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On the Assessment of Complex Cation–Crown Ether Equilibria by Electrospray Mass Spectrometry

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Positive electrospray mass spectrometry (ESI-MS) has been used to examine mixtures of alkali metal and alkaline earth cations with two 18-crown-6 carboxylic acid crown ethers, a bis-crown ether H_2A and a mono crown ether dicarboxylic acid H_2B. A variety of mononuclear and polynuclear complexes were observed as mono, di, and trications. Stoichiometries up to $\{A+4K\}^{2+}$ and $\{A+2Ba\}^{2+}$ were achi-eved at high guest/host ratios. Using published and estimated formation constants, the expected solution speciation was calculated for comparison with the complex ion intensities observed by ESI-MS. Due to the concentration range of the ESI-MS experiment, the complexes observed are minor components of the equilibrium solution. The observed complexes reflected a higher level of protonation than was expected based on previous solution studies. In cases of direct comparison between complex ions of like stoichiometry and charge, the ratio of ion intensities by ESI-MS reflects the solution selectivity of the host. In general however, the electrospray ionization process results in differential production of ions from the equilibrium mixture and comparisons between ions of differing charges or stoichiometries do not correspond to the inherent selectivity of the host in solution.

Keywords: Electrospray mass spectrometry, crown ether, selectivity

Electrospray mass spectrometry (ESI-MS) has a brief but distinguished history in supramolecular chemistry [1]. Using this "soft" ionization technique, it has been possible to observe a variety of non-covalent host-guest complexes in the gas-phase: biomolecular complexes [2], crown ether-cation complexes [3], hydrogen-bonded rosette complexes [4], and so on [5]. The potential of ESI-MS for detecting and characterizing the stoichiometries of host-guest complexes has no parallel among solution-phase techniques to directly detect the stoichiometries of complexes without reference to solution modeling assumptions. Although many authors caution of the potential pitfalls in relating ESI-MS spectra to source solution compositions [6], there is evidence that ESI-MS can be correlated with equilibrium distributions of species in solution in some cases [7–9].

Given the ease of the technique, it is important to evaluate the potential of ESI-MS for the characterization of host-guest complexes, especially for cases involving multiple equilibria.

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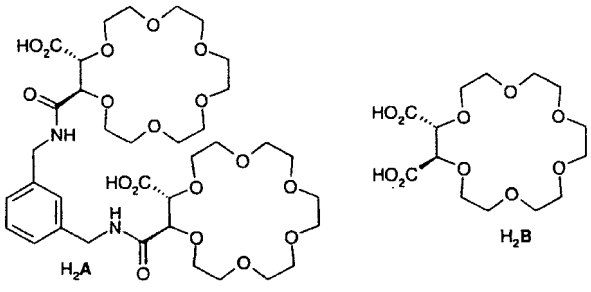
Systems involving mixed host-guest stoichiometries (1:1, 2:1, 1:2) or mixtures of guests competing for a single host should be amenable to ESI-MS analysis, perhaps to give rank-order stability and selectivity information in a single experiment. Our interest in bis-carboxylic acid crown ethers as ion-sensing [10] and as membrane-disrupting agents [11] provided suitable materials (H_2A, H_2B) to explore the potential of ESI-MS for the rapid determination of speciation and selectivity. In addition to 1:1 and 2:1 crown ether-cation complexes expected for bis-crown ethers, the ionization of the carboxylic acids provides a range of protonation states. The stability constants for complex formation are known [10, 12] or can be estimated from a large body of related work [13], thus the likely speciation in solution can be predicted as an aid in the interpretation of the ESI-MS spectra. The crown diacids H_2A and H_2B each contain two ionizable carboxylic acid groups. ESI-MS cannot detect neutral complex species, such as $\{A + Ba\}^0$, but should be able to detect the related protonated complexes such as $\{HA + Ba\}^+$ [14]. The key question is whether a relationship between intensities of cation/crown complexes in the gas phase and the concentrations in the solutions exists even though only a subset of species are directly observed?



SCHEME 1 Structures of crown ethers.

RESULTS

ESI-MS of Complexes of H_2A

Preliminary exploration established that reproducible positive ESI-MS spectra of H_2A with alkali metal and alkaline earth cation salts could be obtained in acetonitrile/water (1:1) solutions at salt concentrations between 10 and 500 μM. The experiments described below used a constant concentration of 50 μM of H_2A as this allowed a suitable range of host/guest ratios within the salt concentration range. Initial experiments used added acetic acid, but the same positive ESI-MS spectra were found with or without this additive, and subsequent experiments omitted additional acid. The crown ether diacid is a stronger acid than acetic acid (pK_1 = 4.06) [8], and metal ion binding decreases the apparent pK_a in proportion to the complex strength [13]. The solutions had an approximate pH of 3.2–3.8 (pH paper), equivalent to the acidity of a similar concentration of a strong acid such as HCl in this solvent system[a]. Preliminary experiments also established that ion intensities varied in expected ways with the solution composition: complex ions increase in intensity with increasing salt concentration and decrease in intensity as competing salts are added.

Figure 1 shows the positive ESI-MS obtained from a solution containing H_2A and a mixture of alkali metal cations. The ratio of the cations added was based on an expectation of the different complexing abilities of alkali metal ions with a typical 18-crown-6 [15]. A series of peaks corresponding to ions of stoichiometry $\{H_2A + M\}^+$ are observed, each with a sequence of isotope peaks to higher masses. The separation between successive isotope peaks is 1 dalton, so all peaks were assigned as monocations. The details of the ion assignments are

[a]A referee queries whether pH paper is a sufficiently "elegant" way to measure pH. It is, as it gives an acid/conjugate base ratio directly without the need for the medium specific buffers required for a pH electrode calibration.

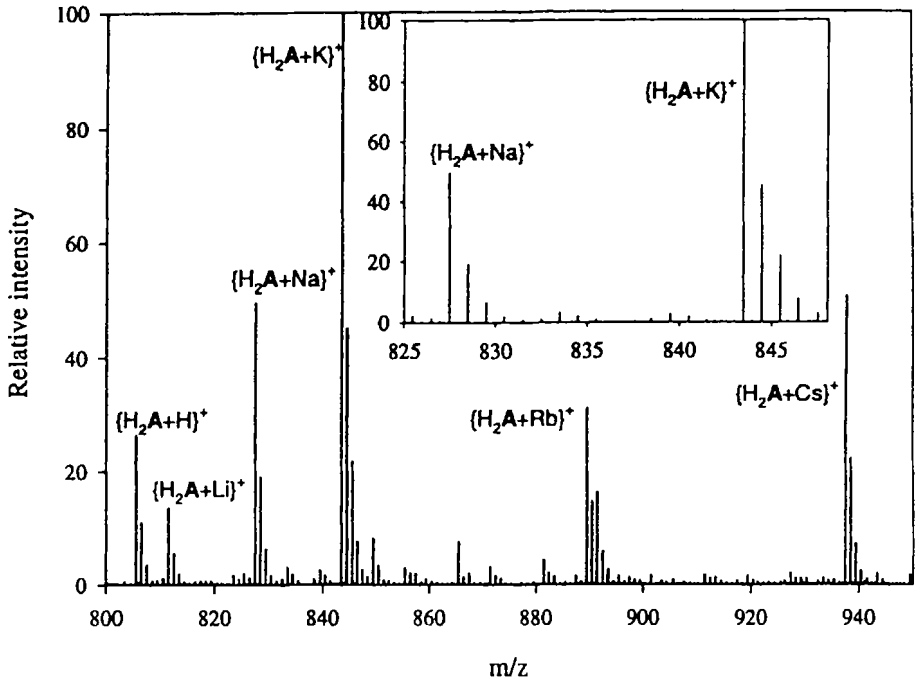


FIGURE 1 ESI-MS spectrum of H_2A and mixed alkali metal cations. Inset: expansion showing unit mass separation of isotope peaks at the base peak. Concentrations are given in the footnote to Table I.

TABLE I ESI-MS ions observed for a matrix of H_2A and alkali metal chlorides[a]

Observed (m/z)	Assigned ions	Calculated (m/z)	Intensity (%)	Apparent selectivity[b]	Calculated $[\{A+M\}^-]$[c]
936.6	$\{H_2A+Cs\}^+$	937.3	56	14	16
888.7	$\{H_2A+Rb\}^+$	889.3	31	31	13
842.8	$\{H_2A+K\}^+$	843.3	100	100	100
826.8	$\{H_2A+Na\}^+$	827.3	48	24	32
810.9	$\{H_2A+Li\}^+$	811.4	12	3	20
805.5	$\{H_2A+H\}^+$	805.3	28		

[a] 50:50 acetonitrile: water; $[H_2A] = [KCl] = [RbCl] = 50\,\mu M$, $[LiCl] = [CsCl] = 200\,\mu M$.
[b] Intensity corrected for the differing salt concentrations; $I_M \times [MCl]/50\,\mu M$.
[c] Model assumed eight complexes at pH 3.5(M^+, lmh, log β)[8, 15, 28]: H^+, 101, 4.08; H^+, 102, 7.78; Li^+, 110, *1.5*; Na^+, 110, 2.0; K^+, 110, 2.8; K^+, 120, 6.3; Rb^+, 110, 1.9; Cs^+, 110, 2.4. Estimated log β values are italicised. [15] Equilibrum concentrations were normalized to 0.14 μM (K^+ 110 complex = 100). Less than 1% of any cation is bound, and less than 10% of the host is bound: $[H_2A]_{eq} = 27.5\,\mu M$; $[HA]^-_{eq} = 17\,\mu M$; $[A^{2-}]_{eq} = 1.3\,\mu M$.

given in Table I, together with the ion intensities of the major isotopic peak. Also given in Table I are the intensities corrected by the source phase concentration factors and normalized to the base peak. Isotopic abundance corrections were not applied as they would not alter the qualitative picture [7]. The values appear to follow the Eisenman III selectivity, typical of alkali metal cations interacting with predominantly neutral oxygen donors, as expected for complexes of a neutral 18-crown-6 [16].

How do these complexes compare to those expected from prior solution studies? Complexes of neutral H_2A were not observed previously: the dominant complexes required to fit pH titration data were complexes of the fully deprotonated A^{2-} ion [10, 14]. Moreover, K^+ complexes of 2:1 K: A^{2-} stoichiometry were

required in the solution equilibrium models [10], but were not detected in the ESI-MS spectra at any level of protonation. The cation complexes observed previously are relatively weak, and the solutions are dilute, so the extent of complex formation is very low. Using the known formation constants, it can be calculated [17] that less than 1% of any cation is bound, and less than 10% of the total ligand is involved in cation complexes. The *ratios* of the concentrations of complexes of $\{A+M\}^-$ stoichiometry, normalised to the K^+ complex, are given in the final column of Table I. If ESI-MS probes solution speciation then these ratios should directly relate to the observed intensities of the individual ions (not corrected for solution composition) on the assumption that complex protonation affects complex stability in a linear way across the series of cations. The agreement is poor. This may simply reflect the accumulated errors of comparing different ions under different solvent conditions. As developed below, it more probably indicates an inherent difference between ESI-MS ion intensities and solution chemistry.

A similar experiment using mixed alkaline earth chlorides and H_2A is illustrated in Figure 2 and summarised in Table II. Although the spectrum is substantially more complex, all observed ions can be assigned. The charge of the ion can be deduced from the mass separations of the isotope peaks: monocations ions give unit mass isotope sequences, dications give half integral spacing, and so on. The insets to Figure 2 illustrate this point. Thereafter, calculated masses for various stoichiometries can be compared with the observed masses, and ion charge states can be confirmed *via* comparison of calculated and observed isotope distribution patterns. The dominant solution species calculated using only known stability constants [10] and the concentration and pH of the experiment are the neutral (unobservable by MS) mononuclear complexes $\{A+Sr\}^0$ and $\{A+Ba\}^0$. The deprotonated 1:2 complexes $\{A+2Ba\}^{2+}$ and

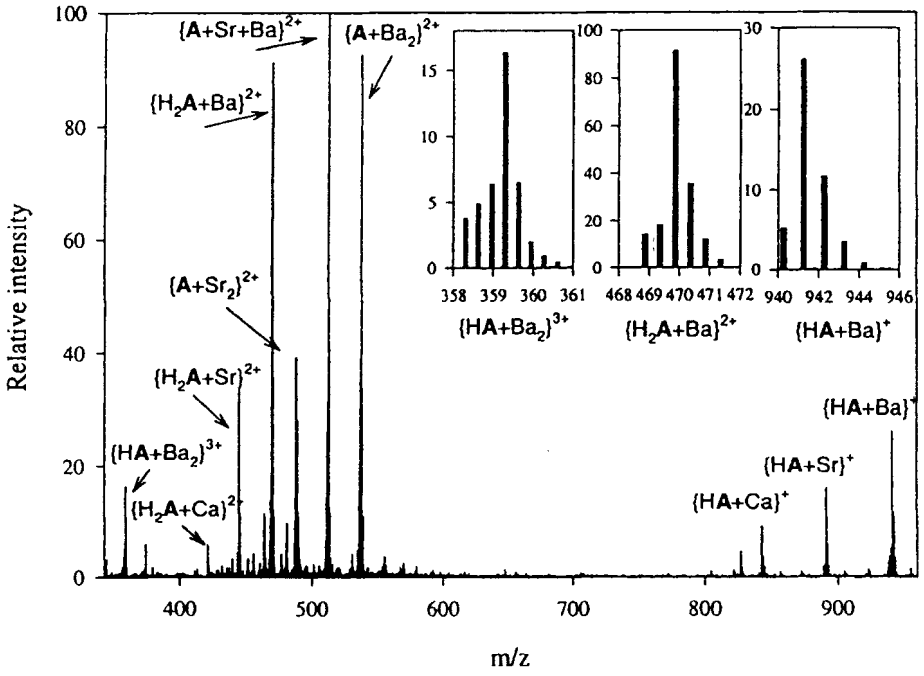


FIGURE 2 ESI-MS spectrum of H_2A and mixed alkaline earth cations. Inset: expansion showing mass separation of isotope peaks for various charge states. Concentrations are given in the footnote to Table II.

TABLE II ESI-MS ions observed for a mixture of H_2A and alkaline earth chlorides[a]

Assigned Ions	Observed m/z	Calculated m/z	Intensity %	Calculated concentration[b] (μM)
$\{HA+Ba\}^+$	941.2	940.3	26	0.91
$\{HA+Sr\}^+$	891.1	890.6	16	0.90
$\{HA+Ca\}^+$	842.9	843.1	10	0.08
$\{A+2Ba\}^{2+}$	537.4	538.3	94	0.13
$\{A+Ba+Sr\}^{2+}$	512.5	513.5	100	0.19
$\{A+2Sr\}^{2+}$	487.7	488.6	38	0.26
$\{H_2A+Ba\}^{2+}$	469.9	470.7	87	0.14
$\{H_2A+Sr\}^{2+}$	445.0	445.8	31	0.14
$\{H_2A+Ca\}^{2+}$	421.2	422.0	7	0.02
$\{HA+Ba\}^+$	359.7	359.2	16	*

[a] 50:50 acetonitrile: water; $[H_2A]=[CaCl_2]=[SrCl_2]=[BaCl_2]=50\,\mu M$.
[b] Model assumed fourteen complexes at pH 3.5 (M^{n+}, lmh, log β) [8, 15, 28]: H^+, 101, 4.08; H^+, 102, 7.78; Ca^{2+} 110, *6.1*; Ca^{2+}, 112, *8.8*; Sr^{2+}, 110, 4.1; Sr^{2+}, 120, *7.5*; Sr^{2+}, 120, *7.5*;. Sr^{2+}, 111, *7.2*; Sr^{2+}, 112–*9.9*; Ba^{2+}, 110, 4.1; Ba^{2+}, 120,*7.2*; Ba^{2+}. 111–*7.2*; Ba^{2+}, 112 *9.9*; mixed $Sr^{2+}+Ba^{2+}$, 1(2)1–*7.35*. Estimated log β values are italicised. [16] About 20% of the host is bound: $[H_2A]_{eq}=24\,\mu M$. $[H\,A]_{eq}=15\,\mu M$; $[A^{2-}]_{eq}=3.9\,\mu M$.

$\{A+2Sr\}^{2+}$ observed have concentrations about an order of magnitude lower. Although not previously detected, the mixed deprotonated complex $\{A+Sr+Ba\}^{2+}$ is also not surprising given the modest Sr^{2+}/Ba^{2+} selectivity of the ligand. None of the protonated complexes observed in the ESI-MS spectra were detected in solution studies. The calculation suggests that about 20% of the host would be associated in cation complexes, with 5% of the Sr and Ba, and 1% of the Ca bound. Using estimated formation constants [18] for the complexes actually observed by ESI-MS allows the final column of Table II to be calculated. The additional complexes do not alter the extent of cation or ligand binding. The comparison of the calculated concentrations with the observed ion intensities is poor overall, although the rank order for any triad of complexes of a defined stoichiometry is correctly reflected by the ESI-MS spectrum.

Table II offers a variety of choices for examining the selectivity of the host for the mixture of guests. Comparisons of ion intensity for ions of like stoichiometry *e.g.*, $\{HA+M\}^+$ gives an apparent Ca:Sr:Ba relative selectivity of 38:61:100 (normalised 'to Ba). In solution, the related monometallic deprotonated complex $\{A+M\}^{2+}$ has a relative Ca:Sr:Ba selectivity of 18:95:100 (also normalised to Ba) [10]. The direct comparison of 1:2 complexes $\{A+2M\}^{2+}$ gives a Sr/Ba selectivity of 0.4 from the ESI-MS while in solution it is 2.0. The general condusion is that H_2A is less selective than would be expected for neutral 18-crown-6 (Ca:Sr:Ba; 0.4:8:100) [15], in accord with a trend usually observed for carboxylate crown ethers [13].

Any mixture of cations with H_2A gives ESI-MS spectra of complex ions. Figure 3 and Table III give the results for an ESI-MS spectrum of H_2A containing one equivalent of $BaCl_2$ and ten equivalents of KCl. The ratio of the concentrations was chosen to reflect the rough order-of-magnitude lower stability of K^+ *versus* Ba^{2+} complexes of the ligand for every stoichiometry observed in solution [10]. The majority of the observed ions have not been previously detected in solution studies, and the calculation of equilibrium concentrations would involve an unacceptably large number of assumptions. Using only the known solution formation complexes [10], the solution was calculated to contain roughly equivalent amounts of 1:1 and 2:1 (M:A) complexes and the dominant ions in the ESI-MS spectrum do indeed contain two metal cations. There are a variety of other two metal ion complexes found among the minor ions together with two examples of ions containing three cations. In this example, as previously,

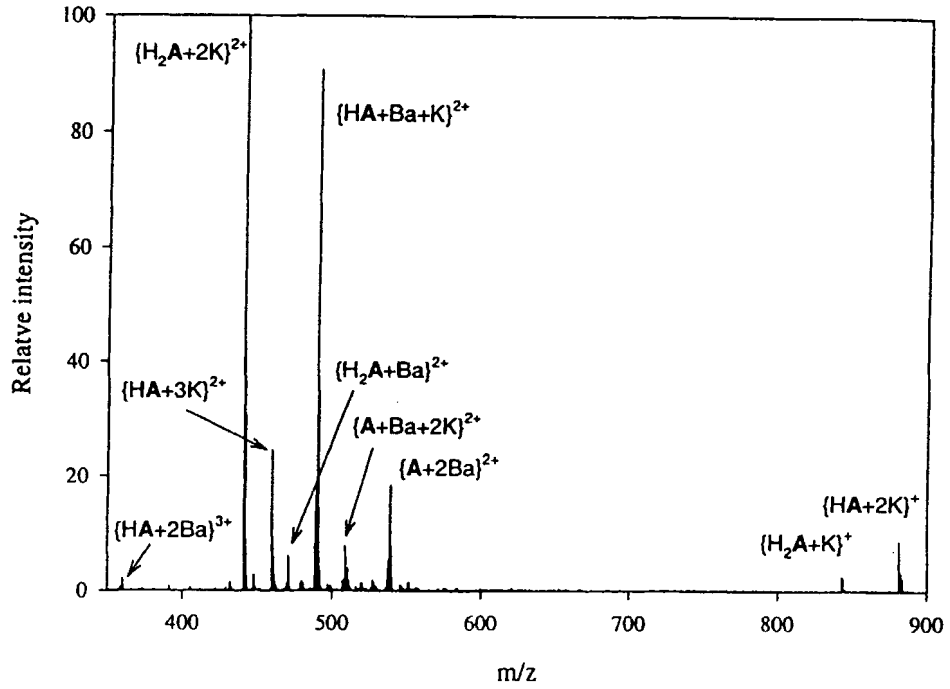


FIGURE 3 ESI-MS spectrum of H_2A, KCl and $BaCl_2$. Concentrations are given in the footnote to Table III.

TABLE III ESI-MS ions observed for a mixture of H_2A, KCl and $BaCl_2$[a]

Assigned Ions	Observed *m/z*	Calculated *m/z*	Intensity
$\{H_2A + K\}^+$	843.3	843.1	3
$\{H_2A + 2K\}^+$	881.4	881.2	9
$\{A + 2Ba\}^{2+}$	539.1	538.3	18
$\{A + Ba + 2K\}^{2+}$	509.1	509.1	8
$\{H_2A + Ba + K\}^{2+}$	490.1	489.7	91
$\{H_2A + Ba\}^{2+}$	471.2	470.7	6
$\{HA + 3K\}^{2+}$	460.1	460.1	24
$\{H_2A + 2K\}^{2+}$	441.1	441.1	100
$\{HA + 2Ba\}^{3+}$	359.8	359.2	2

[a] 50:50 acetonitrile: water; $[H_2A] = [BaCl_2] = 50\,\mu M$, $[KCl] = 500\,\mu M$.

there is a preponderance of protonated complexes, even though these would be expected to be much less abundant than the deprotonated complex ions which are also observed. Also the $Ba^{2+}K^+$ selectivities are apparently lower than expected [10] in any comparison of complexes of comparable stoichiometry.

The higher order complexes observed prompted a qualitative exploration of the stoichiometries which might be accessible at higher salt concentrations. With one equivalent of KCl and H_2A (100 μM), the major component in the ESI-MS spectrum is the 1:1 cation/bis(crown ether) complex $\{H_2A + 3K\}^+$. However, with increasing concentrations of K^+, ions corresponding to one bis(crown ether) complexing with two ($\{HA + 2K\}^+$), three ($\{A + 3K\}^+$), and even four potassium ions ($\{A + 4K\}^{2+}$, at 500 μM KCl) were found in the spectra. Similarly, at one equivalent of $BaCl_2$ (50 μM H_2A), the major component in the ESI-MS spectrum is the 1:1 cation/bis(crown ether) complex $\{H_2A + Ba\}^{2+}$. Increasing concentrations of $BaCl_2$ gave only the 1:2 complex ion $\{A + 2Ba\}^{2+}$. The 1:3 or 1:4 complexes were not detected up to twenty equivalents of $BaCl_2$ added. The triply charged protonated complex $\{HA + 2Ba\}^{3+}$ had a very low intensity (< 2%) and no other triply or quadruply charged ions could be observed. Given the acidity of the solution, it is surprising that complexes of the fully deprotonated ligand can be observed. As discussed below, this is evidence of ion fractionation during ESI-MS. The maximum stoichiometries

observed suggest a limit defined by the extent of electrostatic repulsions between the metal cations. Solution studies did not require the inclusion of higher-order complex species above the deprotonated 2:1 complexes discussed above [10]. Alternatively, tripositive and higher charged ions might not be efficiently produced under the experimental conditions.

Complexes of H_2B

The preponderance of protonated complexes observed in Figures 1–3 prompted a quantitative examination of the ESI-MS spectra of complexes of H_2B, since in this single instance of carboxylate crown ethers, experimental values for the formation constant of the $\{H_2B+M\}^+$ complexes are known from ion selective electrode titrations in acidic solution ($\log K_K = 2.0 \pm 0.3$; $\log K_{Na} = 0.7 \pm 0.4$, pH 1.2 HNO_3, 298 K, 0.5 Me_4NNO_3) [19]. It is therefore possible to calculate equilibrium concentrations for the complexes formed in mixtures of H_2B:NaCl:KCl without the assumptions inherent in the foregoing analysis. An ESI-MS experiment involving fixed concentrations of H_2B and KCl ($[H_2B] = 50\,\mu M$; $[KCl] = 25\,\mu M$) and variable NaCl concentrations (0.1–0.4 mM) gave spectra as illustrated in Figure 4. In the high mass region, only two complex ions are observed, corresponding to $\{H_2B+Na\}^+$ (m/z expt = 375.1; m/z calc. = 375.13) and $\{H_2B+K\}^4$ (m/z expt. = 391.1 m/z calc. = 391.10). These two ions are calculated to account for 0.26% of the total bound ligand, which itself amounts to only 17% of the total ligand available. The intensity of the Na^+ complex ion increases with increasing concentration of NaCl, while the intensity of the K^+ complex ion remains approximately constant. Thus the uttenstiy ratio (I_{Na}/I_K) increase as the concentration ratio $[Na^+]/[K^+]$. The increase is linear (inset Fig. 4) with a slope of

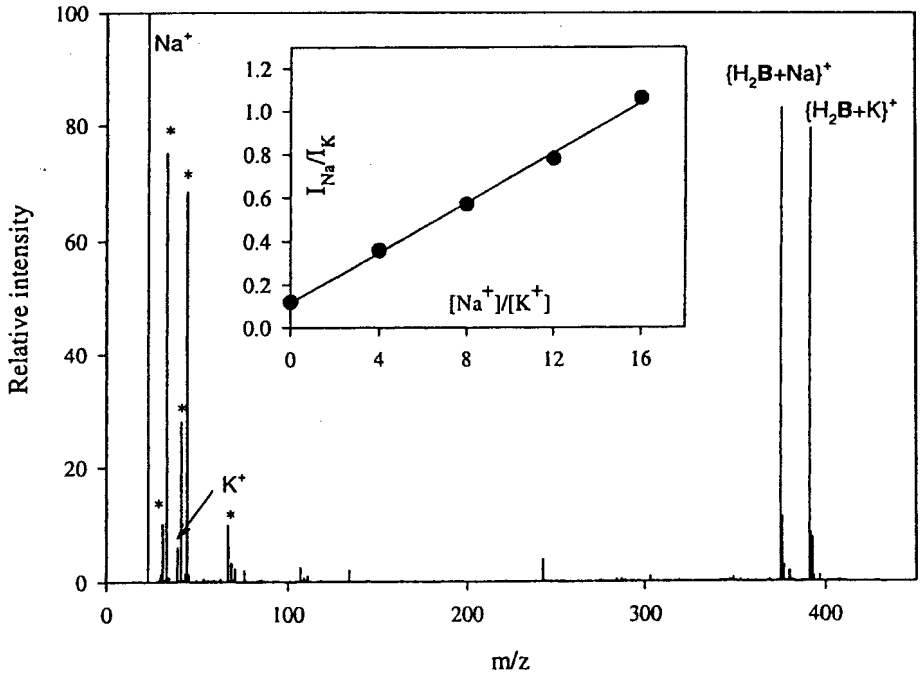


FIGURE 4 ESI-MS of H_2B (50 μM), KCl (25 μM) and NaCl(400 μM) in 50:50 acetonitrile:water. The peaks marked with an asterisk derive from the solvent (see text). Insert: ratio of the intensities of m/z 359 (I_{Na}) and m/z 374 (I_K) as a function of the concentration ratio of Na^+/K^+ in the sample solution.

0.057 ($r^2 = 0.993$). The intercept is positive indicating a low level of sodium contamination in the sample used. Following the assumption that the complex peak intensities are proportional to the concentrations in solution [7–9], this slope corresponds to an apparent K^+/Na^+ selectivity of 18, which can be compared with the solution selectivity found for the same complexes of 20 ± 8 [19].

The low mass region of Figure 4 is more complex than expected. The ions marked with * do not vary in intensity between different solutions, and are apparently related to the solvent and it's impurities. Peaks at the nominal masses of protonated methanol (33.1) and acetonitrile (42.0) are present together with peaks at 31.4, 45.4 and 67.4 m/z. The free Na^+ and K^+ peaks are evident in all spectra in the series. At the low concentrations of the ESI-MS experiment, only a few per cent of the cations are bound, hence the free ion concentrations should closely reflect the total concentrations in the experiment. In Figure 4, the Na^+ intensity is 16.4 times the K^+ intensity, which can be compared to the stoichiometric ratio of 16 for this experiment. The same agreement is not found between the intensities of the Na^+ and $\{H_2B + Na\}^+$ peaks: the former is calculated to be about 550 times more concentrated than the latter, not nearly equivalent as found by ESI-MS.

DISCUSSION

Is there a relationship between the species observed *via* ESI-MS and the equilibrium concentrations of the same species in solution? Some of the results such as the determined Na^+/K^+ selectivity of H_2B, and other cases from the literature [7–9] suggest that the relationships is direct and quantitative. Others, such as the apparently random relationship between ion intensities and calculated concentrations in Table II clearly lead to the opposite conclusion. Some of the selectivity patterns observed by ESI-MS are entirely as expected, others are dramatically attenuated. The principle of jurisprudence known as the "fruit of the tainted vine" asserts that if some portion of a witness' testimony can be shown to be false, then all testimony from that witness should be disregarded. ESI-MS does not suffer the vagaries of memory and motivation that afflict human witnesses, but the principle is sound. If we cannot define the circumstances under which ESI-MS results are reliable, then we will be forced to regard all ESI-MS data with suspicion, and the reported correlations as fortuitous.

Although the detailed mechanism of ion production in ESI-MS remains obscure [1, 20], there are some established features which suggest how the complex ions produced arise from the solution. In positive ESI-MS, the sample solution emerges into the source *via* a needle held at a positive potential relative to a collector plate in the low pressure region. Anions in the solution will migrate towards the needle, while cations will be repelled into the droplets that spray into the source and eventually product the cations observed in the mass spectrometer *via* a process of solvent evaporation and droplet fission [20]. Although we did not observe any chloride adducts in the experiments described and biopolymers frequently give spectra of counter-ion free molecular ions [1], associated anions can be seen in some ESI-MS data [3–5, 7, 9]. The extent of counterion removal will depend on flow rate, solution conductivity, and other operational parameters [20]. To the extent that it occurs, the environment of the drop immediately after it leaves the needle will be significantly different from the sample solution injected.

Consider a simpler version of the equilibria involved with H_2A and H_2B based on a monoacid crown ether HC and a single metal ion complex of the conjugate base MC:

$$HC + H_2O = C^- + H_3O^+ \quad (1)$$

$$C^- + M^+ + X^- = MC + X^- \quad (2)$$

These two equilibria will respond to the electrospray needle potential in different ways. The position of equilibrium (2) may be relatively unaffected by the migration of anions to the needle, especially if there is little interaction of the counterion X^- with the complex MC and little initial ion-pairing with M^+. Equation (1) will be more strongly influenced as C^- could be drawn from the solution by the needle potential. This does not necessarily happen since the equilibrium (1) can shift to the left to remove C^- more rapidly than C^- diffusion and discharge at the needle can occur. The ESI-MS interface is a type of electrolysis cell where the circuit is completed by the ion current between the needle and the collector plate [20, 21]. Both by anion removal and water oxidation, the solution will become more acidic [21]. Thus as the sample solution flows through the needle the complexation equilibrium (2) will shift to the left. As the drop breaks electrical contact with the needle, it will be more acidic than the injected sample, and will have a different equilibrium position than the injected sample.

What evidence is there for this picture of acidification during positive ESI-MS? The spectra presented in Figures 1–4 all contain ions which are more protonated than expected based on equilibrium considerations. Only very stable complexes such as $\{A+2Ba\}^{2+}$ appear in the expected deprotonated forms and even this type also show protonated relatives. Shifts in protonation state as a result of ESI-MS ionisation has been quantified for mixtures of ammonium salts [22], and protonated proteins can be observed by ESI-MS even from basic solution as a result of acidification during ESI-MS ionisation [23]. The acidity of electrospray droplets can be very high as proteins routinely give polycationic charges which exceed by 15–20% those expected on the basis of the lysine + arginine content of the protein [24]. Protein samples use additional acid to assist ion production, but even so it is clear that additional weakly basic sites, such as carbonyls or electron-rich aromatics, must become protonated. The complex ion $\{H_2A+H\}^+$ (Fig. 1) is an example of protonation on a weakly basic site.

Other changes occur during droplet evaporation/fission. Initially the droplets are of the order of micrometers in diameter and maintain their charge as they evaporate [20]. As the droplet shrinks the distance between charges decrease to a point (the Rayleigh limit) where droplet fission occurs. The droplet does not split evenly into two, but typically looses 2% of it's mass and 15% of its charge to about 20 smaller offspring droplets [20, 25]. The parent droplet continues to evaporate, further uneven fission occurs, and the process repeats. Gas-phase ions are produced from very small (nanometer) droplets by mechanisms which are incompletely understood [1, 20]. The uneven fission of the first generation offspring can produce droplets capable of direct gas-phase ion production, so potentially only few fission events are required [20]. The time to the first fission of the parent is several hundred microseconds [19], thus rapid complexation and acid-base equilibria involving cations and crown ethers potentially have sufficient time to occur [26]. The "solution phase" of the parent droplet is far from a well-mixed homogeneous equilibrium as the free charges will tend to migrate to the surface of the droplet to minimise repulsion [20]. There they stand a better chance of being lost to small offspring droplets than do neutrals in the centre of the parent droplet. The subsequent fission of the offspring droplets occurs more rapidly, but still in the microsecond domain, and would be even less likely to reflect equilibrium considerations.

Ion binding by crown ethers is a means to reduce electrostatic repulsion between adjacent cations, thus it is possible that the parent droplet surface could be enriched in crown ether complexes relative to the bulk of the droplet. The crown ether may also possess some surfactant property that would enhance this surface enrichment [27]. This postulated enrichment is

consistent with the spectra obtained from species which are exceedingly dilute in the source solution. It is also consistent with the lack of intensity of discrete cations uch as Na^+ and K^+ as discussed with reference to Figure 4.

Given that the ESI-MS ionization process is complex, it is surprising that the spectra appear to be representative of solution equilibria under any circumstance. The electrospray ionization process provides a unique environment for complexation and acid-base reactions, and the spectra that result are inevitably dominated by the experimental conditions [22–24]. For peptides is it clear that ESI-MS spectra "... do not reflect the equilibrium distribution of solution-phase protonation" [22]. The data in Tables II and III support a similar conclusion for the complexes discussed here. Yet as demonstrated in Figure 4, ESI-MS *can* provide selectivity information which is directly comparable to solution values. How can these two be reconciled? The best examples of ESI-MS correlation with solution here and previously [7–9] involve comparisons between closely related complexes. The inconsistencies all involve comparisons between dissimilar complex ions. As noted above, particularly with reference to Figure 2, direct comparisons of ions of comparable stoichiometry and charge appear to give reliable selectivity information, while comparison of ions of different charges leads to the opposite conclusion.

Consider the case of ions of the same charge competing for a common ligand. In solution they will form some equilibrium mixture of complex ions. Whatever the process which leads to gas-phase ions, the two guests and their complexes will experience much the same electrostatic forces. Thus as they migrate to ever smaller droplets there will be little fractionation to distort the initial ratio of concentrations. In this case the ratio of ion intensities will accurately reflect the solution selectivity of the host. Conversely guests of different charge or complexes of differing stoichiometry will not respond to the electrostatic forces in similar ways and differentiation will occur during the electrospray process. As a result, ion intensities will not reflect solution selectivities.

The bottom line is that the type of experiment illustrated in Figure 3 is always doomed to fail while the type illustrated in Figure 4 is likely to be reliable. Mixed cases such as Figure 2 require cautious interpretation but can yield rank-order selectivities in favorable cases.

EXPERIMENTAL

Electrospray mass spectra were recorded using a Kratos Concept-IH mass spectrometer with Kratos electrospray interface. The electrospray needle was held at +7 kV relative to the electrospray chamber at ground. The accelerator and end-plate potentials were +4.5 kV relative to ground. Nitrogen drying gas was held constant at 25 psi and 50°C. A constant solvent flow of 2 μL/min was delivered *via* syringe pump. Initial experiments used 20 μM gramicidin-S in the bulk solvent flow as an internal mass calibration standard, and for monitoring the resolution and stability of the electrospray. This introduced low levels sodium, so later experiments used metal-free N,N′-ditetradecyl guanidimum chloride [28].

The synthesis of H_2**A** and H_2**B** have been previously described [10, 29]. Residual cation impurities were removed by ion exchange as previously described [12]. Salts were analytical grade and were used as received. An aliquot (100 μL) of the stock solutions of the crown ethers in acetonitrile, and aliquots of the salt solutions in water (total 100 μL) were combined to produce the sample for analysis. Samples (60 nL) were injected into the following stream and spectra were taken continuously. Scans of interest were averaged; typically 10–15 scans were used. Mass calibration used the reference regions which preceded and followed the sample. Data were exported as a list of masses,

intensities, and widths which were subsequently displayed in bar-graph form for the Figures. Replicate injections of the same solutions, or of different preparations of the same solutions, differed by less than 5% in the relative intensities of the complex ions discussed.

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