Critical re-evaluation of FABMS analysis of ligand–cation interactions

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Whilst the use of FABMS in the analysis of ligand–cation interactions has become widespread, there are a number of experimental variables associated with the use of this technique, the effects of which have not previously been addressed. The interactions of 12-crown-4, 15-crown-5 and 18-crown-6 with the alkali metal cations reveal two important observations: (*i*) that the matrix employed for the FAB studies has a dramatic effect on the observed binding behaviour and (*ii*) that the observed relative intensities of protonated ions ($[M + H]^+$) and metallated ions ($[M + Metal]^+$) differ enormously. Determination of the ratios of these signals would allow quantitative arguments to be applied to experiments of this type. Accordingly, previous reports of the use of FABMS for the quantitative study of ligand–cation interactions must be viewed with caution.

Since its inception FABMS has gained universal usage as a soft ionization technique[1] and was quickly applied to the study of the binding of alkali metals to crown ethers.[2,3] In the intervening years many groups have used this technique to determine the binding characteristics of a variety of host–guest systems.[4]

For FABMS studies of ligand–cation interactions, two types of binding experiment are in common usage. (*i*) Competitive binding studies[3] in which a ligand is mixed with a molar equivalent of each of a series of metals (*e.g.* the alkali metals). Since the metals are competing for a limited number of binding sites, the distribution of ion complexes revealed in the mass spectrum should reflect the selectivity of binding. (*ii*) Relative peak intensity measurements[2] where ratios of intensities of the signals resulting from free and complexed ligands are taken as direct indicators of the extent of complexation.

As part of a continuing research programme concerning molecular recognition and inclusion,[5] a series of novel ligands were prepared.[6] We proposed to determine the metal ion binding selectivities of these systems by the literature FAB technique.[3] At this point we noted that there were a large number of variables pertaining to these analyses that had not been previously addressed, *e.g.* background contamination by other cations, the relative ion intensities of $[M + H]^+$ and $[M + Metal]^+$ complexes and the choice and effect of the FAB matrix; the latter has been recently addressed by Giraud *et al.*[7] In order to ascertain which of these factors could have a significant influence on the observed mass spectrum we chose to perform some initial studies on the binding behaviour of the simple crown ethers with the alkali metals.

Results and discussion

Effect of variation of the FAB matrix

The original studies carried out by Johnstone and Rose employed glycerol as the FAB matrix. Their results revealed a close correlation with the known solution metal ion binding behaviour of the simple crown ethers with alkali metal cations.[2,3] Following the subsequent discovery of other useful FAB matrices,[8] these have also been used in studies of this type. Unfortunately there appears to be little consistency or rationale in the choice of matrix used. We have found that the choice of matrix markedly affects the relative intensities of complex ions observed in competitive metal ion binding experiments, a conclusion confirmed by the aforementioned work of Giraud *et al.*[7]

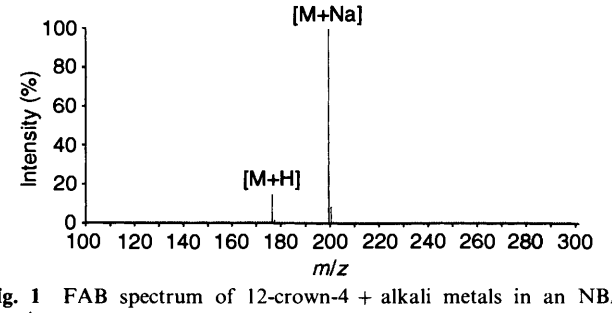

Fig. 1 FAB spectrum of 12-crown-4 + alkali metals in an NBA matrix

The FAB spectra, shown in Fig. 1, were obtained when 12-crown-4 was mixed with stoichiometric amounts of each of the alkali metal nitrates (Li^+, Na^+, K^+, Rb^+ and Cs^+) in MeOH with either glycerol or 3-nitrobenzyl alcohol (NBA) matrix. No ions corresponding to either free crown or ion complexes were detected when a glycerol matrix was used, this observation being in accordance with the original observations of Johnstone and Rose,[3] whereas in the NBA spectrum $[M + Na]^+$ is dominant.

It has been suggested that in the case of glycerol the total suppression of complex ions reflects effective competition for the metal ions by the matrix. However, such an explanation does not account for the lack of the signal corresponding to the protonated crown. This suppression is likely to result from the sample being distributed throughout the bulk of the matrix and not concentrated at the surface.[9]

The results obtained for 18-crown-6, under the same experimental conditions (Fig. 2), demonstrate how the apparent selectivity of complexation may be inverted by changing from one matrix to another. Use of the glycerol matrix reveals intense signals for the potassium ion (*m/z* 303) and rubidium ion (*m/z* 349, 351) crown complexes suggesting preferential binding of these species, whereas when NBA is employed as a matrix, a marked selectivity for sodium ions is apparent.

Competitive binding experiments of the type described above have repeatedly been employed elsewhere to assess the selectivity of complexation of novel ligands.[4a,b,10] The two examples described above have shown how the variation of the matrix can in one case totally suppress the formation of complex ions and in another invert the observed binding selectivity.

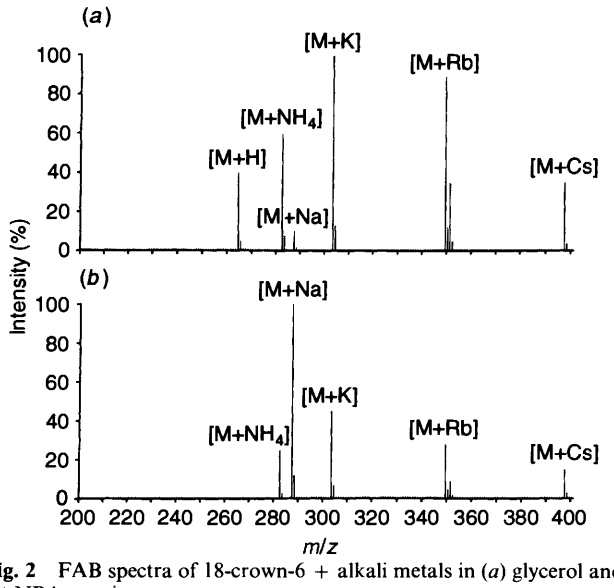

Fig. 2 FAB spectra of 18-crown-6 + alkali metals in (*a*) glycerol and (*b*) NBA matrix

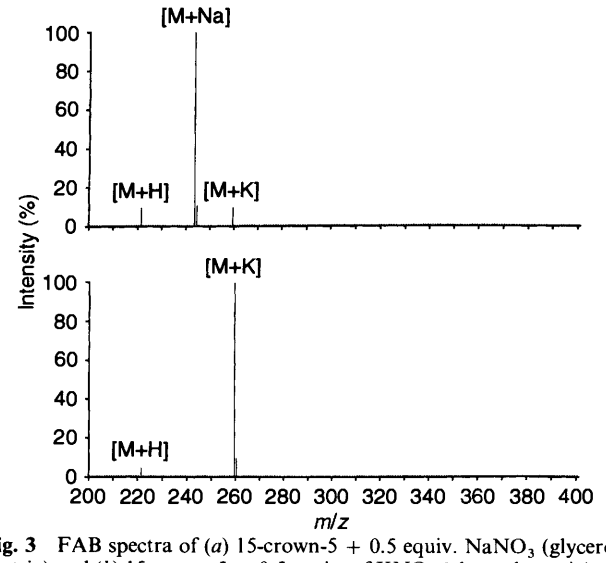

Fig. 3 FAB spectra of (*a*) 15-crown-5 + 0.5 equiv. $NaNO_3$ (glycerol matrix) and (*b*) 15-crown-5 + 0.5 equiv. of KNO_3 (glycerol matrix)

Clearly the nature of the matrix has a profound effect on the binding equilibria under investigation.

It should be noted that the work discussed here is limited to the study of the relatively weak complexes formed between the simple crown ethers and group 1a cations. It has been suggested that a distinction should be made between these systems and complexes which are far more stable in the matrix medium. Indeed, Kataky *et al.* have examined the cation binding selectivity of some strongly ligating polyaza crowns by FABMS,[10b] but concluded that 'the set-up is not appropriate for conclusions to be confidently made about the relative equilibrium stabilities of complexes'. This statement supports our view that the present experimental method is flawed.

Relative ion intensity

Medina and co-workers,[4e] have reported experiments where the relative peak intensities of $[M + H]^+$ to $[M + Metal]^+$ are used as a direct measure of the extent of metal ion binding. This assumes that if $I_{[M+H]^+} = I_{[M+Metal]^+}$ (I = Intensity) then these two species are present in equimolar amounts. Such a statement is at variance with the standard FAB technique of promoting sample cationization by the addition of an alkali metal salt, producing a more intense signal, *i.e.* a more stable 'pseudo-molecular ion'.[11] In addition, early work by Johnstone and Rose proved this assumption to be invalid for the 18-crown-6–K^+ system.[2] Although frequent reference has been made to their work no consideration appears to have been given to this phenomenon in many of the studies published since.

The following simple experiment further undermines the above simplistic assumption. When a mixture of 2 molar equiv. of free crown and 1 molar equiv. of a metal ion are added to the FAB matrix, the observed spectrum should correspond to near equimolar amounts of free crown and bound crown. If the above assumption was correct, then the observed intensities of the $[M + H]^+$ and $[M + Metal]^+$ peaks should be approximately equal. The spectra obtained from two such experiments, in which 15-crown-5 binds sodium and potassium ions, respectively, are shown in Fig. 3. Clearly, the signals for the free and the complexed crown are not equal. However, since these two species should be present in near equal amount it is possible to estimate their relative intensities from the peak heights of these signals, *i.e.* from the ratio of $I_{[M+H]^+} : I_{[M+Metal]^+}$. This affords signal intensity ratios of approximately 1:10 for the sodium case and 1:20 for the analogous potassium complex. Such ratios must be independently measured for each potential host–guest complex in a given FAB matrix since these dramatic differences in intensity will clearly have a significant effect on the interpretation of the results of FABMS binding studies.

Background quantification and the accurate determination of intensity ratios

We have performed calibration experiments, similar to those of Johnstone and Rose,[2a] for the 15-crown-5–Na^+ system, by adding standard volumes of methanolic solutions of sodium nitrate to a methanolic solution of the crown such that the molar ratio of metal ion to crown was varied between 0 and 1. The FAB spectra were then recorded and the resulting data are presented in Table 1. The $[M + K]^+$ peak was used as an internal standard, since this background level of potassium was seen to be constant throughout the series of experiments. Thus, the intensities of both the $[M + H]^+$ and $[M + Na]^+$ signals were divided by the $[M + K]^+$ peak intensity to give the data recorded in Table 2. Plots of these scaled intensities against the molar ratio of added metal ion to crown reveals linear relationships for the decrease in intensity of the $[M + H]^+$ signal and a corresponding increase in that of the $[M + Na]^+$ signal (Fig. 4). The gradients of these lines are a measure of the ion current generated by a given complex ion. The ratio of the gradients is approximately 1:8, *i.e.* the ion current for the sodiated crown is eight times that of the protonated species.

We have compared this technique with the treatment used by Johnstone and Lewis, who observed the changes in intensities of [18-crown-6 + $H]^+$ to [18-crown-6 + $K]^+$ as the ratio of K^+ ions to free crown was varied.[2a] From these experiments, they assigned a signal intensity ratio of 1:4.3 for this system. In our experiments we have determined [crown + $metal]^+$ ion intensities for 15-crown-5 with sodium ions (1:8) and potassium ions (1:22). Such ratios must be measured before any meaningful analysis of the results of FAB metal ion binding studies can be made. Conclusions drawn from data where this phenomenon has not been addressed must be viewed with extreme caution. Further, it is clear that there is great variation in the observed intensity ratios, even with the simple crown ether complexes reported here. In order to interpret the results of a FAB binding experiment these ratios must be measured for each potential metal ion complex. Thus the use of a single binding experiment with a mixture of metal ions as a means of determining quantitative binding selectivity must be invalid.

The calibration experiments described above also allow the

Table 1 Relative peak intensities for varying concentrations of Na^+ to 15-crown-5

Conc./mmol dm^{-3}			Relative peak intensity (%)			
Crown	Metal	Ratio[a]	$I_{[G+H]^+}$[b]	$I_{[M+H]^+}$	$I_{[M+Na]^+}$	$I_{[M+K]^+}$
20	0	0	100	10	5	3
20	4	0.2	100	17	40	7
20	8	0.4	100	10	50	5
20	12	0.6	100	9	77	5
20	16	0.8	100	3	61	3
20	20	1.0	100	1	80	3

[a] Molar ratio of metal ion : crown. [b] Molecular ion corresponding to glycerol.

Table 2 Peak intensities scaled relative to the potassium ion internal standard

Molar ratio	$I_{[M+H]^+}/I_{[M+K]^+}$	$I_{[M+Na]^+}/I_{[M+K]^+}$
0	3.3	1.7
0.2	2.4	5.7
0.4	2.0	10.0
0.6	1.8	15.4
0.8	1.0	20.3
1.0	0.3	26.7

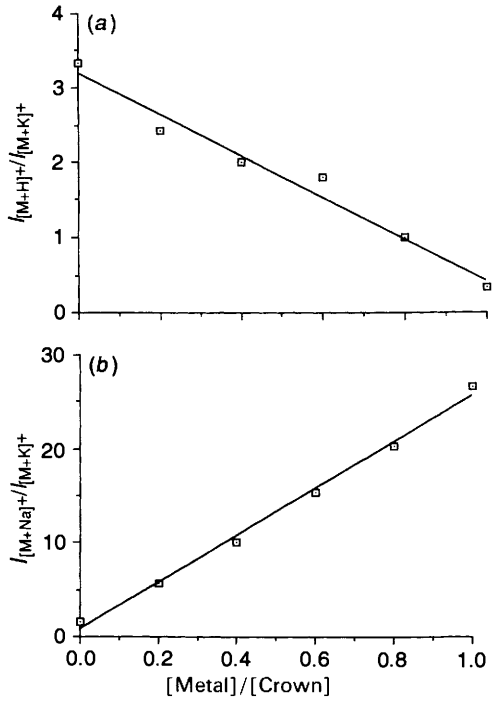

Fig. 4 Plots of (*a*) decrease in intensity of $[M + H]^+$ and (*b*) increase in intensity of $[M + Na]^+$ with increasing molar ratio of metal ion to crown for 15-crown-5

background levels of metal ions to be quantified. Freshly prepared solutions of commercial samples of the simple crown ethers were found to contain significant levels of sodium and potassium ions even when extreme care was observed in the handling and preparation of the samples. These levels were minimized by handling the crown solutions with plastic pipettes and storing the crown solutions in sterile Eppendorf tubes, though with time even these leach alkali metals. Storage of the crown solutions in volumetric flasks resulted in considerable background levels of both sodium and potassium ion complexes reflecting the efficient metal ion stripping capabilities of the crown compounds, Fig. 5. This seemingly trivial point has not been alluded to in any of the work published in this area.

The background concentration of sodium ions may be determined from the calibration experiments where the response to standard additions of sodium ions to the crown has been determined; for the 15-crown-5–Na^+ system described a 1.4 mmol dm^{-3} background concentration of Na^+ ions was present. This background level is relatively small, but not insignificant when quantitative studies are made at low concentration.

The amount of 'free crown' available for binding is further diminished by the presence of (*i*) ammonium adduct ions, the concentration of which decreases rapidly during analysis, suggesting their origin to be of a volatile nature and (*ii*) the formation of sandwich 'dimer' complexes, *i.e.* two ligands binding to one metal (such complexes frequently display markedly different selectivity to the 1:1 species). These 'dimer' peaks are of low intensity with respect to the 1:1 crown–metal complex, but again diminish the overall pool of free crown available. Preliminary experiments suggest that they are independent of sample concentration showing that they are not an artefact of the ionization process. No account of these observations has previously been addressed, though a means of quantification for the sandwich complex is difficult to envisage. We suggest that taking averaged data for the crown–metal complexes after such time that the source of the 'volatile' ammonium ions has significantly diminished should minimize their contribution.

It should also be noted that the intensity ratios determined from the above calibration studies (1:8 for Na^+, 1:22 for K^+) are in good agreement with the values obtained from the single experiments where a half molar equivalent of metal ion is added to the crown (1:10 for Na^+, 1:20 for K^+). This correlation suggests that a single experiment of the former type may be sufficient to allow an estimate of 'relative efficiencies of ionization' to be made. Thus, for a competitive binding experiment, a series of such spectra for each metal to be included in the competitive study would provide a series of relative ionization intensity figures. These factors could then be used to interpret the results of a competitive experiment in a meaningful fashion.

Origin of signal intensity differences

A simple comparison of spectra for solely protonated crown ($[M + H]^+$) and solely sodium complexed crown ($[M + Na]^+$) reveals entirely different fragmentation patterns, Fig. 6. The sodium complex displays little fragmentation whereas the protonated crown species displays fragments corresponding to the step-wise loss of CH_2CH_2O subunits (*m/z* 177, 133, 89 and 44), as well as other peaks derived from the crown.

The implication of this observation is that when measurements of protonated species ($[M + H]^+$) are made, only that proportion of the species that is still intact is actually being measured. The absolute amount of species present can only be determined by making allowance for the various fragment ions also observed in the spectrum. In the example shown, the $[M + H]^+$, *m/z* 221, species accounts for approximately 25% of the observed ion current, whereas for the corresponding sodiated

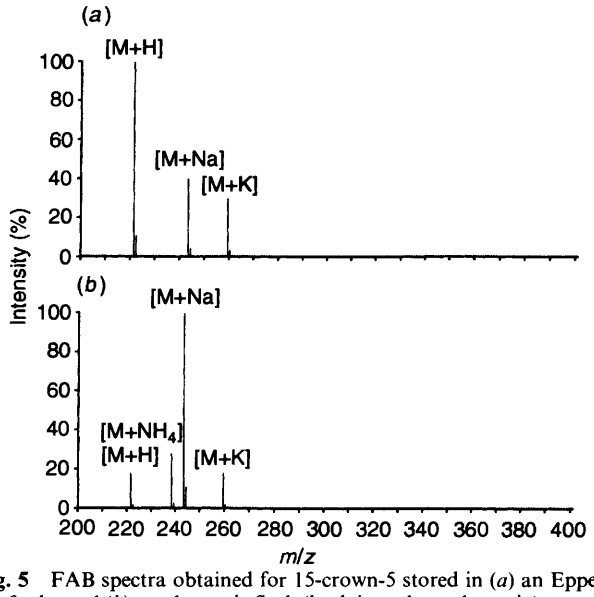

Fig. 5 FAB spectra obtained for 15-crown-5 stored in (*a*) an Eppendorf tube and (*b*) a volumetric flask (both in a glycerol matrix)

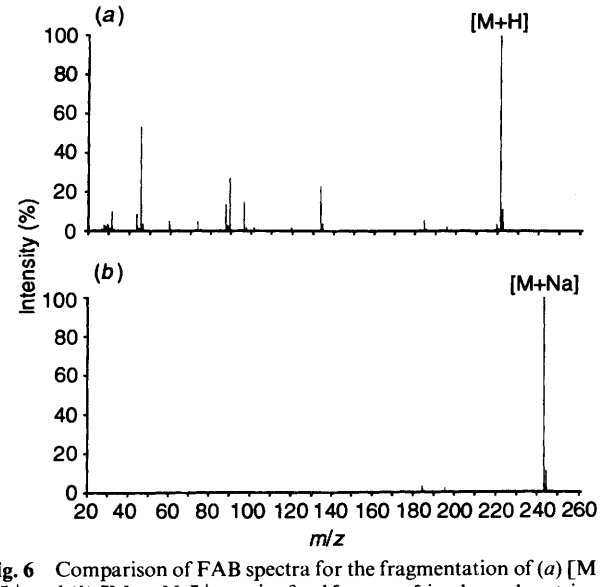

Fig. 6 Comparison of FAB spectra for the fragmentation of (*a*) $[M + H]^+$ and (*b*) $[M + Na]^+$ species for 15-crown-5 in glycerol matrix

species virtually all the complex is observed at *m/z* 243 (95%). This immediately introduces an intensity ratio of 1:4 when comparisons of the peak heights of pseudo-molecular ions are made and is undoubtedly a contributing factor to the overall differences observed in 'ionization efficiency'. A similar trend is also observed when 18-crown-6 is used in place of 15-crown-5. Potassium and cesium complexes display a similar lack of fragmentation.

We have also noted that significant absolute differences in intensity exist between complex ions. This observation is another contributing factor to the overall differences observed between $[M + H]^+$ and $[M + Metal]^+$ signals and further emphasizes the need to calibrate the system under investigation prior to performing any binding experiments.

Conclusions

The technique of studying ligand–cation interactions by FABMS, because of its ease of application and inherent sensitivity, has great appeal. However, two pivotal factors have not been addressed when experiments of this type have been reported in the literature and therefore such reports must be viewed with scepticism. (*i*) The nature of the matrix has a profound effect on the binding equilibria under investigation. (*ii*) The observed 'ionization efficiencies' of the various ligand–cation complex ions vary widely and hence measured ratios of intensities of species give little meaningful information concerning relative extents of binding.

To emphasize this latter point, the assumption that has frequently been made in the previous studies is that if $I_{[M+H]^+} = I_{[M+Na]^+}$ then these species are present in equimolar quantities. This has been shown to be invalid.

To extend this work, a large number of comparative studies are required, examining, for example, the effects of viscosity, acidity and polarity, or the type of anion present; recent work [7] on the dramatic influence of matrix polarity has confirmed such a need. Our work, and the only other quantitative data available, suggest that glycerol is a good solvent in this context, at least for the simple crowns studied here.

It is inescapable that in order for quantitative arguments to be applied to data obtained from these types of experiments, 'ionization efficiencies' for all of the species in question must be determined. This means that an experiment involving one ligand with a series of metals as a means of determining its binding selectivity is invalid, estimates need to be made of the relative 'ionization efficiencies' of the complexes in question. We have shown that a single experiment for each ligand–cation complex may be sufficient to allow the determination of such a series of efficiencies, these can then be applied to the binding selectivity study. It should also be noted that care must be taken when working at low concentrations (< 50 mmol dm^{-3}) due to possible background contamination from alkali metals.

We are currently investigating methods of determining such a set of ionization intensity ratios in a single experiment by adding, *e.g.* 0.1 molar equiv. of Li, Na, K, Rb and $CsNO_3$ to 15-crown-5 such that a 2:1 ratio of crown to total metal ion concentration persists. This excess of crown should ensure that a significant $[M + H]^+$ signal is generated. Ratios of one fifth of this signal intensity to that of each individual $[M + Metal]^+$ peak would then give the relative ionization intensity figures for each crown:metal complex. Initial experiments, as outlined above, have encountered difficulties due to the lack of any signal corresponding to lithiated species, cationized glycerol matrix and relatively high background levels of sodium and potassium (this latter problem may be due to the low concentration of the solutions used). At present, it appears that at least one experiment for each metal to be included in this type of study must be performed in order to assign confidently the necessary signal intensity ratios, though we hope to refine the multi-metal experiment to achieve the one experiment calibration goal. It is at this point that meaningful competitive binding data can be determined.

For purposes of comparison between different host–guest systems it is desirable that a set of protocol becomes established for undertaking studies of this type.

Experimental

All materials were purchased from Aldrich and were used without further purification. Solutions (typically 20 mmol dm^{-3}) of 12-crown-4, 15-crown-5 and 18-crown-6 were prepared gravimetrically in sterile Eppendorf tubes using freshly distilled methanol. Such solutions were used within 24 h of preparation since the background levels of metal ions are seen to increase rapidly with prolonged storage. The nitrates of lithium, sodium, potassium, rubidium and cesium were of the highest grade commercially available and were prepared as methanolic solutions (typically 4–20 mmol dm^{-3}) in volumetric flasks. All mass spectrometric experiments were carried out on a VG Analytical 70-250-SE normal geometry double focussing mass

spectrometer, fitted with an Ion-Tech saddle-field gun. The latter was operated at 8 kV, with the gun current monitored at 1.6 mA, using argon as the bombarding gas. All data were recorded on an 11–250 data system and acquired at 8 kV accelerating voltage, at a scan rate of 3 s/decade over a mass range of 1400–50 amu, with an inter-scan delay of 1 s. In a typical experiment 1 μl (1 μl = 1 mm^3) of crown solution and 1 μl of a metal nitrate solution were loaded onto the stainless steel tip of the FAB probe to which 1 μl of matrix (glycerol or NBA) had been added; in the series of calibration experiments, metal solutions of varying concentration were employed in order to keep the sample volume constant. 20 successive scans were then recorded and scans 5–15 averaged to afford the observed spectrum.

The validity of employing the residual $[M + K]^+$ peak as an internal standard for the calibration of the 15-crown-5–Na^+ system was confirmed by the observation of a consistent ion current resulting from this species.

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