shows how strong is cation solvation by water in the examined system.

In order to determine the value of dissociation rate constant of the complex and to propose a correct equivalent circuit to reproduce the electric properties of the phosphatidylcholine membrane, modified with crown ether, the experimental data were substituted both in Eqns. (14, 15) and (16, 17). The results were found to agree at low frequencies as it has been shown in Fig. 7 for the imaginary part of the impedance.

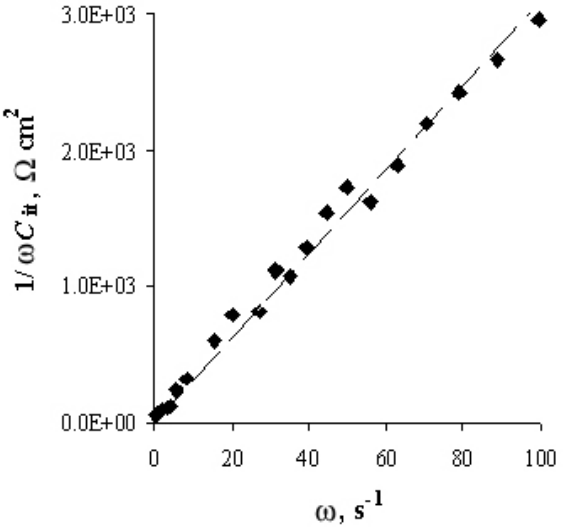


Fig. 7. Frequency dependence of the imaginary part of impedance (f < 15.85 Hz).

This fact was decisive in view that the transfer across interface parameters could be related to the semicircle occurring in the impedance spectrum at low frequencies. The mean $k_D$ value determined from Eqns. (14) and (15) amounts to (133 ± 40) $s^{-1}$. With the dissociation rate constant of the complex being known, the association rate constant was thus calculated from the $k_R = K_h \cdot k_D$ relationship. The resulting value was $(4.56 \pm 1.37) \times 10^5$ $M^{-1} \cdot s^{-1}$. The dissociation and association rate constant values of the complex have not been reported in the available literature of the subject.

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## ИЗМЕРВАНИЯ НА ДВОЙНИ СЛОЕВЕ ОТ ФОСФАТИДИЛХОЛИН СЪДЪРЖАЩИ ДИБЕНЗО-18-КОРОНЕН-6 ЕТЕР ЧРЕЗ ИМПЕДАНСНА СПЕКТРОСКОПИЯ

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(Резюме)

Изследван е ефектът на йонно проводим дибензо-18-коронен-6 етер върху електрохимичните свойства на двуслойна мембрана от фосфатидилхолин чрез импедансна спектроскопия. Експериментите са проведени с различни композиции образуващи разтвор и при различни концентрации на калиевите йони в електролитния разтвор. Като електролит е използван калиев хлорид. На междуфазовата граница липиден двоен слой/електролит се образува комплекс между калиевия йон и молекулата на коронния етер. На основата на математически уравнения са определени константата на хетерогенно равновесие ($K_h$), константите на скоростта на образуване ($k_R$) и на скоростта на дисоциация на комплекса ($k_D$).