

# The use of mass spectrometry to assess complexation phenomena in receptor compounds

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**Abstract:** Numerous methods exist for the assessment of complexation between cation binders such as crown ethers and sodium, potassium, or calcium salts. When complexing agents that have more than one binding site are under study, the assessment problem becomes more complicated. We discuss here the application of mass spectrometric methods to problems of complexation by various receptors.

## INTRODUCTION

Macrocyclic polyether compounds were known for many years (1) prior to Pedersen's disclosure (2) of the compounds he called "crown ethers." The true importance of his discovery was his demonstration that these compounds formed cation complexes with alkali and alkaline earth metals. An enormous field of study has now evolved in the area of crown ethers and cryptands (3) and their cation complexation properties (4). As the structures of novel cation binding ligands have grown more complicated, the measurement of complexation equilibria have become correspondingly more difficult.

The most commonly used methods for assessing cation complexation are the picrate extraction technique, conductometric, NMR, and ion selective electrode methods (5). Because of the increasing complexity of the ligands of interest to us, we have sought alternate methods for assessing cation binding. Although our efforts with ion selective electrodes have been extensive, this method uses a substantial amount of the guest compound and assessments are limited to cations and solutions compatible with the electrodes. Other instrumental methods such as those involving NMR (6) or calorimetry (7) also require a considerable quantity of material for study. Mass spectrometric methods have been relatively little used compared to other techniques in studies of cation complexation but this method is especially valuable when the quantities of material available are limited.

During recent years, the fast atom bombardment mass spectrometry (FAB-MS) method has proved to be a valuable method for assessing cation complexation by macrocyclic ligands (8). The FAB-MS method differs from simple electron impact methods. In FAB-MS, the sample is usually dissolved in a non-volatile matrix (often *m*-nitrobenzyl alcohol) rather than being introduced directly in the pure state on a sample probe. In the FAB-MS method, the sample is desorbed by interaction with a beam of energetic fast atoms. This method of desorption results in relatively low energy transfer and a concomitant lack of fragmentation that characterizes more energetic (*e.g.* EI-MS) mass spectrometric methods. Because of this lower level of fragmentation, molecular ions may be more readily observed.

Since the FAB-MS technique involves relatively lower energies, weakly associated cation-ligand complexes may survive the desorption process and be observed directly. Normally, in the FAB-MS spectrum, the crown-cation complex is observed as a significant ion. The importance of this developing technique is clear from recent reports from Johnstone *et al.* (9), Dearden *et al.* (10), Brodbelt *et al.* (11), and others (12). We have used FAB-MS to probe the binding properties of complex organometallic and multi-ring ligands. Such assessments would have been difficult to accomplish by other means.

## RESULTS AND DISCUSSION

Application of FAB-MS to cation complexation

In early work using the FAB-MS technique (13), Takahashi and coworkers analyzed several simple crowns that could complex various cations with differing stoichiometries. In principle, a crown that has a cavity size similar to a particular cation should complex that cation with a stoichiometry of 1:1. Of course, in solution, there is no guarantee that the particular complex will exhibit maximum stability in any given series for this reason alone (14). Nevertheless, it is expected that as cation size increases relative to crown ether hole size, 2:1 crown:cation complexation will be favored over 1:1 complexation.

The simple crown ether structures shown in the figure were used to assess whether FAB-MS (*m*-nitrobenzyl alcohol matrix) and solution methods gave similar assessments of stoichiometries.

Complexation between the crowns and cations was observed by using the FAB-MS technique. It was interesting to assess stoichiometry in these systems. For the smallest host, dibenzo-14-crown-4, both 1:1 and 2:1 (host:guest) complexes were observed with sodium cation ( $\text{SCN}^-$  anion). As the molar ratio of crown:cation was increased in the alcohol matrix, more of the 2:1 complex was observed. In the cases of benzo-15-crown-5 and dibenzo-18-crown-6, differences were apparent between the  $\text{Cl}^-$  and  $\text{SCN}^-$  anions.

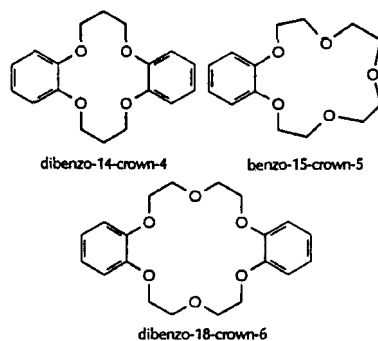
One important question that can be asked concerning any experimental technique is whether the data obtained accurately reflect the chemical phenomena under study. For the FAB-MS method, we undertook an examination of more than 20 crowns for which the cation complexation constants in methanol had been determined. They included 15-crown-5, and derivatives having sidearms such as  $\text{CH}_2\text{OCH}_2\text{CH}_2\text{OR}$  ( $\text{R} = \text{Me}$  or  $\text{Pr}$ ),  $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_2\text{CH}_3$  or  $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_3\text{CH}_2\text{Ph}$ . A similar range of derivatives of aza-12-crown-4, aza-15-crown-5, aza-18-crown-6, and diaza-18-crown-6 were assessed as well (15). Generally, it was possible to correlate binding constants determined in the solution phase with peak intensities observed by FAB-MS. Certain of the variables affecting such studies have recently been appraised (16).

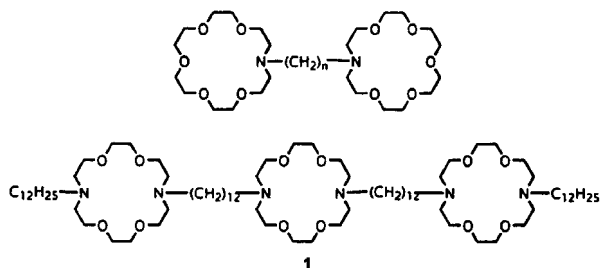
The fast atom bombardment technique has also proved of value in assessing cation complexation by various ligands containing the ferrocenyl moiety. Ferrocenyl amides have been extensively studied by the groups of Hall (17) and Beer (18). We have studied cation binding by ferrocenylcryptands by using this technique (19). The ability to observe multiple-cation complexation using extremely small samples makes mass spectrometry a powerful technique for modern chemical analysis.

Electrospray ionization mass spectrometry

Mass spectral techniques are proving to be increasingly important for the assessment of cation-ligand interactions (20). The development of mass spectrometry as an analytical tool and particularly recent improvements in electrospray ionization mass spectrometry (ESI-MS) (21) have increased the options for assessing complexation interactions. In recent years, the ESI-MS technique has been applied to the study of non-covalent complexes (22), metal salts in aqueous solutions (23), amino acid clusters (24), interactions of peptides with metal ions in solution (25), amino acid-metal complexes (26), natural ionophores binding alkali metals (27), as well as other novel applications (28),(29).

We have recently reported the formation of novel membranes from compounds that contain two polar residues separated by a non-polar chain (30). These may be thought of as two-headed amphiphiles and are often referred to as "bola-amphiphiles" or "bolytes." The FAB mass spectra of the bolytes shown in the figure ( $n = 10, 16, 22$ ) were determined in the usual fashion and evidence was obtained for simultaneous complexation of two cations. In connection with our effort to develop cation conducting channel compounds, we have extended these studies to three-ring macrocycles (1) using the electrospray ionization (ESI-MS) technique. Electrospray is an even "softer" ionization technique than FAB but it is less studied. We successfully demonstrated for the first time that a three-ring ligand (1) could simultaneously bind three alkali metal cations (31).





were dibenzyl ethers of the form  $(4\text{-Y-C}_6\text{H}_4\text{CH}_2)_2\text{O}$  and bis(ferrocenylmethyl) ether. It was possible using peak intensities in the ESI-MS spectra to demonstrate a Hammett correlation between these ethers which have different donicities towards Lewis acids. The Hammett relationship could only obtain if solution and gas phases were providing similar information concerning the binding interactions.

### Anion binding effects in the ESI mass spectra

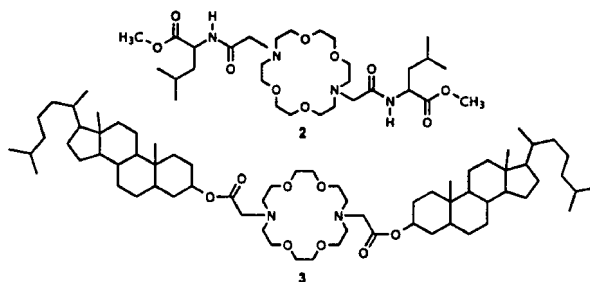
It has been known for some years that under certain conditions the anion associated with a bound cation can influence the overall complexation equilibrium (34). The mass spectrometric method can be used to assess anion interaction but this must be done with a cation that is at least divalent. A monovalent cation complex has a single positive charge; the presence of a mono-anion in the complex would render the system neutral and therefore invisible to a positive ion detector. Divalent calcium complexation of two diaza-18-crown-6 derivatives having glycine present in the sidearms was studied. In one case the sidearm was gly-leu-OMe and, in the other case, glycine was esterified by cholestanol. The compounds are illustrated.

The ESI mass spectrum of **2** obtained in the presence of  $10\ \mu\text{M}$  (in methanol) each of NaCl and  $\text{CaCl}_2$  showed only two peaks at  $m/z$  336.1 and 655.5. These correspond to  $[\mathbf{2}\cdot\text{Ca}]^{2+}$  ( $672/2$ ) and  $[\mathbf{2}\cdot\text{Na}]^+$ . The association of anions with these complexes was apparent in neither case. The positive ion electrospray spectrum of **3** was much more complicated. A solution was studied which contained 18-crown-6 and **3** ( $1\ \text{mM}$  in  $\text{CH}_3\text{OH}$ ) in the presence of  $30\ \mu\text{M}$   $\text{CaCl}_2$ . Some residual  $\text{Na}^+$  was present in the spectrometer as a result of previous runs using this ion. The 18-crown-6 $\cdot\text{Na}^+$  complex was observed at  $m/z$  287 (relative abundance 50%). No peak corresponding to the calcium complex of 18-crown-6 was observed at a relative intensity  $> 10\%$ . The base peak was observed at  $m/z$  1193.9 and corresponded to  $[\mathbf{3}\cdot\text{Ca}\cdot\text{Cl}]^+$ . The divalent calcium complex of the steroidal crown was also observed ( $m/z$  179.5) but its relative intensity of  $\approx 15\%$  made it a minor peak in this spectrum.

### Cation selectivity assessed by ESI-MS

Essentially no chloride anion effect was observed in studies using bis(4-methoxybenzyl) ether ( $1\ \text{mM}$  in  $20\% \text{CHCl}_3\text{:CH}_3\text{OH}$ ) and a mixture of NaCl and KCl ( $0.5\ \text{mM}$ , each). Only five peaks (relative intensity  $> 5\%$ ) were observed in the positive ion ESI-MS spectrum of this simple ether. The base peak was observed at  $m/z$  539.2 and was assigned as  $[\text{ether}_2\text{Na}]^+$ . The corresponding potassium complex was only slightly less intense ( $\approx 90\%$ ) and was observed at  $m/z$  555.2. The single  $[\text{ether}\cdot\text{M}]^+$  complexes were observed for  $\text{Na}^+$  and  $\text{K}^+$  cations at  $m/z$  281.1 and  $m/z$  297.0, respectively. In this case, the  $\text{K}^+$  complex had a greater relative intensity ( $\approx 70\%$ ) than the  $\text{Na}^+$  complex ( $\approx 50\%$ ).

The relative intensity ratio for  $[\text{ether}_2\text{M}]^+ / [\text{ether}\cdot\text{M}]^+$  was 1.92 when M was Na and 1.26 when M was K. By summing the intensities (ion currents) of the peaks attributable to  $\text{Na}^+$  complexes compared to  $\text{K}^+$  complexes, a ratio of  $275/243$  ( $\approx 1.13$ ) was obtained. The apparent ability of these simple ether complexes to capture methanol solvent could be assessed by calculating the following ratios:  $[\text{ether}\cdot\text{Na}]^+ / [\text{ether}\cdot\text{Na}\cdot\text{CH}_3\text{OH}]^+ = 2.26$ ,  $[\text{ether}\cdot\text{K}]^+ / [\text{ether}\cdot\text{K}\cdot\text{CH}_3\text{OH}]^+ = 22.67$ ; and  $[\text{ether}\cdot\text{Na}\cdot\text{CH}_3\text{OH}]^+ / [\text{ether}\cdot\text{K}\cdot\text{CH}_3\text{OH}]^+ = 7.67$ . Although the data included here are obviously limited, the difference in charge density between  $\text{Na}^+$  and  $\text{K}^+$  is apparent in all of the observations.



The sodium cation shows a greater preference for complexation by two ether donors rather than one. The ability of the  $[\text{ether}\cdot\text{M}]^+$  complex to capture a molecule of methanol solvent is greater when the cation is more charge dense. In the present case, the involvement of the chloride anion is obscured by the fact that it neutralizes the monovalent cation to which it might be attached. Such ions as  $[\text{ether}\cdot\text{M}^+\cdot\text{Cl}^-]$  would bear zero net charge and be invisible to a positive ion mass detector.

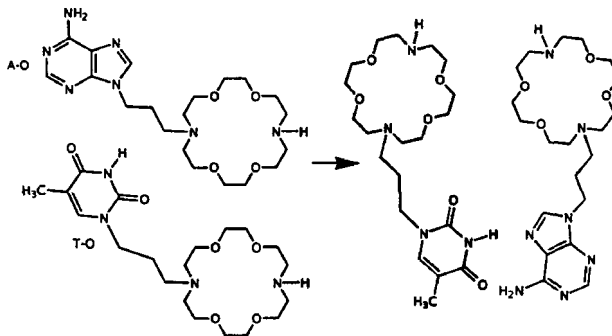
#### Lariat ethers having nucleotide-terminated sidearms

We have previously examined the solution phase association of lariat ether compounds which have sidearms terminated by nucleotide bases (35). These heterocycles can form hydrogen bonds of either the Watson-Crick or Hoogsteen types and, in the present context, potentially lead to dimer formation.

The electrospray mass spectrum was obtained of a  $\text{CHCl}_3:\text{CH}_3\text{OH}$  (2:1) solution containing A-O and T-O (each 3.33 mM) and 1.5% acetic acid.

The major peaks observed in the spectrum were 429.1 (base peak) which corresponds to  $[\text{T-O}\cdot\text{H}]^+$ . A peak with a relative intensity of  $\approx 55\%$  was observed at  $m/z$  438.1 and corresponds to  $[\text{A-O}\cdot\text{H}]^+$ . Peaks are observed at  $m/z$  857.7 and 866.8 but their intensities are  $\approx 10\%$  and  $\approx 30\%$ , respectively. The larger peak corresponds to  $[\text{A-O}\cdot\text{T-O}\cdot\text{H}]^+$  and the latter to  $[(\text{T-O})_2\text{H}]^+$ . An even smaller peak is observed

(relative intensity  $< 10\%$ ) at  $m/z$  875.7 that can be assigned to  $[(\text{A-O})_2\text{H}]^+$  dimer. Although  $\text{Na}^+$  cation was not deliberately added to these solutions, peaks corresponding to sodium complexes were observed due to the presence of residual salt in the spectrometer. Thus, low relative intensity ( $\approx 5\text{-}10\%$ ) peaks were observed at 451 and 460. The intensity of the former was approximately twice that of the latter as expected since the thymine sidearm has a well-placed urea carbonyl group that can, as judged from models, interact with a ring-bound sodium cation. In summary, peaks corresponding to dimers were observed and of the possibilities,  $[\text{A-O}\cdot\text{T-O}\cdot\text{H}]^+$  appeared to be more stable than either  $(\text{T-O})_2\text{H}^+$  or  $(\text{A-O})_2\text{H}^+$ . The dominant ions, however, were attributable to the protonated monomers rather than to any aggregates.

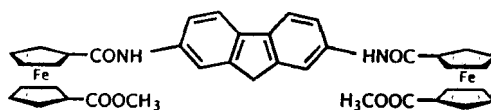


#### Ferrocenyl receptor molecules

We have designed and prepared a series of receptor molecules of the "molecular cleft" type (36). These are based upon the versatile ferrocene unit. Because the two cyclopentadiene rings rotate freely with respect to each other, we have referred to iron as an atomic ball bearing (37). These receptors contain two carboxyl groups which may rotate to face each other and bind diamines of appropriate molecular dimension. The compound shown in the figure possesses two carboxymethyl groups in the 1'-rings. In our previous studies with diamines, these functional groups were free carboxyl groups which could form salt bridges with the amines. The mass spectrometric method is limited by the requirement that a charged particle be observed. In the reaction of a dicarboxy receptor with a diamine, salt bridge formation results in the production of two positive and two negative charges. Unfortunately, the overall particle is neutral. In the present studies, we examined the interaction of the dimethyl ester with diammonium salts. Interaction between the carbonyl groups and the diammonium salts could lead to complexes which would be doubly-charged and therefore readily detectable by positive ion mass spectrometry.

Two separate experiments were undertaken.

In both cases, the receptor (R) was present at a concentration of 1mM in  $\text{CH}_3\text{OH}$ . The diammonium salts,  $[\text{H}_3\text{N}(\text{CH}_2)_n\text{NH}_3]\text{Cl}_2$ , were added at concentrations of 1 mM each. In the first experiment, the mixture contained ammonium salts having  $n = 3, 4, 5,$  and  $6$ . In the second experiment,  $n = 6, 7, 8,$  and  $9$ . In the first experiment, nine peaks were observed which had relative



intensities > 10%. The base peak ( $m/z$  825.5) corresponds to the diammonium salt complex having  $n = 4$  although the  $n = 3$  analog is nearly as intense (83%). The pentanediammonium and hexanediammonium salt complexes both give peaks with relative intensities of 35%. The third major peak in the spectrum (76%), however, is observed at  $m/z$  795.5 which corresponds to  $[\text{H}_3\text{N}(\text{CH}_2)_6\text{NH}_3 \bullet \text{R}_2]^{2+}$ . The peak attributable to  $[\text{H}_3\text{N}(\text{CH}_2)_5\text{NH}_3 \bullet \text{R}_2]^{2+}$  had a relative intensity of only 12%. Complexes including two propane- or butanediammonium salts were not observed at relative intensities greater than 10%.

When the longer chain series of diammonium salts was examined in a competitive experiment, single receptor (*i.e.* 1:1) complexes were observed but none had a relative intensity greater than 30%. The base peak was observed at  $m/z$  816.3 and was attributed to  $[\text{H}_3\text{N}(\text{CH}_2)_9\text{NH}_3 \bullet \text{R}_2]^{2+}$ . Only two other peaks were observed in this spectrum to have relative intensities > 30%. These were  $m/z$  809.5 (75%) and 802.2 (48%), These peaks correspond to  $[\text{H}_3\text{N}(\text{CH}_2)_8\text{NH}_3 \bullet \text{R}_2]^{2+}$  and  $[\text{H}_3\text{N}(\text{CH}_2)_7\text{NH}_3 \bullet \text{R}_2]^{2+}$ , respectively. The results presented here clearly do not represent a comprehensive study of this bis(ferrocene) receptor system, but complexes are observed by mass spectrometric analysis and some selectivity is apparent in each competition study.

## CONCLUSIONS

The present paper reports the use of electrospray ionization mass spectrometry to assess complex formation in several different receptor systems. This method permits one to probe complexation using a variety of solvent systems and in quite dilute solution. Direct competition experiments are particularly appropriate as ions corresponding to several different complexes may be simultaneously observed. Recent evidence suggests that for some systems the gas phase spectra accurately reflect the solution situation although controls must be done for each given situation.

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