$^1$H NMR and spectrophotometric study of alkaline metal ion complexes with N-dansyl aza-18-crown-6

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Abstract: Formation of complexes of A18C6-Dns and metal cations (Ca$^{2+}$, Sr$^{2+}$, Ba$^{2+}$ and Mg$^{2+}$) in acetonitrile has been studied by NMR, absorption and fluorescence spectroscopy and PM5 semi-empirical methods. A18C6-Dns forms stable complexes with Ca$^{2+}$, Sr$^{2+}$ and Ba$^{2+}$ cations. The stability constants of various complexes are determined by different methods and their structures are visualised by the PM5 semi-empirical calculations.

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1 Introduction

Chromoionophores are an important group of supramolecular ligands whose absorption and fluorescence spectra change upon complexation [1–8]. A typical fluorescent moiety used in chemistry for labelling different classes of compounds is the dansyl (5-dimethylamino-1-naphtyl) fragment. Usually dansyl is introduced into the structure via a sulphonamide bond with a primary amino group. This yields the ligands which could interact with metal ions by coordination of the amino group, followed by abstraction of sulphonamide hydrogen atom [9]. This paper reports NMR, absorption and fluorescence
spectroscopy studies of complex formation between a dansyl substituted aza-18-crown-6 macrocyclic ligand (Fig. 1) and alkaline metal ions. The usage of the crown ether as a matrix binding the metal ions enabled us to examine the correlation between the geometrical properties of the inclusion cavity and radii of the alkaline earth metal cations with a pendant fluorophore.

![Fig. 1 Structure of the A18C6-Dns studied.](image)

2 Experimental

The A18C6-Dns was synthesized according to the procedure described by Sulowska [4, 10] using 1.93 mmol 1,4,7,10,13-pentaoxa-16-azacyclodecane, 2.00 mmol of 5-dimethylamine-1-naphthylensulphonic chloride and 2 mmol anhydrous caesium carbonate.

The perchlorates were purchased from Aldrich and were dried in vacuum. The final water contents in all salts were less than 0.05 mol per 1 mol of MClO$_4$.

2.1 Absorption and fluorescence spectroscopy

The absorption spectra were recorded on a Lambda 40P (Perkin Elmer) spectrometer, whereas the fluorescence spectra were recorded using a LS-50B (Perkin Elmer) spectrometer using 340 nm excitation wavelength in the range 400 – 750 nm with a spectral band width 4.5 nm for excitation and emission at 25 °C. The solutions of the compound studied and metal cations were prepared directly before measurements. The titration with the spectroscopic methods was performed by adding to solution of A18C6-Dns (usually around 1.5·10$^{-4}$ mol·dm$^{-1}$ for absorption spectroscopy and 2·10$^{-5}$ mol·dm$^{-1}$ for fluorescence spectroscopy) a solution of metal salt (their concentration was 15-30 times high than this of ligand). The stability constants of the complexes were determined using the
software EQUID [11], by minimisation of the differences between the theoretical model and the experimental data, following the method of Gauss-Newton-Marquart for non-linear equations.

2.2 $^1$H NMR

$^1$H NMR spectra were recorded on a Varian Gemini 300 spectrometer, operating at frequency of 300 MHz, at the spectral width of 4500 Hz; acquisition time 4 s; pulse width 60$^\circ$; temperature 293 K. The samples were dissolved in anhydrous acetonitrile-$d_3$ under inert atmosphere. Perchlorates were carefully dried and handled under nitrogen. The concentration of ligand was set to 0.02 mol·dm$^{-3}$ and the metal salt solution was added to the solution of A18C6-Dns to obtain various metal/ligand ratios. The spectra were recorded for metal ion concentrations varying from 0 to 0.06 mol·dm$^{-3}$ with the step equal to 0.002 mol·dm$^{-3}$. The stability constants were determined from the three or four of the most representative value of chemical shifts of protons. The log$K$ values were obtained from simultaneous fitting of all protons taken for analysis.

2.3 Theoretical calculation

The geometric structures were calculated on PC class computers using the software CAChe Work System Pro, version 5.03, by the semiempirical method PM5 from the packet MOPAC 2002 [12–16]. The complex energy was calculated by the RHF method, while the partial charges by the Mulliken population analysis. The starting conformations were calculated by CONFLEX module, analysing the molecule to find the structures of energy the closest to the global energy minimum. The procedure gave a series of conformations of representative systems subjected to further analysis. Barium is not fully parameterised in this version of program, so for its ions the calculations were not done.

3 Results and discussion

The molecule of A18C6-Dns ionophore is composed of two fragments. The first is the aza crown ether, capable of complexation with ions in solutions, and the second fragment is responsible for the fluorescence of the molecule. Multifunctional systems of this type are particularly important in biological and medical researches as well as in construction of molecular machines [17].

3.1 Absorption spectroscopy

The absorption spectrum of A18C6-Dns in acetonitrile reveals two maxima at $\lambda = 338$ nm ($\varepsilon = 4100$ dm$^3$·mol$^{-1}$·cm$^{-1}$) and $\lambda = 253$ nm ($\varepsilon = 11800$ dm$^3$·mol$^{-1}$·cm$^{-1}$) and is similar to the spectra of other dansyl derivatives previously reported [18]. The interaction of Ca$^{2+}$, Sr$^{2+}$ and Ba$^{2+}$ ions with A18C6-Dns ionophore induces changes in the absorption band
positions, however, no changes are caused by the presence of Mg$^{2+}$ ions. The absorption spectra of the complexes with the ions Ca$^{2+}$, Sr$^{2+}$ and Ba$^{2+}$ show a characteristic band at $\lambda = 352$ nm ($\varepsilon = 3900$ dm$^3$·mol$^{-1}$·cm$^{-1}$), red-shifted by 14 nm corresponding to free ligand. Fig. 2 presents the absorption spectrum of acetonitrile solution of A18C6-Dns and the ionophore with an addition of calcium perchlorate.

![Absorption spectra recorded in acetonitrile solution containing A18C6-Dns (1.5×10$^{-4}$mol dm$^{-3}$) and calcium perchlorate (0-1.27×10$^{-3}$ mol dm$^{-3}$).](image)

**Fig. 2** Absorption spectra recorded in acetonitrile solution containing A18C6-Dns (1.5×10$^{-4}$mol dm$^{-3}$) and calcium perchlorate (0-1.27×10$^{-3}$ mol dm$^{-3}$).

To determine the stability constants of A18C6-Dns ionophore complexes with metal ions, the ligand studied were titrated, recording the spectral changes at 338 nm. Fig. 3a shows a dependence of absorbance at 338 nm for A18C6-Dns as a function of molar ratio $c_{Ca^{2+}}/c_L$. The linear dependence of the absorbance at 338 nm to the absorbance at 380 nm obtained for all studied arrangements confirms only 1:1 complex formation [19]. Fig. 3b presents an exemplary diagram of this correlation plotted for A18C6-Dns with a calcium perchlorate mixture in acetonitrile.

The stability constants determined from the absorption spectra are collected in Table 1. For the A18C6-Dns complexes with the ions Ca$^{2+}$, Sr$^{2+}$ and Ba$^{2+}$ the stability constants do not differ significantly and increase in the sequence Ba$^{2+}$<Ca$^{2+}$<Sr$^{2+}$. Their values are directly related to the degree of fit of the ions to the macrocycle cavity. The changes in the dansyl absorption band position observed in the spectra of all compounds studied prove that the cation complexation has some effect on the intramolecular charge transfer in the fluorophore. The bathochromic shift is a result of the interaction of the metal ion binding to the crown ether with the naphthalene ring being a donor group. This conclusion is confirmed by the changes in the chemical shifts of the signals assigned to the aromatic protons of A18C6-Dns caused by the presence of the alkali earth metal ions.
**Fig. 3a** Dependence of absorbance at 338 nm for A18C6-Dns as a function of molar ratio $\frac{C_{Ca^2+}}{C_{A18C6-Dns}}$.

**Fig. 3b** Absorbance-absorbance diagram for measurements of A18C6-Dns with calcium perchlorate in acetonitrile solution.
3.2 Fluorescence spectroscopy

A18C6-Dns in acetonitrile shows green fluorescence typical of dansyl derivatives [20, 21] at $\lambda = 521$ nm, (Fig. 4a and 4b).

![Fluorescence spectra](image)

**Fig. 4a** Spectrofluorimetric titration of A18C6-Dns in acetonitrile solution ($2.1 \times 10^{-5}$ mol dm$^{-3}$) with calcium perchlorate ($0-4.1 \times 10^{-4}$ mol dm$^{-3}$).

Its quantum yield, determined by the reference standard method with quinine solution in sulphuric acid as a standard [22], is 0.24. The changes in the emission spectra of the compounds effected by the metal ions are more differentiated than those in the absorption spectra. The presence of metal ions is responsible for a decrease in the intensity of dansyl fluorescence and a small bathochromic shift. The effect is the smallest for magnesium ions, while for calcium, strontium and barium ions it reaches significant values up to 85 %. Addition small amount of water (1-3 mol%) have no effect on the spectroscopic properties of a ligand and ligand-metal ions system. The stability constants of bivalent ions complexes with A18C6-Dns are given in Table 1. The values are slightly different that those obtained from UV-Vis and NMR spectroscopy. The comparison is difficult because this method considers the excited states for which the binding constants could differ.
Fig. 4b Fluorescence intensity at 522 nm as a function of molar ratio $C_{Ca^2+}/C_{A18C6-Dns}$.

| Complexes                | log$K_{UV}$ spectroscopy | log$K_{fluorescence}$ | 1H NMR |
|--------------------------|--------------------------|-----------------------|--------|
| (A18C6-Dns + Mg)$^{2+}$  | nd                       | nd                    | 3.1(± 0.6) |
| (A18C6-Dns + Ca)$^{2+}$  | 5.56 (± 0.03)            | 5.70 (± 0.05)         | 5.5 (± 0.5) |
| (A18C6-Dns + Sr)$^{2+}$  | 5.91 (± 0.08)            | 5.67 (± 0.04)         | 5.6 (± 0.8) |
| (A18C6-Dns + Ba)$^{2+}$  | 5.30 (± 0.04)            | 5.51 (± 0.01)         | 5.3 (± 0.7) |

Table 1 The stability constants of complexes of A18C6-Dans with Mg$^{2+}$, Ca$^{2+}$, Sr$^{2+}$ and Ba$^{2+}$ in acetonitrile solution determined by the UV spectroscopy, spectrofluorimetry and 1H NMR.

3.3 1H NMR studies

1H NMR chemical shifts of free A18C6-Dns and its complexes with magnesium, calcium, strontium and barium metal ions are presented in Table 2.

In the spectra of the A18C6-Dns complexes studied, the significant chemical shift changes, induced by ion complexation, were observed especially for H-2, H-4 and H-8 signals. The resonance line of protons in the para position (H-4) and of protons in the ortho position (H-2) to the sulphonamide group shift downfield upon complexation. The signal of the protons in the para position to the dimethylamine group (H-8) is shifted upfield. Similar trends are observed for complexes with calcium, strontium and barium
Table 1

| Compounds     | N-CH$_2$ | O-CH$_2$ | N-CH$_3$ | H-2  | H-3  | H-4  | H-6  | H-7  | H-8  |
|---------------|----------|----------|----------|------|------|------|------|------|------|
| A18C6-Dns     | 2.91     | 3.50-3.70| 2.80     | 8.54 | 7.58 | 8.17 | 7.26 | 7.59 | 8.27 |
| A18C6-Dns + Mg$^{2+}$ | 2.93     | 3.50-3.70| 2.83     | 8.58 | 7.63 | 8.05 | 7.30 | 7.63 | 8.26 |
| A18C6-Dns + Ca$^{2+}$ | 2.94     | 3.50-3.70| 2.81     | 8.67 | 7.70 | 8.32 | 7.31 | 7.66 | 7.92 |
| A18C6-Dns + Sr$^{2+}$ | 2.98     | 3.50-3.70| 2.81     | 8.68 | 7.72 | 8.40 | 7.31 | 7.67 | 7.78 |
| A18C6-Dns + Ba$^{2+}$ | 2.93     | 3.50-3.70| 2.81     | 8.66 | 7.69 | 8.31 | 7.31 | 7.66 | 7.97 |

Table 2 $^1$H NMR chemical shifts of compounds studied (for complexes limiting chemical shifts were given).

For the magnesium complexation process the shift changes are much smaller than for other ions. Moreover, the H-4 line moves downfield, contrary to the shift in the spectra of the other complexes. An increase in the chemical shifts of the signals assigned to almost all aromatic protons on complexation can indicate a decrease in the electron density over the whole naphthalene ring. The results of $^1$H NMR titration of A18C6-Dns with the ions Mg$^{2+}$, Ca$^{2+}$, Sr$^{2+}$ and Ba$^{2+}$ permitted determination of the complex stability constants on the basis of chemical shift changes. The NMR titration curves reveal a single inflection point for all systems, which proves that the systems tend to the same equilibrium. Results of modelling of equilibria by the software EQUID [23] have confirmed the formation of 1:1 stoichiometric complexes in the systems studied. The stability constants, calculated from NMR measurements are very close to those obtained from absorption spectroscopy. However, they also indicate that the ligand complexes with strontium ions are more stable than those of the calcium and barium complexes (Table 1). The $^1$H NMR spectra of aromatic protons of the ligand studied, recorded for a metal to ligand ratio varying from 0 to 2, are shown in Fig. 5(a). The Ca$^{2+}$ and Sr$^{2+}$ cations, whose ion diameters (0.99 and 1.13 Å respectively) are similar to that of the macrocyclic cavity (about 1.3 Å), form relatively stable complexes with the A18C6-Dns ligand. The Ba$^{2+}$ ion is too large (1.35 Å) and could not fit into the ligand macrocyclic ring. Any evidence of the 1:2 sandwich complex formation for Ba$^{2+}$ ions was not found. For this case the ion is placed slightly over the ring plane. In the case of magnesium (0.65 Å), there are two reasons for weak metal binding by the compound studied. The first is the difference of the sizes of the ion and the cavity and the second is the relatively weak interaction between Mg$^{2+}$ ions and polyethers. For the complexes with calcium and strontium ions, strong signal broadening was observed in the spectra recorded at a metal/A18C6-Dns ratio less than 1. This is due to strong ion binding and, as a consequence, a slow exchange between free and complexed species. For the complexes of barium and magnesium ions, the exchange processes are fast and the resonance lines are narrow.
3.4 Theoretical calculation

The results of PM5 semiempirical calculations of HOF values for the free ligand and its complexes with metal ions studied and the values of the energy of stabilisation (defined as a difference between HOF of complex and the sum of isolated ligand and metal cation) are presented in Table 3.

For the complexes with the Mg$^{2+}$ and Ca$^{2+}$ ions, the dansyl group does not fully isolate
Fig. 5 (continued) $^1$H NMR spectra of the aromatic region of A18C6-Dns as the function of metal/ligand ratio with various ions: a. A18C6-Dns + Mg$^{2+}$; b. A18C6-Dns + Ca$^{2+}$; c. A18C6-Dns + Sr$^{2+}$; d. A18C6-Dns + Ba$^{2+}$.

the metal ion in the cavity from interactions with the solvent, while the isolation of Sr$^{2+}$ ions in the complex is the best. The strontium atom is coordinated in the macrocycle cavity and closed with the dansyl fragment. As follows from these results the size and electron structure of the crown ether moiety of A18C6-Dns ligand is best filled by Sr$^{2+}$ ions (Fig. 6-9). Our theoretical model does not account for the destabilising effects of the solvent. Therefore, in this case, we cannot make simple correlations between thermodynamical gas phase stability of the complex and experimental data.
Table 3 Heats of formation (H.o.f.) and stabilization energies (E_{stab}) for the complexes between A18C6-Dans and Mg^{2+}, Ca^{2+}, Sr^{2+}.

| Complex                  | H.o.f. (kcal mol\(^{-1}\)) | E_{stab} (kcal mol\(^{-1}\)) |
|--------------------------|----------------------------|------------------------------|
| (A18C6-Dns + Mg)^{2+}    | 35.0                       | 304.7                        |
| (A18C6-Dns + Ca)^{2+}    | -113.9                     | 354.6                        |
| (A18C6-Dns + Sr)^{2+}    | -173.5                     | 381.9                        |

Fig. 6 The optimized structure of A18C6-Dns.

In all complexes studied the distances between one of the oxygen atoms of the sulphonic group and the metal ion inside the macrocyclic cavity varied from 2.137 to 4.282 Å, which points to a possibility of formation of a coordination bond between the metal ion and the oxygen atom. It is reasonable to suppose that formation of bonds between the metal ion and the oxygen atom of the sulphonic group enhances stabilisation of the ion in the crown ether. Analysis of results for the series of complexes studied has shown that the shortest bond occurs for the complexes with Ca and Sr, 2.137 Å and 2.352 Å, respectively, while the bond for the complexes with Mg (4.322 Å) were longest. As follows from analysis of the interatomic distances in the complex molecules, the mean Me-O distances in the crown are the shortest for the complex with Mg (2.086 Å) and they increase up to 2.332 Å in the complex with strontium ions. In the series Mg, Ca, Sr the role of
Fig. 7 The optimized structure of (A18C6-Dns + Mg)$^{2+}$ complex.

Fig. 8 The optimized structure of (A18C6-Dns + Ca)$^{2+}$ complex.
the side moiety in stabilisation of the metal complex increases, which is manifested as an increase in the mean length of the M-O(crown) bond and the M-O(S) distance (Table 4).

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\begin{array}{cccc}
\text{Complexes} & M^{2+}-N_{\text{crown}} & M^{2+}-O_{\text{crown}} & M^{2+}-S & M^{2+}-O_{S} \\
(A18C6-Dns + Mg)^{2+} & 2.251 & 2.056; 2.120; 2.094; 2.101; 2.061 & 3.446 & 3.632; 3.433 \\
(A18C6-Dns + Ca)^{2+} & 3.604 & 2.316; 2.288; 2.304; 2.284; 2.262 & 3.386 & 3.916; 2.137 \\
(A18C6-Dns + Sr)^{2+} & 2.522 & 2.336; 2.307; 2.397; 2.280; 2.341 & 2.920 & 4.282; 2.352 \\
\end{array}
\]

Table 4 The distances (Å) between cations and donor atoms in A18C6-Dns complexes with metal ions.

Introduction of the metal ion into the macrocyclic molecule leads to significant changes in the charge density distribution over the whole molecule. The calculated charge density values on the fragments of the A18C6-Dns molecule are given in Table 5.

The formation of the complex brings about profound changes in the partial charge distribution. The changes are particularly well manifested in the complexes with Ca and Sr in which the total charge of the ion is delocalised over the dansyl fragment. The charge delocalisation over the side arm is the strongest in the complex A18C6-Dns with Sr$^{2+}$ ion and little less in the complex with Ca$^{2+}$ ion. In the complex with Mg$^{2+}$ ion the charge introduced with the ion is accumulated on the macrocyclic part of the molecule and to a less degree on the side arm. The greatest changes in the electronic structure of dansyl group are manifested as strong reduction of the emission band at $\lambda = 522$ nm and a shift of the absorption band at $\lambda = 330$ nm assigned to the dansyl fragment. Introduction of Mg$^{2+}$ ions into the acetonitrile solution of A18C6-Dns practically does not affect on the absorption and emission spectra.
Table 5 Changes in charge distribution of the selected fragments of A18C6-Dans induced by the complex formation. Mulliken changes have been used for the analysis.

4 Conclusion

The formation of complexes of A18C6-Dns and Ca$^{2+}$, Sr$^{2+}$, Ba$^{2+}$ and Mg$^{2+}$ in acetonitrile was studied by $^1$H NMR, absorption and fluorescence spectroscopy and PM5 semi-empirical methods. The stability constants for the A18C6-Dns complexes with the ions Ca$^{2+}$, Sr$^{2+}$, Ba$^{2+}$ and Mg$^{2+}$ determined from the three studied methods increase in the sequence Mg$^{2+}$ < Ba$^{2+}$ < Ca$^{2+}$ < Sr$^{2+}$. Their values are directly related to the degree of the fit of the ions to the macrocycle cavity. The optimized structures of (A18C6-Dns + metal ions)$^{2+}$ complex by theoretical methods indicated formation of a coordination bond between the metal ion and the oxygen atom in crown ethers and changes in the partial charge distribution on atoms of the complexes.

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