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Short Communication

### THE EFFECT OF THE PRESENCE OF CROWN ETHER ON ION TRANSPORT ACROSS THE LIPID BILAYER

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**Abstract:** We studied the electric properties of phosphatidylcholine bilayers modified with crown ether (dibenzo[18] crown-6). The studies were carried out for various crown ether concentrations in forming solutions and various potassium ion concentrations in electrolyte solutions. The presence of crown ether in the membrane influences the membrane's impedance; there is a reduction in its resistivity, a decrease in its resistance of phase transfer and an increase in its capacity of phase transfer with an increase in crown ether concentration in the bilayer and in K<sup>+</sup> ion concentration in the electrolyte solution.

**Key Words:** Impedance Spectroscopy, Lecithin, Dibenzo[18]crown-6.

#### INTRODUCTION

A group of chemical compounds called crown ethers has aroused a great interest in recent years because of their characteristics, which are of importance in preparative chemistry and in biology. New fields of application in research and potential uses in industry have been found for them [1-3]. The crown ethers were the first neutral synthetic compounds which were found to be able to form stable complexes with alkali metal ions. The structures of some macrocyclic polyethers are similar to those of certain naturally-occurring macrocyclic antibiotics, such as valinomycin, which affect cation transport across biological and artificial membranes. The first discovered and most versatile of the aromatic crown compounds is dibenzo[18]crown-6 (Fig. 1) yielding 1:1 complexes with the potassium ion.

The action of the crown ether in the lipid bilayer can be presented as follows: stage 1: the binding of the ion and the stripping of its hydration layer near the surface of the membrane; stage 2: diffusion across the membrane in the form of a complex with the ion; stage 3: the liberation of the ion on the opposite side of the membrane,

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where the ion undergoes hydration; and stage 4: diffusion of the free ionophore to the original membrane surface, closing the ion carrier cycle in the membrane [4].

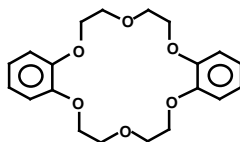


Fig. 1. The structure of 2,3,11,12-dibenzo-1,4,7,10,13,16-hexaoxacyclooctadeca-2,11-diene (dibenzo[18]crown-6).

In this study, through an investigation of the effect of dibenzo[18]crown-6 on the electric properties of the lipid bilayer, we want to demonstrate that the ionophore antibiotics can be replaced by crown ethers in membrane transport processes.

## MATERIALS

3-sn-phosphatidylcholine (lecithin) and dibenzo[18]crown-6 (both of Fluka) were used in the experiment. The phosphatidylcholine was dissolved in chloroform to prevent oxidation; the solvent was evaporated in an atmosphere of argon and the residue was dissolved in a hexadecane solvent. The crown ether was dissolved in chloroform. The forming solutions contained lecithin or crown ether-lecithin mixtures of 1:40, 1:50, 1:60, 1:70 and 1:80 weight ratios. Potassium chloride solutions were used as the electrolytes. The solutions were prepared from mili-Q water and KCl (POCh). KCl was calcinated to remove organic impurities.

All experiments were carried out at room temperature (20-22°C).

## MEASUREMENTS

The model 273A apparatus from the PAR company was used in the impedance measurements; its block diagram was presented in [5]. The measuring vessel used in the studies of the electric properties of lipid bilayer membranes was described exactly in [6]. The electrochemical cell in the four-electrode configuration contained two identical reversible silver-silver chloride electrodes and two identical current platinum electrodes. Bilayer membranes in the form of a bubble built of lipids were produced. The use of hexadecane as the solvent allowed us to obtain membranes of thickness and capacity values similar to those of membranes formed of monolayers [7], whereas short-chain hydrocarbons used as solvents would have increased membrane thickness and would have lowered its capacity. The bilayers were polarized with an alternating current of 4 mV amplitude in the 0.1-10000 Hz frequency range. Data analysis was performed by means of software using a nonlinear least square fit (Equivcrt.Pas) elaborated by B. A. Boukamp [8].

## RESULTS AND DISCUSSION

In the absence of the ion carrier, the impedance diagrams exhibit very simple profiles, showing a capacitive semicircle in the analyzed frequency range, indicating that the lipidic membrane behaves as an isotropic sheet (Fig. 2).

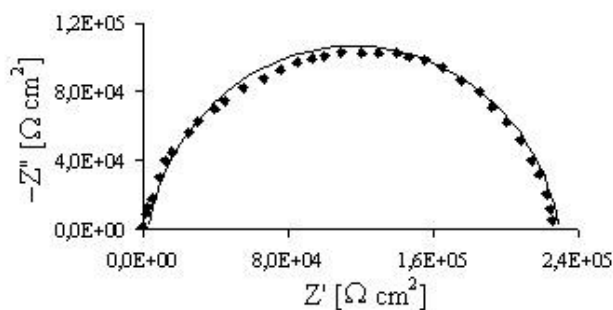


Fig. 2. Nyquist diagrams of phosphatidylcholine membranes in 0.0001M KCl.

The electric properties of the pure lipid membrane are described by electric circuit No. 1, presented in Fig. 3. The possibility of misinterpretation of the recorded data is reduced by simplicity of the circuit [9]. The capacity and resistance of the membrane are represented by  $C_m$  and  $R_m$ , respectively.  $R_0$  is the electrolyte solution resistance; it was assumed to be of ohmic nature and not to perturb the membrane properties. In the 0.0001-0.1M KCl concentration range,  $C_m$  and  $R_m$  were found to be independent of electrolyte concentration; they were equal to  $230 \pm 20 \text{ k}\Omega \text{ cm}^2$  and  $0.62 \pm 0.02 \text{ }\mu\text{F/cm}^2$ , respectively. The parameters are slightly different in the 1 M KCl solution (the resistance is lower and the capacity is higher); this is connected with the entry of some quantity of potassium ions into the interior of the bilayer.

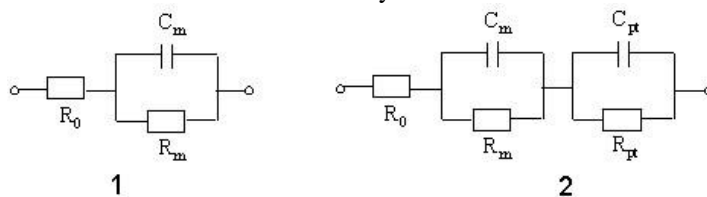


Fig. 3. Equivalent circuits representing the electrical properties of pure phosphatidylcholine membrane (1) and phosphatidylcholine membrane containing dibenzo[18]crown-6 (2).

The frequency response is drastically different when crown ether is present in the membrane. The impedance spectrum of the bilayer modified with dibenzo[18]crown-6 exhibits capacitive contribution at high frequencies, with the indication of a second semicircle at low frequencies (Fig. 4). The appearance of

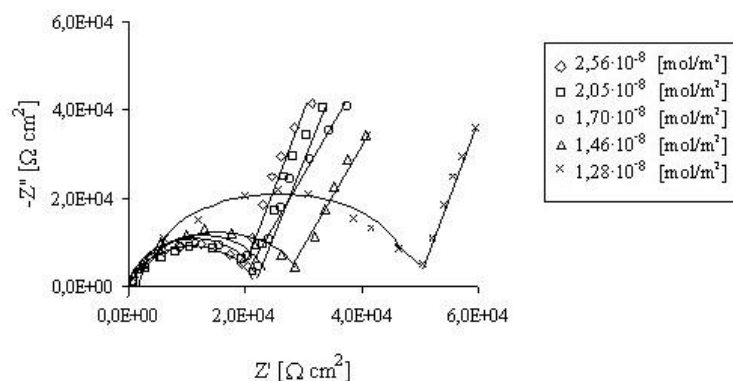


Fig. 4. Nyquist diagrams of phosphatidylcholine membranes modified with crown ether of various analytical concentrations in 0.0001M KCl.

the second semicircle in the impedance spectrum is due to the potassium ion transport caused by the presence of the crown ether in the membrane.

Scheme 2 of Fig. 3 presents a simple model of the membrane in which the impedance components of the membrane and the impedance representing the interface are taken into account.  $C_{pt}$  and  $R_{pt}$  (pt stands for phase transfer) are the capacity and the resistance, respectively, of the membrane-electrolyte solution phase transition [9].

The experimental  $R_m$ ,  $R_{pt}$ ,  $C_{pt}$  parameters are presented in Figs. 5-7 as functions of the  $K^+$  ion concentration in solution and of crown ether concentration in the membrane. The  $C_{pt}$  and  $R_{pt}$  values were not determined for 1M KCl because the formation of the second semicircle was observed to start at a KCl concentration as high as 0.1M KCl.

The presence of the crown ether in the membrane and of the  $K^+$  ion in the solution have no significant effect on membrane capacity, which varies in the  $0.6 \mu\text{F}/\text{cm}^2 < C_m < 0.8 \mu\text{F}/\text{cm}^2$  range. This can be explained by the higher water content in the bilayer, which enhanced electric permittivity, and thus, the capacity of the membrane. The mean values of the measured parameters were obtained from ten independent measurements of the lipid bilayer. The standard deviations are not shown in the figures for the sake of clarity. Deviations were up to 5% of the mean capacity and up to 10% of the mean resistance (the scatter of the results increased with increasing crown ether concentration as the membrane stability was then poorer).

It can be concluded that an increase in potassium ion concentration at constant dibenzo[18]crown-6 concentration provokes a marked decrease in  $R_m$  (Fig. 5) and in  $R_{pt}$  (Fig. 6) and a marked increase in  $C_{pt}$  (Fig. 7). Similarly, both resistances decrease and capacitance increases with increasing crown ether concentration at constant  $K^+$  ion concentration (increasing membrane conductivity).

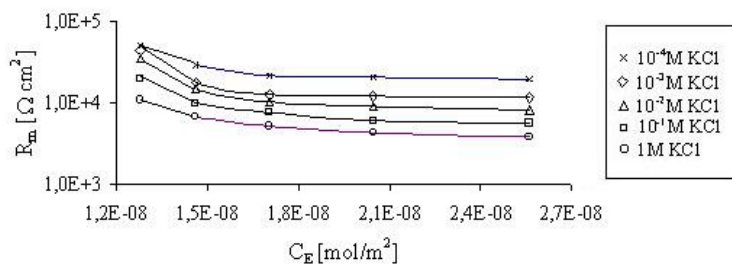


Fig. 5. The dependence of the resistance of the membrane on the analytical crown ether concentration at various electrolyte concentrations

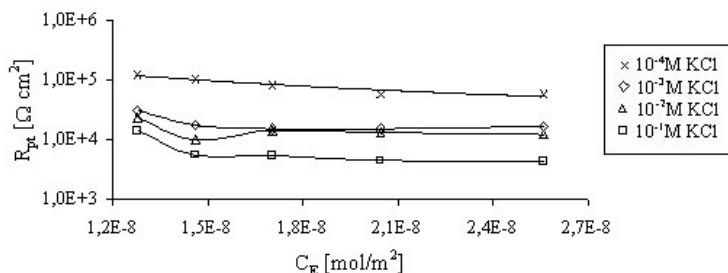


Fig. 6. The dependence of the resistance of transfer on the analytical crown ether concentration at various electrolyte concentrations.

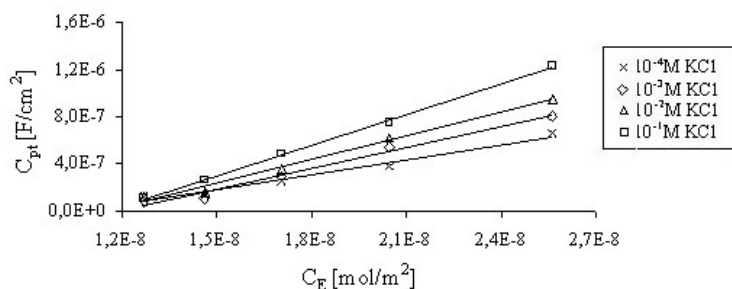


Fig. 7. The dependence of the capacitance of the transfer on the analytical crown ether concentration at various electrolyte concentrations.

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 is the hydrated potassium ion itself. 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 kinetics studies on biphasic extraction [10 and literature cited there in].

There are several classes of macrocyclic compounds which can yield marked changes in the selective  $K^+$  permeability of the lipid membranes. 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. 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 valinomycin. Enniatin B and dibenzo[18]crown-6 affect the membrane resistance to markedly weaker extent than valinomycin and the polyene antibiotic, monactin-dinactin, with a 32 atom ring. Polyethers with less than 18 ring atoms provoke a greater permeability increase across the lipid bilayer for the sodium ion than for the potassium ion. Although the substituents on the rings vary in these different compounds, they are 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 [11].

### CONCLUSIONS

The impedance due to ion transport consists of three elements: the resistance  $R_m$ , characterizing ion flow through the membrane phase, and an interfacial barrier, represented by  $C_{pt}$  and  $R_{pt}$ . The data presented in this paper clearly show the decrease in the  $R_m$  and  $R_{pt}$  resistances with increasing crown ether concentration in the membrane and with increasing potassium ion concentration in the electrolyte solution. The results confirm that it is possible to use dibenzo[18]crown-6 as a phospholipid membrane modifier to increase membrane permeability. It has a great importance in practice, because it permits the creation of an artificial, physicochemical equivalent of biological transport systems.

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