An electrospray ionization tandem mass spectrometric study of \( p\)-tert-butylcalix[6]arene complexation with ammonium hydroxide, and ammonium and sodium ions

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The formation of complexes involving \( p\)-tert-butylcalix[6]arene with neutral and charged species has been investigated by tandem mass spectrometry combined with electrospray ionization. Complexes of \( p\)-tert-butylcalix[6]arene with \( \text{NH}_4^+ \) ions were observed in the ratios 1:1, 2:1, and 3:1, together with the complexes of \( p\)-tert-butylcalix[6]arene with \( \text{NH}_4\text{OH} \) and \( \text{Na}^+ \) ions in the ratios 1:1:1, 2:1:1, and 3:1:1. A single 1:1 complex of \( p\)-tert-butylcalix[6]arene with \( \text{Na}^+ \) ions was observed. In addition, a doubly charged complex of \( p\)-tert-butylcalix[6]arene with \( \text{NH}_4\text{OH}, \text{Na}^+, \) and \( \text{NH}_4^+ \) ions in the ratio 6:1:1:1 was observed. The identity of each complex was determined by mass analysis of product ions formed by the application of a declustering potential over the range 20–220 V and by observation of product ion mass spectra wherein the collision energy was varied from 5 to 50 eV. Fragmentation of the complexes is characterized by the facile loss of the ammonia molecule, sodium and ammonium ions, loss of neutral \( p\)-tert-butylcalix[6]arene, and successive neutral losses of \( \text{C}_6\text{H}_6 \) from the six \( \text{tert-butyl} \) groups in each \( p\)-tert-butylcalix[6]arene molecule. Copyright \( \odot \) 2008 John Wiley & Sons, Ltd.

Calixarenes are macrocyclic molecules that are derived from phenolic monomers connected via methylene bridges in such a manner as to incorporate a hydrophobic cavity into which a variety of molecules and ions can be occluded (Fig. 1).\(^1\) There is a wide range of application of such compounds. An early application of a calixarene in separation science was the use of the hexacarboxylated derivative of \( p\)-sulfonated calix[6]arene as a ‘super-uranophile’ for the selective extraction of uranium from sea water in the presence of an excess of other metal ions\(^2\) and in association with a \( \pi\)-diketone.\(^3\) In addition, calixarenes have been used for the extraction of long-lived radionuclides from acidic or high salinity media generated from the reprocessing of waste nuclear fuels.\(^4\)

Several studies have been carried out with the objective of combining solvent extraction with efficient separation and concentration processes of metal ions. For example, the efficacy of using cyclic host calixarene compounds, such as carboxyl derivatives of \( p\)-tert-butylcalix[\( n\)]arenes, combined with the extraction behavior of alkaline, alkaline-earth, rare earth and transition metals in liquid-liquid systems, has been investigated.\(^5\) A further application is the monitoring of the accumulation of volatile bases by means of a color change of a calix[4]arene-based dye.\(^8\)

Calix[\( n\)]arenes are the starting materials for the preparation of supermolecules that have been used as building blocks for new supramolecular materials. Some of these materials, which often take the form of thin films, coatings and supported materials, have been designed for use in sensors due to their binding abilities.\(^9\) Because the properties of calixarenes can be modulated by introduction of appropriate moieties or by the choice of a particular conformation, these compounds possess great versatility as selective receptors for metals ions and they have found use as ion-selective electrodes and solid-state sensors.\(^11\)

The electrospray ionization (ESI) process has an enormous potential for speciation studies in solution because many of the ions transferred to the gaseous phase retain the same structure and charge of the species in solution.\(^12\) When a specific ion is isolated in a tandem mass spectrometer and then subjected to collision-induced dissociation (CID) followed by mass analysis of the product ions, the resulting product ion mass spectrum can permit some conclusions to be drawn concerning the structure of the mass-selected ion species and the elucidation of the fragmentation pathways.

ESI combined with mass spectrometry (MS) has been employed for the investigation of macrocyclic complexes,
including calixarenes. For example, ESI-MS was used to probe the formation of host-guest complexes involving calixarenes and alkylammonium ions.\textsuperscript{13} Crown ethers, another type of macrocycle, have been studied with respect to their propensity to form ammonium complexes. For those crown ether–ammonium complexes that are strongly bound due to multiple hydrogen bonds, retention of the ammonium moiety was observed in product ions formed by CID. On the other hand, a weakly bound complex such as 12-crown-5-ammonium dissociated readily by loss of the ammonia molecule.\textsuperscript{15–17} Tandem mass spectrometry (MS/MS) has been used for the evaluation of hydrogen-bonding interaction related to host-guest affinity. Investigation of the complexation behavior of 18-crown-6 with various ammonium ions in solution using ESI ion trap mass spectrometry revealed a reasonable correlation between their stability constants and those derived from other methods.\textsuperscript{18}

There have been several reports on the study of calixarene–metal ion interactions by ESI-MS. For example, a quadrupole ion trap equipped with an ESI source was employed for the determination of the alkali-metal ion binding selectivity of a series of calixarenes; it was found for tert-butylcalix[4]arenes-tetraacetic acid tetraethyl ester that complexation of Na\textsuperscript{+} was favored over K\textsuperscript{+}, corroborating the results obtained by other methods.\textsuperscript{19} In an ESI-MS investigation of the formation of supramolecular complexes between 25,27-diphosphoryloxycalix[4]arene and a series of amino acids and metal salts, it was shown that these compounds have both high affinity and strong selectivity for the formation of the ternary complex with Zn\textsuperscript{2+}-His.\textsuperscript{20}

There is mass spectrometric evidence that calixarenes may form oligomeric aggregates through intermolecular hydrogen bonding.\textsuperscript{10} One such study showed that calixarenes can aggregate as dimer/monomer and/or trimer/monomer, and the ratios in which they combined have been measured by secondary ion mass spectrometry (SIMS).\textsuperscript{21}

Here, we report on an ESI-MS/MS study of complexes of tert-butylcalix[6]arene of molecular weight (MW) 972.6 with ammonium ions, with ammonium hydroxide and sodium ions, and with ammonium hydroxide and both ammonium ions and sodium ions. The present work is part of a mass spectrometric study being carried out in this laboratory into the interactions of macrocyclic cavities and molecular species.

**EXPERIMENTAL**

**Chemicals and preparation of standard solution**

Analytical-grade p-tert-butylcalix[6]arene (95% purity) was purchased from Aldrich (Gillingham, UK); aceton (purity >99.5%) was obtained from Baker (Deventer, The Netherlands); and acetonitrile was purchased from Fluka (Buchs, Switzerland). Ammonium hydroxide P.A. was purchased from Baker. Water used for these experiments was purified by an EASYpure RF system (Barnstead, Dubuque, IA, USA).

A stock solution of 20 mg L\textsuperscript{-1} of p-tert-butylcalix[6]arene in acetone was prepared. All experiments were performed using a standard solution of 1.0 ng µL\textsuperscript{-1} in acetonitrile/water (50:50, v/v), containing 10 mol L\textsuperscript{-1} ammonium hydroxide.

**Mass spectrometry**

Experiments were performed in an API 4000\textsuperscript{TM} triple-stage quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Concord, Canada) using an ESI source (TurbolonSpray\textsuperscript{®}). The mass spectrometer was tuned in the positive ion mode by infusion of a polypropylene glycol (PPG) solution. The electrospray needle was maintained at 5500 V and operated at room temperature. The horizontal and vertical probe positions were set at 5 mm and 10 mm, respectively, from the orifice. The nebulizer and the curtain gas pressures were both set at 10 psi, and the entrance and declustering potentials were 10 and 50 V, respectively. Air was used as the nebulizer gas and nitrogen was used as the curtain gas and as the collision gas. The pressure of the collision gas used for CID was set at 6 arbitrary units. The sample was introduced by direct infusion at the rate of 10 µL min\textsuperscript{-1} using the syringe pump of the mass spectrometer.

**RESULTS AND DISCUSSION**

**Monomer complexes**

Mass scans were obtained of the sample using the first quadrupole mass filter, Q1, in conjunction with declustering potentials (DPs) of 20, 150 and 220 V, and are shown in Figs. 2(a), (b), and (c), respectively.

At a DP of 20 V, two complex ions were observed. The base peak, m/z 990.7, of the mass spectrum shown in Fig. 2(a) is identified as the complex [M[6] + NH\textsubscript{4}\textsuperscript{+}], where M[6] represents the calixarene molecule. The ion of m/z 1030.8 is identified as the [M[6] + NH\textsubscript{4}\textsuperscript{+} + Na\textsuperscript{+}]\textsuperscript{+} complex. The ion of m/z 666.5 is identified as the [M[6] + NH\textsubscript{4} – M[2]\textsuperscript{+}]\textsuperscript{+} product ion corresponding to the loss of 324 Da from the base peak; the complementary radical cation, M[2]\textsuperscript{+}, of m/z 324, is also observed.

As the DP is increased to 150 V (Fig. 2(b)), the [M[6] + H\textsuperscript{+}]\textsuperscript{+} ion is observed together with protonated M[2], [M[2] + H\textsuperscript{+}]\textsuperscript{+}, of m/z 325.3. At a DP of 220 V, the Na\textsuperscript{+} and K\textsuperscript{+} ions present as impurities in solution give rise to the observation of the [M[6] + Na\textsuperscript{+}] and [M[6] + K\textsuperscript{+}]\textsuperscript{+} complexes of m/z 995.7 and 1011.8, respectively (Fig. 2(c)). The relative abundances of the m/z 990.7 and 1030.8 ions drop to 41.9% and 87.5%, respectively, when the DP is increased from 150 to 220 V, and the base peak is then [M[2] + H\textsuperscript{+}]\textsuperscript{+} of m/z 325.3.

![Figure 1. Structural formula of p-tert-butylcalix[6]arene (MW 972.6) showing the basic phenolic unit, M[1], which has a MW of 162.1.](image-url)
Figure 2. Positive ion ESI mass spectra of the standard solution of \( p \)-tert-butylcalix[6]arene at 1.0 ng \( \mu \)L\(^{-1}\) in 50:50 (v/v) acetonitrile/water containing 10 mM ammonium hydroxide with declustering potentials of (a) 20 V, (b) 150 V, and (c) 220 V.
Figure 3 shows a product ion mass spectrum of the [M + NH₄]⁺ complex, m/z 990.7, acquired in multiple channel acquisition (MCA) mode to sum 10 product ion mass spectra obtained at different collision energies (CEs). In this experiment, the CE was ramped from 5 to 50 eV with CE steps of 5 eV. Initially, at a CE of 5 eV, two product ions, [M + H]⁺ (m/z 973.8) and [M[2] + H]⁺ (m/z 325.4), were observed. As the CE was increased from 10 to 40 eV, a series of product ions was observed at m/z 917.8, 861.5, 805.5, 749.5, 693.5 and 637.3; this series corresponds to six successive losses of 56 Da, which is a 2-methylpropene unit, C₄H₈, formed by rearrangement of the p-tert-butyl moiety and subsequent fragmentation of the upper part of the calixarene molecule shown in Eqn. (1):

$$\frac{[C_4H_9 - C_6H_3(OH)(CH_2)]_n + H^+}{[C_4H_9 - C_6H_3(OH)(CH_2)]_{n-1} + \left[\frac{[C_6H_3(OH)(CH_2)] + H^+ + (CH_2)CHCH_2}{2}\right]} \quad (1)$$

These product ions have the general formula [M[6] + H - n(56)]⁺, where n is the number of 2-methylpropene units lost. At a CE of 25 eV, the product ion of m/z 325.4 fragments to yield m/z 269.3 and a 2-methylpropene molecule. Another short series of product ions that differ by 56 m/z units is observed at m/z 713.5, 659.7 and 599.1, where m/z 713.5 may be due to [M[6] + H - M[2] - 28]⁺.

At a CE of 30 eV, the mass-selected molecular complex has been replaced as the base peak by the [M[2] + H]⁺ ion. At a CE higher than 35 eV, a product ion of m/z 175.2 starts to appear that may be [M[1] + CH]⁺.

Product ion mass spectra (not shown here) of [M[6] + H]⁺ were also obtained over the range of CE from 5 to 50 eV. Once more, a series of product ions was observed at m/z 917.8, 861.5, 805.5, 749.5, 693.5 and 637.3, corresponding to successive C₄H₈ losses.

The product ion mass spectrum of m/z 1030.9 obtained at a CE of 35 eV is shown in Fig. 4. The precursor ion is identified as a complex with ammonium hydroxide and a sodium ion, [M[6] + NH₄OH + Na]⁺. In order to verify the formation of this complex, a calixarene solution at the same concentration was prepared where the ammonium hydroxide was replaced with ammonium acetate. In this case this complex was not detected, confirming the presence of ammonium hydroxide in the m/z 1030.9 ion.

**Dimer complexes**

In the mass range from m/z 1950 to 2100 of the full scan of the calixarene solution, two clusters of ions with m/z 1964.3 and 2003.4 were clearly distinguished.

A scan of the isotopic cluster of the [2M[6] + NH₄]⁺ ion is shown in Fig. 5(a) with unit mass resolution. The product ion mass spectrum of m/z 1963.3 (Fig. 5(b)) was obtained with the CE ramped from 5 to 50 eV; the principal product ions observed were m/z 990.8, identified as [M[6] + NH₄]⁺, and the complementary m/z 973.5, i.e., the protonated molecule [M[6] + H]⁺. It should be noted that product ions corresponding to successive losses of 56 Da from the protonated calixarene molecule are observed here also. Upon consideration of these data, the observed isotopic cluster shown in Fig. 5(a) can be attributed to the formation of...
Figure 4. Product ion mass spectrum of the \([M + \text{NH}_4\text{OH} + \text{Na}]^+\) complex, \(m/z\) 1030.9, obtained with a collision energy of 35 eV.

Figure 5. (a) Q1 mass scan of the standard solution of \(p\text{-tert}-\text{butylcalix}[6]\)arene at 1.0 ng \(\mu\text{L}^{-1}\) showing the isotopic cluster of the \([2M + \text{NH}_4]^+\) complex. (b) Product ion mass spectrum of the \([2M[6] + \text{NH}_4]^+\) complex, \(m/z\) 1964.5, accumulated with the collision energy ramped from 5 to 50 eV.
Figure 6. (a) Q1 scan of the standard solution of \( p \)-tert-butylcalix[6]arene at 1.0 ng \( \mu \)L\(^{-1} \) showing the isotopic cluster of the \([2M + \text{NH}_4\text{OH} + \text{Na}]^+\) complex. (b) Product ion mass spectrum of the \([2M + \text{NH}_4\text{OH} + \text{Na}]^+\) complex, \( m/z \) 2003.4, accumulated with the collision energy ramped from 5 to 50 eV.

Figure 7. Q1 scan of the standard solution of \( p \)-tert-butylcalix[6]arene at 1.0 ng \( \mu \)L\(^{-1} \) showing \([3M[6] + \text{NH}_4]^+\), \([6M[6] + \text{NH}_4\text{OH} + \text{Na}]^{2+}\) and \([3M[6] + \text{NH}_4\text{OH} + \text{Na}]^+\) complexes obtained at low mass resolution.
a 2:1 complex of calixarene molecules with an ammonium ion, [2M[6] + NH₄⁺].

A scan of the isotopic cluster of the [2M[6] + NH₄OH + Na⁺] ion is shown in Fig. 6(a) with unit mass resolution. The product ion mass spectrum of m/z 2003.4 obtained at a CE of 45 eV is shown in Fig. 6(b). The principal product ions observed in the mass spectrum of m/z 2003.6 were [M[6] + NH₄OH + Na⁺] (m/z 1031.0), [M[6]⁺] (m/z 972.9) and...
[M[2] + H]⁺ (m/z 325). On the basis of these observations, the complex is identified as being formed by two calixarene molecules together with an ammonium hydroxide molecule and a sodium ion, [2M[6] + NH₄OH + Na]⁺.

The complex containing two calixarene molecules has an isotopomeric pattern that was confirmed by computational simulation using the Calculator Tool from Analyst³⁴ version 1.4 software (Applied Biosystems/MDS Sciex).

It should be noted that ESI-MS is more suitable for the observation of calixarene complex ions than is SIMS. In a SIMS study of monodeoxycalix[4]arene, a complex ion containing a calixarene dimer was observed; however, the reported value of 0.1% for the ratio of dimer/monomer signal intensity at the high concentration of 0.01 mol L⁻¹ in chlorobenzene was low. In contrast, with ESI, the ratio of the dimer/monomer ammonium complexes observed in this work was 3.4%, reflecting partially the dimer structure in solution, with a calixarene concentration as low as 1.0 μmol L⁻¹. Fukazawa et al. also observed the formation of monodeoxycalix[4]arene dimer in solution using H-NMR spectroscopy. They concluded that this structure has an arrangement of two cones facing the respective narrower rim of each other.

**Trimers and hexamer complexes**

From a scan of the mass range m/z 2900–3000, three peaks were detected, although unit resolution could not be achieved. Due to the low abundance of these peaks in the full scan spectrum, the quadrupole resolution was changed to low resolution in order to obtain more signal intensity of the trimers and doubly charged complexes formed in the electrospray process. The three cluster ions can be seen in the vicinity of m/z 2937.7, 2957.8 and 2977.6 (Fig. 7). In order to determine the compositions of the complexes, MS/MS was performed. Each of the precursor ions was isolated in turn and subjected to CID using ramped CE from 5 to 45 eV in order to observe their dissociation pathway as a function of the variation in CE. The product ion mass spectrum of the m/z 2937.5 ion showed [M[6] + NH₄]⁺ (m/z 991.6) and [2M[6] + NH₄]⁺ (m/z 1964.7) product ions that correspond to losses of 2M[6] and M[6], respectively, from the precursor ion (Fig. 8). Thus the precursor ion of m/z 2937.5 is identified as the trimer complex, [3M[6] + NH₄]⁺.

The product ion mass spectrum of the m/z 2958.2 ion showed [M[6] + H]⁺ (m/z 973.6), [M[6] + NH₄]⁺ (m/z 990.4) and [M[6] + NH₄OH + Na]⁺ (m/z 1030.8) product ions (Fig. 9). Thus the precursor ion of m/z 2958.2 is identified as a hexameric complex with the composition [6M[6] + NH₄OH + Na]²⁺. Dissociation of the complex occurs by charge separation with the loss of 4M[6] to form [M[6] + NH₄]⁺ (m/z 990.4) and [M[6] + NH₄OH + Na]⁺ (m/z 1030.8) product ions with almost equal signal intensities. The mass spectral evidence indicates that, in the complex, NH₄⁺ is associated with one M[6] monomer and NH₄OH + Na⁺ is associated with another monomer.

The product ion mass spectrum of the m/z 2977.5 ion showed [M[6] + H]⁺ (m/z 973.9), [M[6] + NH₄]⁺ (m/z 990.4), [M[6] + NH₄OH + Na]⁺ (m/z 1030.9), [2M[6] + NH₄]⁺ (m/z 1965.9) and [2M[6] + NH₄OH + Na]⁺ (m/z 2003.6) product ions (Fig. 10). The product ion of m/z 1309.7 was not identified. On the basis of the observed product ions, the precursor of m/z 2977.5 was identified as a trimeric complex with the composition [3M[6] + NH₄OH + Na]⁺.

CONCLUSIONS

Data presented here show the complementation of the mentioned species as monomer, dimer, trimer and hexamer complexes observed in the gas phase of p-tert-butylcalix[6]arene, NH4⁺, NH4OH and Na⁺. The monomer complexes of p-tert-butylcalix[6]arene were observed in ratios of 1:1 with NH4⁺ and Na⁺, and in ratios of 1:1:1 with NH4OH and Na⁺. The dimer and trimer complexes of p-tert-butylcalix[6]arene with NH4⁺ in ratios of 2:1 and 3:1 were also detected together with NH4OH and Na⁺ in ratios of 2:1:1 and 3:1:1. A hexameric doubly charged complex of p-tert-butylcalix[6]arene with NH4OH, Na⁺ and tert-butylcalix[6]arene were observed in ratios of 1:1 respectively, relative to complex/monomer ratios of 3:1 for tert-butylcalix[6]arene, ammonium hydroxide, ammonium and sodium ions observed in the gas phase, suggesting that these complexes could be present in the liquid phase.

REFERENCES