COMPLEXATION CHARACTERISTICS OF MEMBRANE-ACTIVE CROWN-ETHERS

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Sharafutdinova U., Toyirov U., Salakhutdinov B., Tashmukhamedova A., Mirkhodjaev U., 2011: Complexation characteristics of membrane-active crown-ether (*Charakterystyka kompleksowania błono-aktywnych eterów koronowych*), Monitoring Środowiska Przyrodniczego, Vol. 12, s. 125–131, Kieleckie Towarzystwo Naukowe, Kielce.

Abstract: Crown ethers are the large class of membrane-active organic compounds which are intensively studied due to their complexon, ionophoric and channel forming activity. The present study describes the investigations of the possible interactions between sulfo-derivatives of DB18C6 with ions of K^+, Na^+, Ca^{2+} in water and ethanol solutions using conductance-measuring method. The analysis of thermodynamic characteristics of interaction, particularly free Gibbs energy (ΔG) alteration, shows that the process of interaction of crown-ethers and uni- and bivalent ions is held spontaneously. In this case the interaction is supposed to proceed due to forming ion bounds between ions of dissociated salt and charged regions of crown-ethers. The obtained results for the sulfo-derivatives of DB18C6 show no ability to form stable interactions with ions of uni- and bivalent metals neither in water nor in ethanol solutions. It allows us to make a conclusion about non-inophoric nature of investigated compounds and proves the idea of their ability to change membrane permeability by forming ion-transporting structures.

Key words: crown ethers, sulfo-derivatives of DB18C6, ions interactions, conductometric analysis.
Słowa kluczowe: *etery koronowe, sulfopochodne DB18C6, oddziaływania z jonami, analiza konduktometryczna.*

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1. Introduction

Crown ethers represent a large class of compounds which relatively can be easily synthesized and modified, what essentially widens quantity of membrane-active substances. Study of influence of crown ethers on the artificial and biological membranes depending on their molecular constitution. It is essential for a revealing of the rules between the structure and the membrane activity of these compounds to the purposeful synthesis of crown ethers with preset properties.

Crown-ethers reveal complexon, ionophoric and channel forming activity (Sharafutdinova et al., 2007). It was recently shown that sulfo-derivatives of dibenzo-18-crown-6 (then DB18C6) are able to modify the ion permeability of planar lipid bilayers by forming single ion channels permeable to different cations (Yarishkin et al., 2004). The purpose of the present study was to investigate the possible interactions between sulfur-derivatives of DB18K6 with ions of K^+, Na^+, Ca^{2+} in water and ethanol solutions using conductance-measuring method.

2. Materials and methods

Conductometric analysis was used to study complexation characteristics of membrane-active crown-ethers (Andreev et al., 1971) which were synthesized and kindly provided by the laboratory of Tashmukhamedova (Grebenyuk et al., 2001). Structures of crown-ethers are given below (Fig. 1).

The crown-ethers studied in the present investigations were as follows: 4′-tert-butyl-4′′(5′′)-dibenzo-18-crown-6-sulfoacid; 4′-acetyl-4′′(5′′)-dibenzo-18-crown-6-sulfoacid; 4-dibenzo-18-crown-6-sulfoacid; 4′-4′′(5′′)-dibenzo-18-crown-6-disulfoacid. Distilled water and ethanol were used as solvents to prepare crown-ethers stock solutions (0.5 mM). KCl, NaCl, CaCl2 of the highest purity available, were purchased from Reakhim (Mikhailovsk, Russia). We used Student statistic (Rassandrova, Lebedev, 1970).

Crown-ethers were dissolved in water to obtain 0.5mM concentration. Active metal salts were dissolved in water to the 1mM concentration. In the latter case crown-ethers and salts of metals of the first and second group of periodic table were dissolved in ethanol to 0.5mM and 1mM concentration, respectively. The conductivity measurements were carried out at room temperature (20°C) by gradual dilution of DB18C6 water or ethanol solutions with certain volumes of water or ethanol solutions of salt.

The interaction constant was calculated using the equation suggested by Sharafutdinova et. al. (2007).

$$K = \frac{\frac{\Delta\chi}{\delta}10^3}{\left(C - \frac{\Delta\chi}{\delta}10^3\right)\cdot\left(L - \frac{\Delta\chi}{\delta}10^3\right)}$$

Where:

$$\delta_{1,2} = \frac{(L_1 + C_1 - L_2 - C_2) \pm \sqrt{(L_1 + C_1 - L_2 - C_2)^2 - 4(C_1L_1/P_1 - C_2L_2/P_2)\cdot(P_1 - P_2)}}{2(C_1L_1/P_1 - C_2L_2/P_2)},$$

is used to provide that there is no the plateau region on experimental curve.

C1, *L1* and *C2*, *L2* – the concentrations of alkali-earth metal and DB18C6 solutions respectively. In this case C and L characteristics were selected randomly.

$P = \Delta\chi \cdot 10^3$; $\Delta\chi$ – average conductivity of DB18C6 solution (Yarishkin et al., 2004).

3. Results and discussion

Addition of metal salts dissociating to univalent and bivalent cations (KCl, NaCl, CaCl2) in the experimental chamber with presence of dibenzo-18-crown-6's sulfo-derivatives caused the growth of solution's conductivity both in water and ethanol as solvents (Fig. 2, 3, 4, 5). In the case of acetyl-DB18C6, tertbutyl-DB18C6, DB18C6-disulfo-acid ions the conductivity grew correspondingly to increasing of salt concentration in the chamber. Their initial conductivity did not reach the values higher than 1 (Fig. 2A, B, D). Whereas the DB18C6-monosulfo-acid showed the high initial conductivity which grew almost linearly (Fig. 2C).

In the experiments with ethanol as solution only three sulfo-derivatives were used. The Figs. 4, 5 represent the changes of solution conductivity with the presence of ethanol as diluent. Apparently it did not affect the characteristics of conductometric curves considerably. Along titration curve the quantitative characteristics of the interaction of DB18C6 with uni- and bivalent cations in water and ethanol: interaction constant ($K \times 10^4$) and Gibbs free energy (ΔG) in kJ were calculated (Table 1).

Figure 6 shows the correlation of complexation constants changes by K^+/Na^+ ratio depending on the structure of crown-ethers dissolved either in water or ethanol, respectively.

As it is shown in Figure 5 the complexation constant by Ca^{2+} ions changes with respect to DB18C6-sulfo-derivatives' structure.

K^+/Na^+ selectivity of complexation observed for 4'-DB18C6-sulfoacid dissolved in ethanol was 4.2 and has a significant importance as a biological matter.

Previously we reported that DB18C6-sulfoderivatives possess amphiphilicity to a variable degree and non-polar affinity decrease in the following order: 4′-tret-butyl-4′′(5′′)-dibenzo-18-crown-6-sulfoacid; 4′-acetyl-4′′(5′′)-dibenzo-18-crown-6-sulfoacid; 4-dibenzo-18-crown-6-sulfoacid; 4′-4′′(5′′)-dibenzo-18-crown-6-disulfoacid (Andreev et al. 1971).

The highest Ca_{2+} selectivity of complexation was observed for 4′-acetyl-4′′(5′′)-dibenzo-18-crown-6-sulfoacid when crown-ethers were dissolved in ethanol, and for 4′-tert-butyl-4′′(5′′)-dibenzo-18-crown-6-sulfoacid when dissolved in water.

The analysis of our results suggests that practically all the carried out conductometry experiments with K^+, Na^+ and Ca^{2+} complexations are more efficient when crown-ethers are being dissolved in ethanol. These parameters are several orders higher in comparison to those received from crown-ethers dissolved in water. The only exception was in Ca^{2+} complexation experiments when 4′-tret-butyl-4′′(5′′)-dibenzo-18-crown-6-sulfoacid was dissolved in ethanol and complexation constant was 10 times lees than when dissolved in water.

Thus it can be seen from figure 7 that the stability of 4′-acetyl-4′′(5′′)-dibenzo-18-crown-6-sulfoacid and

Ca_{2+} ions complex differs from the stability of other complexes of DB18C6-sulfoderivatives. As it was mentioned above stability constant represents the measure of caloricity and entropy alterations at complexation reaction because complex stability grows with the growth of caloricity and the entropy. Apparently it happens when 4′-acetyl-4′′(5′′)-dibenzo-18-crown-6-sulfoacid forms complexes with Ca2+ ions in ethanol.

On the other hand the structure of 4′-acetyl-4′′(5′′)-dibenzo-18-crown-6-sulfoacid proposes that its sulfonate group, which works as acceptor, may turn into donor that in its case leads to reactivity growth of the second benzyl nucleus. There are appropriate conditions for converting the benzenoid into chinnoid structure that results in formation of absolute positive charge on catechol oxygen and consequently formation of "three-leg" structure of macrocycle (Fig. 8) (Tashmukhamedova et al., 1998).

The other 5 oxygens of macrocycle have a couple of free electrons that are able to bind Ca^{2+} ions. Similar effects we observed before while investigating the complexation of DB18C6-diacylderivatives (Yarishkin et al. 2003). Hence, the chinnoid structure formation leads to significant conformational changes of 4′-acetyl-4′′(5′′)-dibenzo-18-crown-6-sulfoacid in ethanol solution. That proves once more that entropy term distributes more into complexation reaction of this crown-ether in comparison with other observed DB18C6-sulfoderivatives.

Addition of uni- and bivalent salts (K^+, Na^+, Ca^{2+}) in the experimental chamber with dibenzo-18-crown-6's sulfo-derivatives revealed that DB18C6 are not able to form stable complexones with ions of metals both in water and ethanol solutions. The analysis of curves of conductometric titration showed that 4′-tert-butyl-4′′(5′′)-dibenzo-18-crown-6-sulfoacid, 4′-acetyl-4′′(5′′)-dibenzo-18-crown-6-sulfoacid and 4′-4′′(5′′)-dibenzo-18-crown-6-disulfoacid didn't make the significant contribution to the conductivity changes and the growth of conductivity had come about through the presence of dissociated salt ions. On the contrary the linear increasing of the conductivity of 4-dibenzo-18-crown-6-sulfoacid solution may be the result of formation the ion bonds between dissociated salt cations and charged groups in DB18C6 molecule. Thus, received data indicate that in symmetrical molecules the process of interaction is prohibited by the presence of additional functional groups such as acetyl, tribute and extra sulfo residues. However, similar curves are observed in the experiments with weak complexones (Scrachitelli, 1963).

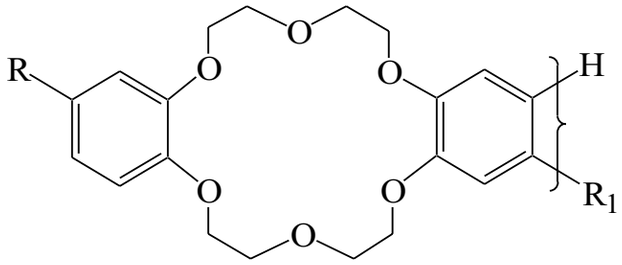
Complexation capability alters dependently on changes of energy of interaction between cations and oxygen donor atoms of macrocycle as substitutive atoms at lateral benzoid cycles definitely cause charge distribution in cycle that means the certain effect on electrostatic interaction between cation and ligand. Solvation of initial reagents and their complexes also has undoubted deposit. That explains such an obvious dependence of complexation in solution from enthalpy change, the thermodynamical function that the best reveals the salvation processes of initial reagents as well as the energy of binding with donor atoms of macrocycle.

By the example of DB18C6-sulfoderivatives it is possible to say that one should not neglect the probable conformational changes in the molecule of crown-ether according to the nature of substitutive atom. Obviously exactly these sorts of conformational changes in crown-ether molecules at complexation increase the entropy distribution to termodynamical process.

4. Conclusions

The analysis of thermodynamic characteristics of interaction, particularly free Gibbs energy (ΔG) alteration, shows that the process of interaction of crown-ethers and uni- and bivalent ions is held spontaneously. In this case the interaction is supposed to go due to forming ion bounds between ions of dissociated salt and charged regions of crown-ethers. The characteristics of interaction constants were up to dissolvent's nature and higher when dissolved in ethanol that points to the possibility of the dissolvent to take pert in the interaction process. However the interaction constant values were 1-2 orders lower than it was observed before for effective complexones (Sharafutdinova et al., 2007; Scrachitelli, 1963; Mirkhodjaev, 1986).

The obtained results for the sulfo-derivatives of DB18C6 show no ability to form stable interactions with ions of uni- and bivalent metals neither in water nor in ethanol solutions acting as almost weak electrolyte in the both of solvents. It allows us to make a conclusion about non-inophoric nature of investigated compounds and proves the idea of their ability to change membrane permeability by forming ion-transporting structures.



R = –C(CH$_3$)$_3$; R$_1$ = –SO$_3$H	4''-tret-butil-4''(5'')-DB18C6-sulfoacid (1)
R = –COCH$_3$; R$_1$ = –SO$_3$H	4'-acetil-4''(5'')-DB18C6-sulfoacid (2)
R = –H; R$_1$ = –SO$_3$H	4'-DB18C6-sulfoacid (3)
R = –SO$_3$H; R$_1$ = –SO$_3$H	4',4''(5'')-DB18C6-disulfoacid (4)

Fig. 1. Chemical structures of studied macrocyclic sulfoderivatives
Ryc. 1. Struktura chemiczna badanych sulfopochodnych makrocyklicznych

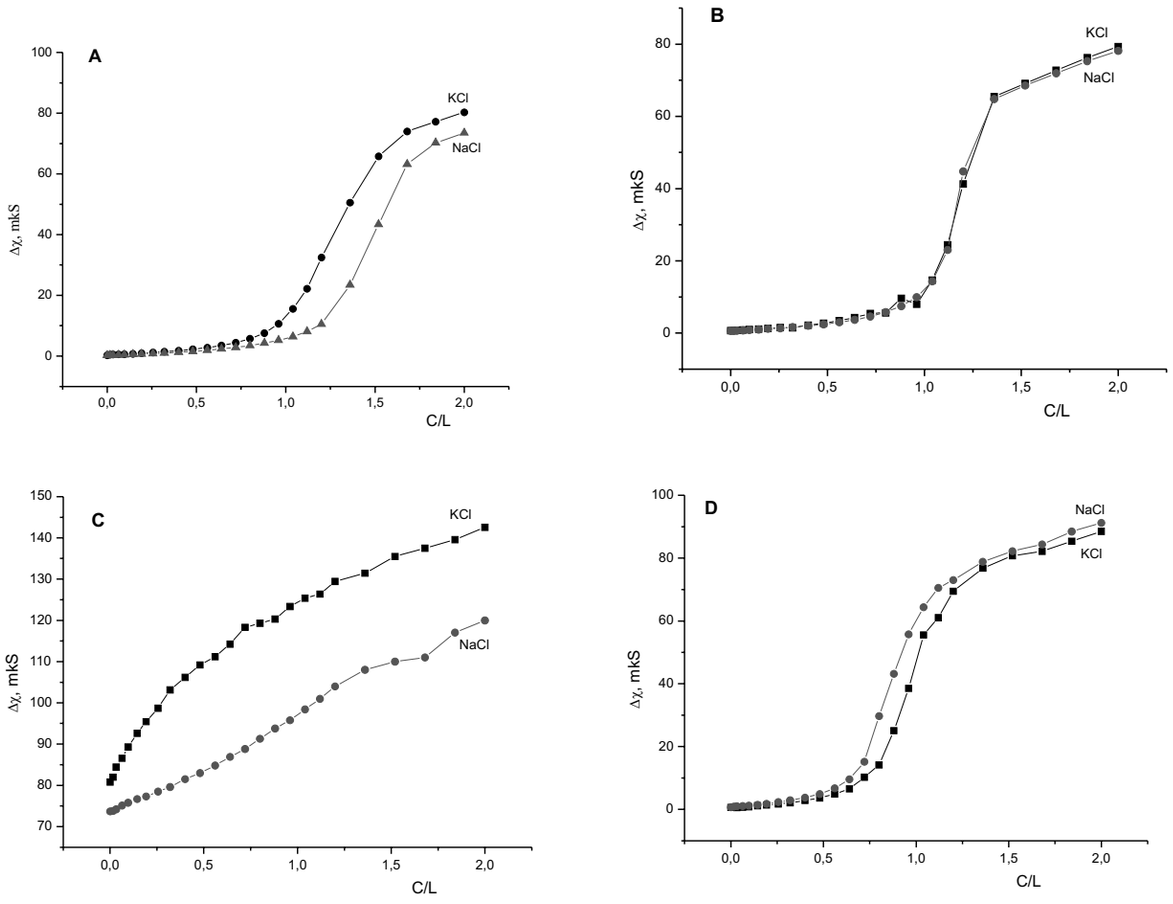


Fig. 2. Curves of conductometric titration of sulfo-derivatives of DB18C6 in water with salts of univalent cations: K^+, Na^+. Δχ is conductivity values of DB18C6 solutions in presence of univalent salt solutions (in mkS). C/L is the ratio of concentration DB18C6 sulfo-derivatives to concentration of salts, respectively
A – 4′-tert-butyl-4′′(5′′)-dibenzo-18-crown-6-sulfoacid; **B** – 4′-acetyl-4′′(5′′)-dibenzo-18-crown-6-sulfoacid; **C** – 4-dibenzo-18-crown-6-sulfoacid; **D** – 4′-4′′(5′′)-dibenzo-18-crown-6-bisulfoacid.
Ryc. 2. Krzywe miareczkowania konduktometrycznego sulfopochodnych DB18C6 w wodzie z solami jednowartościowych kationów: K^+, Na^+. Δχ oznacza wartości konduktywności roztworów DB18C6 w obecności roztworów soli kationów jednowartościowych. C/L oznacza stosunek stężeń sulfopochodnych DB18C6 do stężenia soli

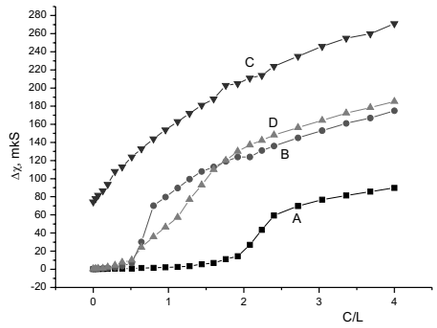


Fig. 3. Curves of conductometric titration of sulfo-derivatives of DB18C6 in water with salts of bivalent cations of Ca^{2+}. Δχ is conductivity values of Db18C6 solutions in presence of bivalent salt solutions (in mkS). C/L is the ratio of concentration DB18C6 sulfo-derivatives to concentration of salt. **A** – 4' tert-butyl-4′′(5′′)-dibenzo-18-crown-6-sulfoacid; **B** – 4′-acetyl-4′′(5′′)-dibenzo-18-crown-6-sulfoacid; **C** – 4-dibenzo-18-crown-6-sulfoacid; **D** – 4′-4′′(5′′)-dibenzo-18-crown-6-bisulfoacid

Ryc. 3. Krzywe miareczkowania konduktometrycznego sulfopochodnych DB18C6 w wodzie z zawartością soli kationów dwuwartościowych. Δχ oznacza wartości konduktywności roztworów DB18C6 w obecności roztworów soli kationów dwuwartościowych. C/L oznacza stosunek stężeń sulfopochodnych DB18C6 do stężenia soli

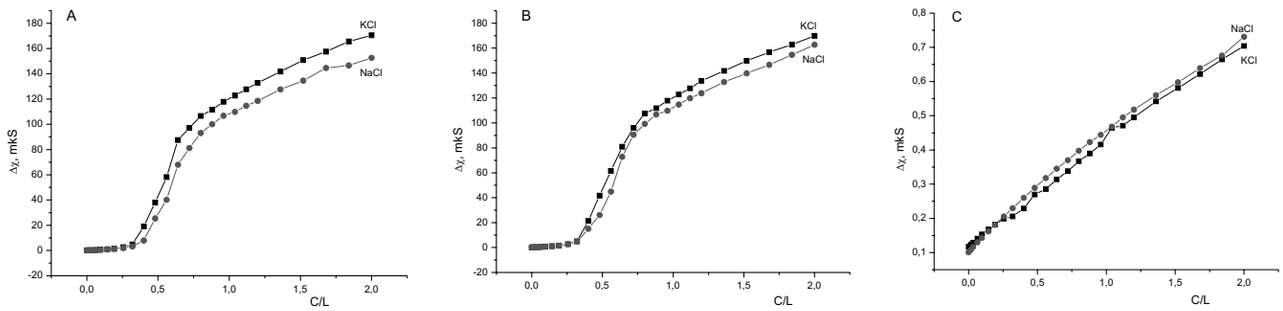


Fig. 4. Curves of conductometric titration of sulfo-derivatives of DB18C6 in ethanol with salts of univalent cations: K^+, Na^+. Δχ is conductivity values of Db18C6 solutions in presence of univalent salt solutions (in mkS). C/L is the ratio of concentration DB18C6 sulfo-derivatives to concentration of salts, respectively

A – 4′-acetyl-4′′(5′′)-dibenzo-18-crown-6-sulfoacid; **B** – 4-dibenzo-18-crown-6-sulfoacid; **C** – 4′-4′′(5′′)-dibenzo-18-crown-6-bisulfoacid.

Ryc. 4. Krzywe miareczkowania konduktometrycznego sulfopochodnych DB18C6 w etanolu z solami jednowartościowych kationów: K^+, Na^+. Δχ oznacza wartości konduktywności roztworów DB18C6 w obecności roztworów soli kationów jednowartościowych. C/L oznacza stosunek stężeń sulfopochodnych DB18C6 do stężenia soli

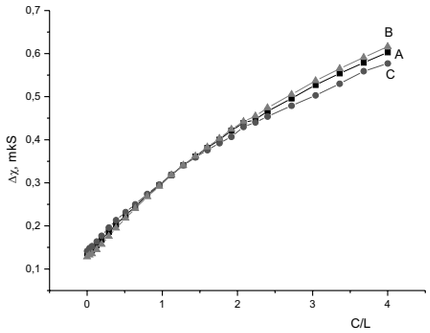


Fig. 5. Curves of conductometric titration of sulfo-derivatives of DB18C6 in ethanol with salts of bivalent cations of Ca^{2+}. Δχ is conductivity values of DB18C6 solutions in presence of bivalent salt solutions (in mkS). C/L is the ratio of concentration DB18C6 sulfo-derivatives to concentration of salt

A – 4′-acetyl-4′′(5′′)-dibenzo-18-crown-6-sulfoacid; **B** – 4-dibenzo-18-crown-6-sulfoacid; **C** – 4′-4′′(5′′)-dibenzo-18-crown-6-disulfoacid

Ryc. 5. Krzywe miareczkowania konduktometrycznego sulfopochodnych DB18C6 w etanolu z solami dwuwartościowych kationów Ca^{2+}. Δχ oznacza wartości konduktywności roztworów DB18C6 w obecności roztworów soli kationów dwuwartościowych. C/L oznacza stosunek stężeń sulfopochodnych DB18C6 do stężenia soli

Tab. 1. The quantitative characteristics of the interaction of DB16C6 with univalent and bivalent cations in water and ethanol solutions.
Tab. 1. Ilościowa charakterystyka oddziaływania DB16C6 z jednowartościowymi i dwuwartościowymi kationami w roztworach wodnych i etanolowych

	Cations	$K \times 10^4$		ΔG (kJ)	
		Water	Ethanol	Water	Ethanol
4'-tertbutyl-4''(5'')-DB18C6-sulfo-acid	K^+	1.63	–	-23.64	–
	Na^+	2.42	–	-24.65	–
	Ca^{2+}	9.93	–	-28.03	–
4'-acetyl-4''(5'')-DB18C6-sulfoa-cid	K^+	0.97	92.11	-22.37	-33.45
	Na^+	11.34	100.08	-28.35	-33.65
	Ca^{2+}	1.41	174.48	-23.29	-35.02
4'-DB18C6-sulfo-acid	K^+	1.0	131.44	-16.82	-34.31
	Na^+	0.10	54.85	-22.70	-26.25
	Ca^{2+}	0.30	42.81	-19.15	-31.59
4-4''(5'')-DB18C6-disulfo-acid	K^+	1.22	80.74	-22.92	33.13
	Na^+	0.93	214.75	-22.24	-35.51
	Ca^{2+}	0.83	7.45	-21.98	-27.33

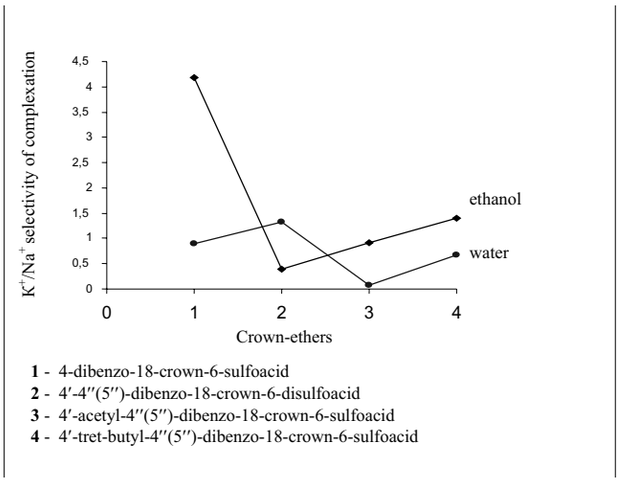


Fig. 6. The variability of K^+/Na^+ selective complexation – DB18C6-sulfoderivatives relationship
Ryc. 6. Zmienność selektywnego kompleksowania K^+/Na^+ – oddziaływanie sulfopochodnych DB18C6

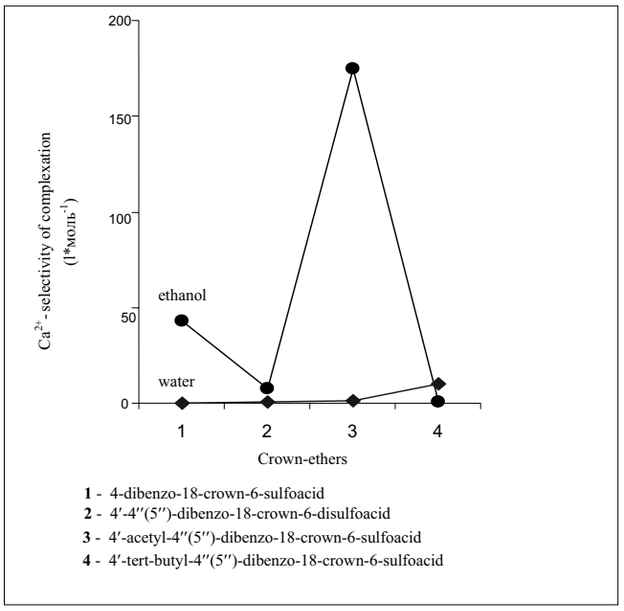


Fig. 7. The variability of Ca2+ selectivity complexation – DB18C6-sulfoderivatives relationship
Ryc. 7. Zmienność selektywnego kompleksowania Ca2+ - oddziaływanie sulfopochodnych DB18C6

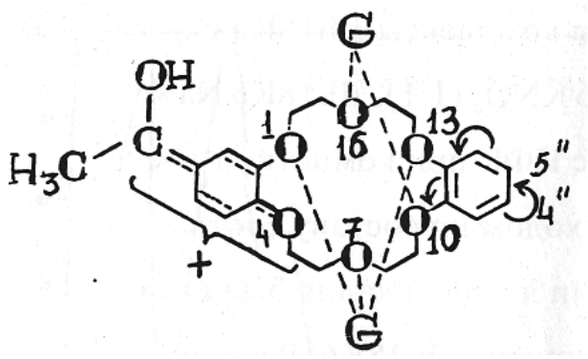


Fig. 8. The assumed structure of the oxonium cation
Ryc. 8. Przypuszczalna struktura kationu oksoniowego

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CHARAKTERYSTYKA KOMPLEKSOWANIA BŁONO-AKTYWNYCH ETERÓW KORONOWYCH

Streszczenie

Etery koronowe stanowią dużą grupę błonowo-aktywnych związków organicznych, które są intensywnie badane ze względu na swoją aktywność kompleksującą, jonoforową i możliwość tworzenia kanałów jonowych. Niniejszy artykuł opisuje możliwe oddziaływania między sulfopochodnymi DB18C6 z jonami K^+, Na^+, Ca^{2+} w roztworach wodnych i alkoholowych, badane metodami konduktometrycznymi. Analiza oddziaływań termodynamicznych wykazała, że proces oddziaływania eterów koronowych z jonami jedno- i dwuwartościowymi przebiega spontanicznie. Oddziaływania te polegają na tworzeniu wiązań jonowych między jonami zdysocjowanych soli z naładowanymi rejonami eterów koronowych. Wyniki otrzymane dla sulfopochodnych DB18C6 nie wykazały możliwości tworzenia stabilnych oddziaływań z jonami jedno- i dwuwartościowymi ani w roztworach wodnych, ani alkoholowych. Pozwala to na wyciągnięcie wniosku dotyczącego niejonoforowego charakteru badanych związków i potwierdza sugestię dotyczącą ich zdolności do zmiany przepuszczalności błon w wyniku tworzenia struktur transportujących jony.