

# Calibration of Ion Spray Mass Spectra Using Cluster Ions<sup>†</sup>

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Mass calibration for ion spray mass spectrometry can be achieved by using cluster ions formed by flow injection of solutions of alkali metal salts in aqueous acetonitrile into the liquid flowing to the ion spray needle. Source contamination is thereby reduced to a minimum. For quadrupole mass analyzers, sodium iodide provides an ideal compromise between undesirable spectral complexity and spacings between calibrant mass peaks sufficiently close that interpolation errors are negligible. When much closer spacings are required, protonated water clusters provide an excellent calibration up to about  $m/z$  1000. If higher mass ranges are required with a large number of calibrant peaks, a solution of mixed alkali metal iodides does provide the expected spectra but intensities are poor at higher  $m/z$  values. For liquid chromatography with on-line mass spectrometry (LC/MS) the mass calibration may be checked without changing the mobile phase by post-column flow injection of a cesium carbonate solution, since the carbonate anion is wholly displaced by the anion of the mobile phase acid modifier, resulting in no mixed clusters. The metal salt calibrants have the additional advantage of being useful over a wide range of tuning parameters in the atmospheric pressure ionization source, covering those appropriate to both relative molecular mass determinations of large proteins and to LC/MS of small analyte species.

## INTRODUCTION

Spray ionization techniques have revolutionized the direct coupling of liquid chromatography to mass spectrometry (LC/MS). The first such technique to gain widespread acceptance was the thermospray interface introduced by Blakley and Vestal.<sup>1</sup> More recently, techniques based upon ion evaporation from charged micro-droplets at atmospheric pressure, developed from early experiments of Iribarne and co-workers,<sup>2</sup> Aleksandrov *et al.*<sup>3</sup> and Fenn and co-workers,<sup>4</sup> have extended the range of analytes accessible to LC/MS to much larger and more labile molecules. The electrospray technique<sup>3,4</sup> relies on electrical instability of the charged liquid effluent to achieve the nebulization required, but its efficient operation is restricted to liquid flow-rates in the range 5–10  $\mu\text{l min}^{-1}$  even when additional precautions are taken<sup>5</sup> to optimize this process. The introduction of ion spray (pneumatically assisted electrospray) by Henion and co-workers<sup>6</sup> has permitted extension of the ion-evaporation phenomenon to flow-rates of up to 200  $\mu\text{l min}^{-1}$  of highly aqueous solutions, more compatible with standard HPLC columns operated under gradient conditions. More recently it was shown by Allen and Vestal<sup>7</sup> that use of elevated temperatures as a means of desolvation permits operation of their electrospray source at flow-rates up to 80  $\mu\text{l min}^{-1}$ , although with a sensitivity about 5–10 times less

than that obtained at 10  $\mu\text{l min}^{-1}$ . Ion-evaporation techniques have also found application<sup>8–14</sup> in accurate measurements of relative molecular masses (RMM) of proteins, by exploiting the phenomenon of multiple protonation.

All of these spray techniques have suffered from the lack of a convenient mass calibration standard. An ideal calibrant would give a mass spectrum in which the peaks are sufficiently closely spaced that interpolation errors are negligible, but not so closely spaced as to render the spectrum so confusing that it is difficult to identify individual peaks. The widespread use of perfluorokerosene as a mass calibrant for electron impact ionization mass spectrometry reflects the fact that its mass spectrum satisfies both criteria. In fast atom bombardment (FAB) mass spectrometry, the use of alkali metal halide cluster ions permits the coverage of a wide mass range. However, when using magnetic sector instruments for which the scan time–mass relationship is highly non-linear and unpredictable (although reproducible), serious interpolation errors can arise if the calibrant mass peaks are too widely spaced. Vékey<sup>15,16</sup> has demonstrated that the use of mixed halide calibrants greatly improves the mass accuracy, while still providing standard spectra which are simple enough that no confusion can arise in peak identification.

Polypropylene glycol (PPG) and polyethylene glycol (PEG) are widely used as mass standards for spray ionization techniques, despite difficulties in identifying individual PPG or PEG oligomer peaks in the complex spectra of the mixtures used. In addition, it is well known that serious source contamination can arise from the use of these compounds in thermospray

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sources, although this problem appears to be much less serious in the case of the ion spray source used in this work. A disadvantage of PPG and PEG calibrants for the ion spray source is that the required potential difference between the API orifice plate and the r.f.-only quadrupole, which funnels the ions to the first mass filter, falls within the range of values used in LC/mass spectrometric experiments on smaller analytes but is much lower than that appropriate to experiments on multiply protonated proteins. This potential difference, which in practice is controlled by variations of the float potential on the r.f.-only quadrupole rods while leaving the orifice potential unchanged, is required to remove solvent molecules clustered to the ions derived from the analyte. Variations in this float potential have been observed to cause small but significant shifts of the  $m/z$  scale of the first quadrupole mass filter. These shifts are associated with mass spectral peak broadening and distortion; when the interface has been optimized for large multiply protonated proteins, the shifts can be as large as 1  $m/z$  unit in the worst case for ions of small molecular mass (as opposed to  $m/z$ ), and thus appear to be a function of ion velocity. Accordingly, it is desirable to find calibrants for ion evaporation mass spectrometry which satisfy the two spectral criteria discussed in the preceding paragraph, and whose use minimizes requirements for re-tuning the atmospheric pressure ionization (API) interface parameters between calibration and experiment.

Previously it was shown<sup>17</sup> that post-column injections of cesium iodide solutions into the effluent from a reversed-phase HPLC column yielded excellent mass spectra dominated by the familiar  $Cs_n I_{n-1}^+$  cluster ions, but also containing ions resulting from progressive replacement of iodide by trifluoroacetate anions derived from the acid commonly used to improve chromatographic performance in reversed-phase HPLC. The resulting mass spectra were readily interpreted and provided a sufficient number of peaks for successful calibration of the quadrupole mass filter used in this earlier work.<sup>17</sup> Moreover, injection of only a few  $\mu\text{l}$  of the CsI solution into the mobile phase flow of typically  $50 \mu\text{l min}^{-1}$  resulted in no deleterious effects on the ion source.

This work is an extension of the previous investigation.<sup>17</sup> The objective was to find calibrants suitable for both positive- and negative-ion mass spectrometry, preferably with small enough spacings between mass peaks that interpolation errors for magnetic sector instruments<sup>15,16</sup> could be minimized. To this end many metal salts were surveyed using a quadrupole mass spectrometer equipped with an ion spray ionization source. In addition, an entirely different approach exploiting protonated water clusters was also investigated. Unfortunately, no magnetic sector instrument equipped with an electrospray or ion spray ionization source was available for this work, so the effect of the much larger acceleration potentials involved could not be assessed. Finally, although recent work of Ikonomou *et al.*<sup>18</sup> has shown that the differences between electrospray and ion spray ionization are not trivial, it is believed that the potential calibrants investigated here for an ion spray source should be applicable, with only minor adjustments, to electrospray sources.

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## EXPERIMENTAL

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All experiments used a SCIEX (Thornhill, Ontario, Canada) API III triple quadrupole atmospheric pressure ionization mass spectrometer, equipped with an ion spray interface. A simple syringe pump (Harvard Instruments) was used for infusion experiments (flow-rates  $3\text{--}5 \mu\text{l min}^{-1}$ ) and a Hewlett-Packard Model 1090 liquid chromatograph was used for flow injection experiments ( $0.5 \mu\text{l}$  sample volume injected into flow of  $10\text{--}50 \mu\text{l min}^{-1}$ ). At the lower flow-rates (sample flowing into the ion source for  $\sim 6$  s) it was possible to record mass spectra by conventional full-scan operation of the mass spectrometer. The detection system of the API III instrument utilizes a pulse-counting strategy. As a result, the instrument is scanned in a series of discrete steps, characterized by a step size (typically in the range  $0.1\text{--}1.0 m/z$  unit) and by a dwell time per step. These two parameters, together with the required scan range, determine the total scan time. Full-scan spectra were obtained using this scan mode in order to survey each potential calibrant. The API III instrument can also be operated using a piecemeal scan function, in which disconnected short  $m/z$  ranges are scanned while the intervening ranges are omitted altogether. Such a scan mode for quadrupole mass filters is not possible for magnetic sector instruments, but minimizes the required scan time in cases for which preliminary survey scans have established the ranges of interest. In turn, this feature facilitates calibration over a wide  $m/z$  range with minimum consumption of calibrant and consequently minimum contamination of the ion source.

The salts used were obtained from Pfaltz and Bauer (Waterbury, CT, USA) and were used in solutions in aqueous acetonitrile ( $\sim 50\%$ , v/v) at a concentration of  $0.1 \text{ mol l}^{-1}$ .

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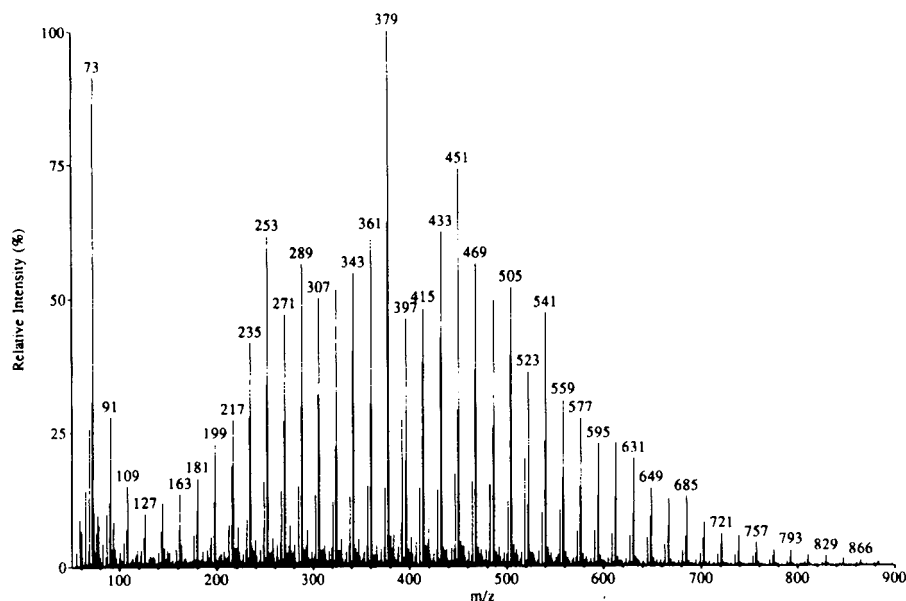
## RESULTS AND DISCUSSION

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### Protonated water clusters

The nature of cluster ions of the type  $H^+(H_2O)_n$ , first reported by Searcy and Fenn,<sup>19</sup> has been the subject of elegant studies by Castleman and co-workers,<sup>20,21</sup> who demonstrated that the particularly stable cluster  $H^+(H_2O)_{21}$  has a clathrate-like structure, with 20 water molecules arranged as a pentagonal dodecahedral cage surrounding a trapped hydronium ion. Protonated water clusters have been observed to be formed by electrospray ionization,<sup>22</sup> and were used for mass calibration of a magnetic sector mass spectrometer with an electrospray source by Aleksandrov and co-workers.<sup>3,23,24</sup> It was shown later by Chowdhury and Chait<sup>5</sup> that electrospray ionization of a 1% solution of acetic acid in water could yield a mass spectrum of protonated water clusters up to about  $m/z$  1000 ( $n = 55$ ).

The spectrum obtained in this work by ion spray ionization of a 0.1% solution of formic acid is shown in Fig. 1, and is generally similar to the earlier result<sup>5</sup> obtained using a quadrupole mass spectrometer, even



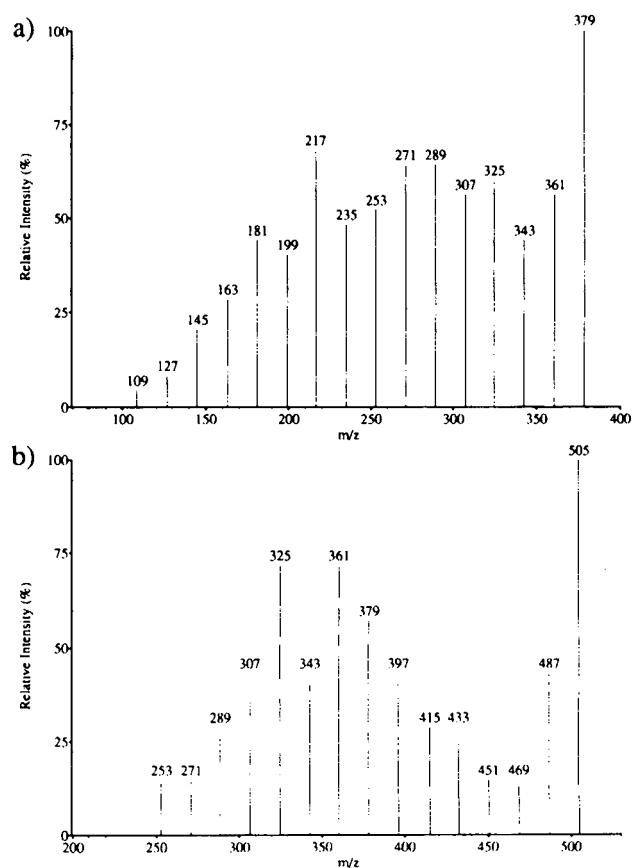
**Figure 1.** Mass spectrum generated by ion spray ionization of an aqueous solution (0.1%) of formic acid, introduced by continuous infusion at  $10 \mu\text{l min}^{-1}$ . Full mass scan, step size  $0.25 m/z$  units, dwell time per step 1 ms. The  $m/z$  annotations are values rounded off to the nearest integer.

though the API source designs were very different. As before, the 'magic number' cluster ion  $\text{H}^+(\text{H}_2\text{O})_{21}$  is especially prominent at  $m/z$  379. This spectrum could be easily reproduced by careful control of the potential difference applied between the API source orifice plate and the rods of the r.f.-only quadrupole funnel. In this API interface region considerable collision-induced dissociation (CID) can occur, a phenomenon exploited to good effect in general practice in order to dissociate solvent molecules clustered to analyte ions. The API interface conditions which optimized the intensity of the protonated water clusters were found to correspond to those typically used in LC/mass spectrometric analyses of small molecules ( $\text{RMM} < 1000$ ), as for PPG and PEG calibrants, but a much higher potential difference (in practice a lower float potential on the r.f.-only quadrupole funnel) was required for observation of multiply protonated proteins. Thus the protonated water clusters provide an ideal calibrant for LC/mass spectrometric experiments on compounds of  $\text{RMM}$  up to 1000. However, both their limited mass range (Fig. 1) and a requirement for retuning the API interface, with associated small shifts in the mass scale of the mass filter, make this approach unsuitable for relative molecular mass determinations of large proteins.

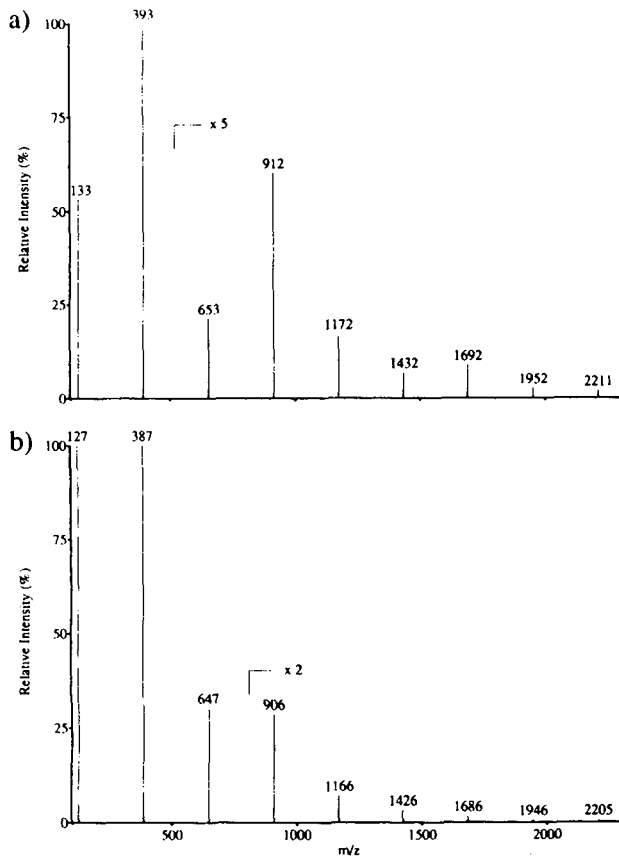
The  $\text{H}^+(\text{H}_2\text{O})_n$  ions can also be used to calibrate the second mass analyser in a tandem mass spectrometer, since their CID reactions proceed by simple stepwise losses of water molecules exemplified in Fig. 2. It is of interest that  $\text{H}^+(\text{H}_2\text{O})_{21}$  ( $m/z$  379) is not especially prominent in the CID spectra of the higher cluster ions [e.g. Fig. 2(b)], emphasizing the kinetic nature of CID experiments compared with the equilibria established in the API source. Similar water cluster spectra have also been generated previously by field ionization and related techniques,<sup>25</sup> and also by thermospray ionization.<sup>26</sup>

Although protonated water clusters provide excellent calibration up to about  $m/z$  1000, it is desirable to

extend this range to  $m/z$  2000 or greater. It has been shown<sup>27</sup> that thermospray ionization of an acetic acid-ammonium acetate buffer solution produces ions of the type  $[(\text{CH}_3\text{COOH})_x \cdot (\text{NH}_3)_y \cdot (\text{NH}_4^+)]$ , which provide



**Figure 2.** CID spectra of  $\text{H}^+(\text{H}_2\text{O})_n$  protonated water clusters. Laboratory frame collision energy, 35 eV. Argon collision gas, target thickness  $3.5 \times 10^{14}$  molecules  $\text{cm}^{-2}$ . (a) CID spectrum of  $\text{H}^+(\text{H}_2\text{O})_{21}$ ,  $m/z$  379; (b) CID spectrum of  $\text{H}^+(\text{H}_2\text{O})_{28}$ ,  $m/z$  505.

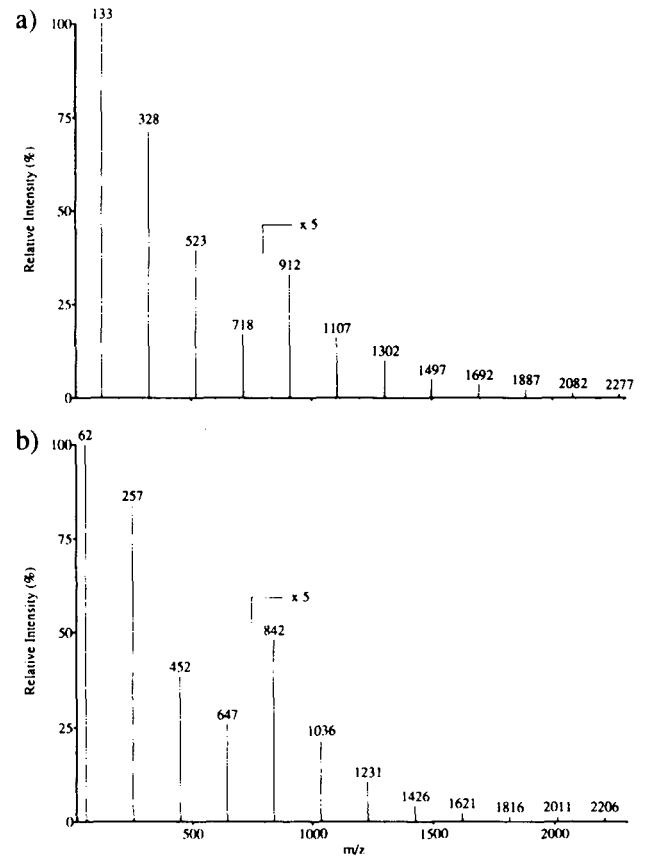


**Figure 3.** Mass spectra generated by ion spray ionization of 0.1 mol l<sup>-1</sup> solution of cesium iodide in aqueous acetonitrile. Flow injection of 0.5 μl into a 30 μl min<sup>-1</sup> flow of aqueous acetonitrile (50%, v/v). (a) Positive ions; (b) negative ions. The *m/z* annotations are values rounded off to the nearest integer.

an excellent calibration up to *m/z* 1000. Replacement of acetic acid by perfluorinated alkyl acids<sup>28,29</sup> permits calibration of thermospray mass spectra up to *m/z* 4000 for positive ions and up to *m/z* 2000 for negative ions. This approach was tried in this work for ion spray ionization. Although spectra of this kind were indeed generated (not shown), in our hands it was not possible in this way to improve the calibration range over that achievable using protonated water clusters (Fig. 1). The same was true when ammonium acetate alone was used, as described recently.<sup>30</sup>

### Metal salt cluster ions

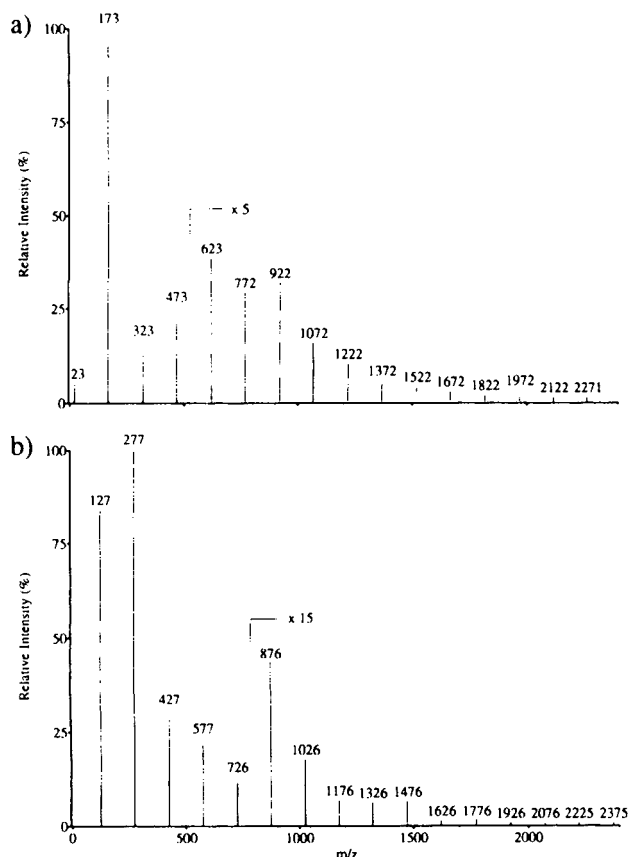
As described previously,<sup>17</sup> flow injection of a solution of cesium iodide provides an excellent ion spray yield of Cs<sub>*n*+1</sub>I<sub>*n*</sub><sup>+</sup> ions together with mixed cluster ions (Cs<sub>*n*+1</sub>I<sub>*n*-*x*</sub>A<sub>*x*</sub>)<sup>+</sup> if a competitive anion A<sup>-</sup> (e.g. trifluoroacetate, TFAc<sup>-</sup>) is also present. Use of aqueous acetonitrile, rather than pure water, as the solvent was the key to the successful generation of these spectra; previous attempts<sup>18</sup> to generate such alkali metal halide cluster ions by both electrospray and ion spray ionization of methanol solutions also failed. Figure 3 compares the positive- and negative-ion mass spectra resulting from ion spray ionization of a CsI solution with no com-



**Figure 4.** Mass spectra generated by ion spray ionization of a 0.1 mol l<sup>-1</sup> solution of cesium nitrate in aqueous acetonitrile. Flow injection of 0.5 μl into a 30 μl min<sup>-1</sup> flow of aqueous acetonitrile (50%, v/v). (a) Positive ions; (b) negative ions. The *m/z* annotations are values rounded off to the nearest integer.

peting anions present. In both cases calibration up to *m/z* 2200 is readily obtained, although the inter-peak spacings (260 u) are too large for a fully satisfactory calibration if a magnetic sector instrument is to be used.<sup>15,16</sup> Use of RbI provided similar results (not shown), except that the distribution of ion current over the isotopic variants of each cluster significantly reduced the available intensities.

The strategy of using a lower mass anion was tried, and CsNO<sub>3</sub> yielded excellent spectra (Fig. 4) up to *m/z* 2300, for both positive and negative ions, with inter-peak spacings of 195 u. Use of NaI, however, provided useful intensities up to *m/z* 2300 in both the positive- and negative-ion modes (Fig. 5) with inter-peak spacings of only 150 u. Potassium iodide worked almost as well. Although the use of NaI represents an improvement over the performance obtainable using CsI and indeed is now the standard ion spray calibration procedure used in this laboratory for quadrupole analysers, for which only minor deviations are observed from the ideal linear time-to-mass scan law, the spacings are still too large for optimum mass assignments using magnetic sector instruments. Results obtained using NaF (spacings only 42 u) were disappointing since the mass spectra petered out above *m/z* 900 in the positive-ion mode and above about *m/z* 1300 for negative ions. This disappointing result was also obtained for KF, suggest-



**Figure 5.** Mass spectra generated by ion spray ionization of a 0.1 mol l<sup>-1</sup> solution of sodium iodide in aqueous acetonitrile. Flow injection of 0.5  $\mu$ l into a 30  $\mu$ l min<sup>-1</sup> flow of aqueous acetonitrile (50%, v/v). (a) Positive ions; (b) negative ions. The  $m/z$  annotations are values rounded off to the nearest integer.

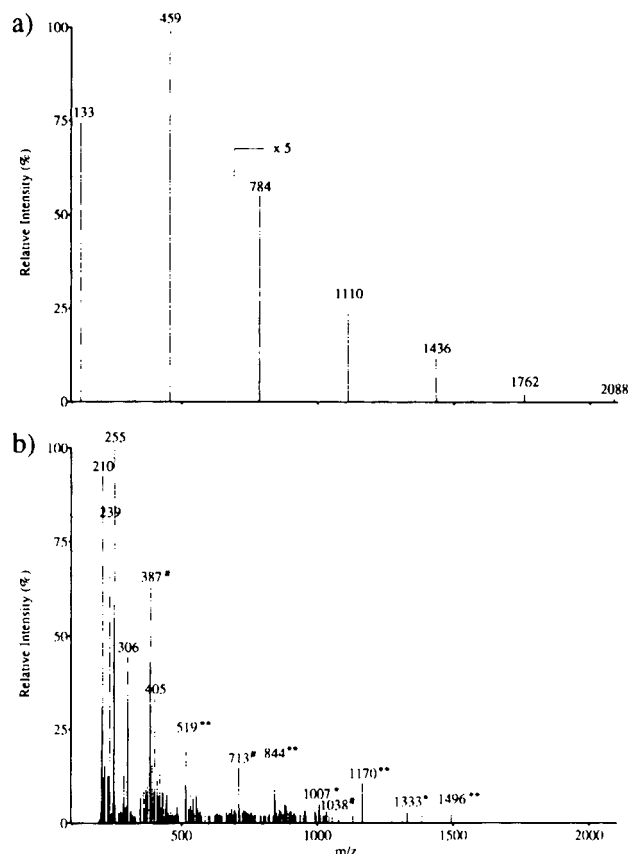
ing that the decrease of intensity with increasing mass, evident in Figs 3–5, is due not only to roll-off of quadrupole transmission efficiency but also to a parallel decrease in probability of alkali metal halide cluster formation with increasing cluster size.

In FAB mass spectrometry, the standard method of obtaining more closely spaced calibrant mass peaks is to use a mixture of the alkali metal iodides, thus obtaining many mixed cluster ions. This approach was attempted in this ion spray work, using a mixture of the mono-isotopic alkali metal iodides NaI, KI and CsI. The resulting spectra (not shown) did contain the expected mixed cluster ions, but again the distribution of the total ion intensity over a large number of ionic species resulted in poor signal-to-noise ratios at higher  $m/z$  values. This could possibly have been improved by injecting larger amounts. However, it is important to bear in mind that serious contamination of the ion spray source with metal salts can be avoided only by injecting minimum amounts. It might be possible to use a sequential, rather than a simultaneous, approach whereby sequential injections of 0.5  $\mu$ l each of solutions of NaI, KI and CsI yield three simple spectra which are then superimposed by the computer to give a single combined data file.

Finally, some experiments were conducted using cesium carbonate, particularly with a view to facile replacement of the carbonate by anions of acids strong-

er than carbonic present in chromatographic mobile phases. The positive-ion mass spectrum obtained by ion spray ionization of Cs<sub>2</sub>CO<sub>3</sub> in aqueous acetonitrile, with no acid in the mobile phase, showed only the expected series of cluster ions [(Cs<sub>2</sub>CO<sub>3</sub>)<sub>n</sub>Cs]<sup>+</sup> [Fig. 6(a)], with peak spacings of 326 u. However, the corresponding negative-ion mass spectrum [Fig. 6(b)] was much more complex. The ion series [(Cs<sub>2</sub>CO<sub>3</sub>)<sub>k</sub>(CsCO<sub>3</sub>)]<sup>-</sup> is apparent, together with a series of ions attributed to doubly charged clusters of the type [(Cs<sub>2</sub>CO<sub>3</sub>)<sub>j</sub>(CO<sub>3</sub>)<sub>2</sub>]<sup>2-</sup>. It may be shown by simple algebra that the  $m/z$  values for cluster ions of these two types must be equal provided that  $j = 2k + 1$ . This implies that the doubly charged cluster ions with even values of  $j$  may be uniquely assigned as such, but that the peaks at  $m/z$  values corresponding to odd values of  $j$  also contain contributions from the singly charged clusters. This result is valid for negative clusters formed from any salt C<sub>2</sub>A, where C and A denote cation and anion, respectively, and also applies to positive cluster ions of salts CA<sub>2</sub>. A third series of negative ions, corresponding to [(Cs<sub>2</sub>CO<sub>3</sub>)<sub>n</sub>(HCO<sub>3</sub>)]<sup>-</sup> is also observed in Fig. 6(b).

When the cesium carbonate solution was injected into flows of aqueous solutions of acids commonly used



**Figure 6.** Mass spectra generated by ion spray ionization of a 0.1 mol l<sup>-1</sup> solution of cesium carbonate in aqueous acetonitrile. Flow injection of 0.5  $\mu$ l into a 30  $\mu$ l min<sup>-1</sup> flow of aqueous acetonitrile (50%, v/v). (a) Positive ions; (b) negative ions. The  $m/z$  annotations are values rounded off to the nearest integer. The ion series in (b) are identified as follows: \* [(Cs<sub>2</sub>CO<sub>3</sub>)<sub>n</sub>(HCO<sub>3</sub>)]<sup>-</sup>; \*\* [(Cs<sub>2</sub>CO<sub>3</sub>)<sub>j</sub>(CO<sub>3</sub>)<sub>2</sub>]<sup>2-</sup>; \*\* [(Cs<sub>2</sub>CO<sub>3</sub>)<sub>j</sub>(CO<sub>3</sub>)<sub>2</sub>]<sup>2-</sup> plus [(Cs<sub>2</sub>CO<sub>3</sub>)<sub>k</sub>(CsCO<sub>3</sub>)]<sup>-</sup>.

in HPLC mobile phases, the  $\text{Cs}_2\text{CO}_3$  cluster ions were wholly suppressed at the expense of the cesium clusters with the corresponding anions. For example, Fig. 7 shows positive-ion spectra thus obtained by injecting  $\text{Cs}_2\text{CO}_3$  solution into flows of aqueous trifluoroacetic acid [Fig. 7(a)] and acetic acid [Fig. 7(b)]. In both cases calibration was possible up to  $m/z$  2300, almost the usable upper limit of the mass range of the quadrupole mass analyzer used. Post-column flow injection of cesium carbonate solutions is thus ideal for checking mass calibration experiments for LC/mass spectrometric experiments without having to change the composition of the mobile phase. Since carbonic acid is such a weak acid no mixed clusters are formed, and the resulting calibrant peaks (192 u spacing in the case of acetic acid) are unambiguous and sufficiently intense (Fig. 7).

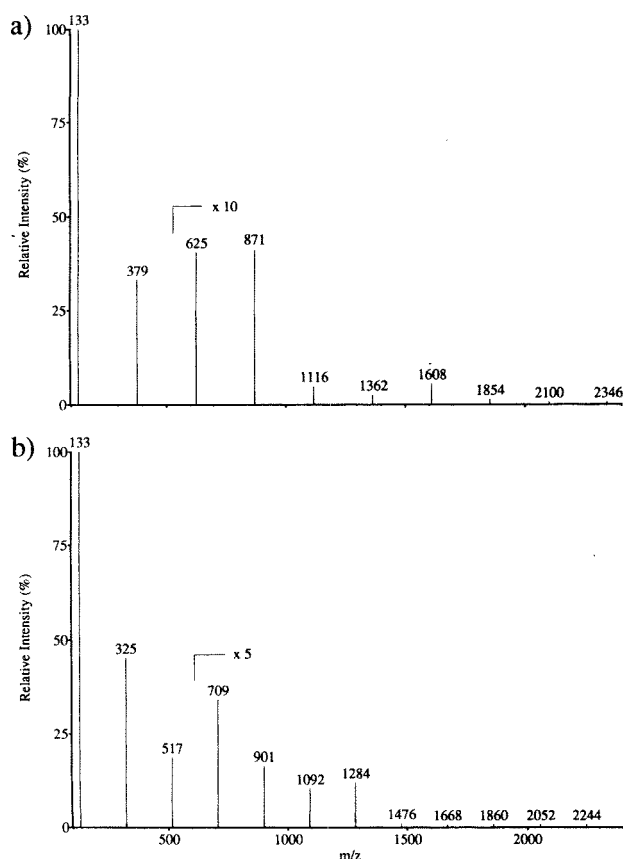
Although source contamination by metal salts can be a serious problem, the present approach of flow injection of small amounts has not led to any noticeable shortening of the useful source lifetime in practice. Moreover, all of the salts recommended here gave excellent spectra, providing satisfactory calibrations over the full range of API interface conditions, including the float potential applied to the r.f.-only quadrupole funnel. Hence it is possible to calibrate the mass spectrometer under the conditions to be used in the desired experi-

ment, whether those appropriate to small molecules (low interface potential difference) or to large biomolecules (high potential difference). This alone represents a significant advantage of the metal salt calibrants over PPG and PEG, in addition to the much simpler spectra thus obtained.

## CONCLUSIONS

Protonated water clusters, generated by ion spray ionization, provide an ideal means of calibration for LC/MS over a limited mass range (up to about  $m/z$  1000), and indeed have been exploited for this purpose using a magnetic sector instrument by Aleksandrov and co-workers.<sup>3,23,24</sup> The use of metal salt clusters ions to calibrate ion spray mass spectra is possible, without serious source contamination, provided that the amounts used are strictly limited by use of flow-injection techniques. Sodium iodide provides excellent results up to  $m/z$  2300, with inter-peak spacings of 150 u, in both positive- and negative-ion modes. An important API interface tuning parameter, viz. the potential difference between the orifice plate and the r.f.-only quadrupole which funnels the ions to the mass analyzer, is not critical to the success of the metal salt calibrants which are thus equally applicable to both small (RMM < 1000) or large (e.g. protein) analytes. In the case of LC/mass spectrometric experiments in which the mobile phase contains an appreciable concentration of acid to improve chromatographic performance, alkali metal carbonates are ideal calibrants for post-column injection since no mixed clusters are formed. In our hands, the accuracy and precision of relative molecular mass measurements of proteins, using on-line LC/mass spectrometric<sup>17</sup> or capillary electrophoresis/mass spectrometric<sup>31</sup> techniques in conjunction with alkali metal salt calibrations, is within 0.01%; this uncertainty is appreciably reduced when the protein solution is introduced by continuous infusion, but at the cost of greater consumption of protein sample.

Although the aim of this work was the purely practical objective of investigating improved mass calibration standards, some of the results obtained are of more fundamental interest. It was pointed out by Ikonomou *et al.*<sup>18</sup> that experiments on alkali metal halide solutions can provide an experimental test to distinguish between rival views of how ions in charged droplets are converted into gas-phase ions. The theoretical treatment of Iribarne and Thomson<sup>32</sup> views this process as direct evaporation of solvated ions from small ( $\ll 1 \mu\text{m}$ ) droplets whose charge-to-volume ratio is almost large enough that electrohydrodynamic instability will result in droplet disintegration (the Rayleigh stability limit). This proposal has been criticized by Röllgen and co-workers,<sup>33</sup> on the basis of theoretical arguments to the effect that the field strength required for ion evaporation<sup>32</sup> almost always exceeds that for droplet break-up by the Rayleigh mechanism. Röllgen and co-workers<sup>33</sup> suggested that the ultimate gas-phase ions are produced predominantly by solvent evaporation from extremely small droplets ( $\sim 1 \text{ nm}$  diameter), each of which contains just one excess ion. If Röllgen and



**Figure 7.** Positive-ion mass spectra generated by ion spray ionization of  $0.1 \text{ mol l}^{-1}$  solution of cesium carbonate in aqueous acetonitrile. Flow injections of  $0.5 \mu\text{l}$  into a  $30 \mu\text{l min}^{-1}$  flow of (a) aqueous acetonitrile with trifluoroacetic acid (0.1%) and (b) aqueous acetonitrile with acetic acid (0.1%). The  $m/z$  annotations are values rounded off to the nearest integer.

co-workers' mechanism is essentially correct, positive potential electrospray or ion spray of solutions of alkali metal halides (AX) should result in abundant small droplets containing a single excess  $A^+$  ion together with several ( $A^+X^-$ ) pairs. Ikonomou *et al.*<sup>18</sup> pointed out that solvent evaporation from such droplets must lead to  $A_{n+1}X_n^+$  clusters, and argued that their failure to observe these species using methanol solutions represents strong evidence against Röllgen and co-workers' mechanism.<sup>33</sup>

The present results, however, cast some doubt on the validity of this conclusion. The most obvious difference between the present experiments and those of Ikonomou *et al.*<sup>18</sup> lies in the use of aqueous acetonitrile instead of methanol as solvent. The present choice of the former solvent was somewhat fortuitous, and was dictated primarily by its widespread use in reverse-phase HPLC under gradient conditions; the actual solvent composition (50%, v/v) for the salt solutions was a compromise between increased ionization efficiency and decreasing salt solubilities at higher acetonitrile content.

In summary, the present results appear to cast doubt on the validity of the production (or not) of alkali metal halide cluster ions as a distinguishing test between the rival mechanisms,<sup>32,33</sup> at least until the role of the aqueous acetonitrile has been clarified. Any meaningful distinction between the highly solvated 'evaporated' ions (with diameters slightly less than 1 nm) of Iribarne and Thomson<sup>32</sup> on the one hand, and Röllgen and co-workers' nm-sized droplets containing a single excess ion<sup>33</sup> on the other, can refer only to their mechanisms of formation. In the latter case<sup>33</sup> the mechanism involves a cascade of droplet divisions via Rayleigh instability, terminating at singly charged droplets where no further division of charge is possible. The model of Iribarne and Thomson<sup>32</sup> essentially proposes that later stages of this cascade can be avoided by droplet disintegrations involving a highly asymmetric division such that one of the product droplets is much smaller than the other and contains a single excess ion. The possibility that the two rival views might be mechanistically indistinguishable was raised previously by Smith *et al.*<sup>22</sup>

## REFERENCES

1. C. R. Blakley and M. L. Vestal, *Anal. Chem.* **55**, 750 (1983).
2. B. A. Thomson, J. V. Iribarne and P. Dziedzic, *Anal. Chem.* **54**, 2219 (1982).
3. M. L. Aleksandrov, L. N. Gall, N. V. Krasnov, V. I. Nikolaev, V. A. Pavlenko and V. A. Shkuzov, *Dokl. Akad. Nauk SSSR* **277**, 379 (1984).
4. C. M. Whitehouse, R. M. Dreyer, M. Yamashita and J. B. Fenn, *Anal. Chem.* **57**, 675 (1985).
5. S. K. Chowdhury and B. T. Chait, *Anal. Chem.* **63**, 1660 (1991).
6. A. P. Bruins, T. R. Covey and J. D. Henion, *Anal. Chem.* **59**, 2642 (1987).
7. M. H. Allen and M. L. Vestal, *J. Am. Soc. Mass Spectrom.* **3**, 18 (1991).
8. C. M. Whitehouse, R. N. Dreyer, M. Yamashita and J. B. Fenn, *Anal. Chem.* **57**, 675 (1985).
9. M. L. Aleksandrov, G. I. Baram, L. N. Gall, N. V. Krasnov, Yu. S. Kusner, O. A. Mirgorodskaya, V. I. Nikolaev and V. A. Shkurov, *Biorg. Khim.* **11**, 700 (1985).
10. S. F. Wong, C. K. Meng and J. B. Fenn, *J. Phys. Chem.* **92**, 546 (1988).
11. C. K. Meng, M. Mann and J. B. Fenn, *Z. Phys. D* **10**, 361 (1988).
12. T. R. Covey, R. F. Bonner, B. I. Shushan and J. D. Henion, *Rapid Commun. Mass Spectrom.* **2**, 249 (1988).
13. J. A. Loo, H. R. Udseth and R. D. Smith, *Anal. Biochem.* **179**, 404 (1989).
14. M. Mann, C. K. Meng and J. B. Fenn, *Anal. Chem.* **61**, 1702 (1989).
15. K. Vékey, *Rapid Commun. Mass Spectrom.* **2**, 213 (1988).
16. K. Vékey, *Rapid Commun. Mass Spectrom.* **24**, 183 (1989).
17. S. Pleasance, P. Thibault, P. G. Sim and R. K. Boyd, *Rapid Commun. Mass Spectrom.* **5**, 307 (1991).
18. M. G. Ikonomou, A. T. Blades and P. Kebarle, *Anal. Chem.* **63**, 1989 (1991).
19. J. Q. Searcy and J. B. Fenn, *J. Chem. Phys.* **61**, 5282 (1974).
20. X. Yang and A. W. Castleman, Jr, *J. Am. Chem. Soc.* **111**, 6845 (1989).
21. S. Wei, Z. Shi and A. W. Castleman Jr, *J. Chem. Phys.* **94**, 3268 (1991).
22. R. D. Smith, J. A. Loo, C. G. Edmonds, C. J. Barinaga and H. R. Udseth, *Anal. Chem.* **62**, 882 (1990).
23. M. L. Aleksandrov, L. N. Gall, N.V. Krasnov, V. I. Nikolaev, V. A. Shkurov, G. I. Baram, M. A. Grachev and Yu. S. Kusner, *Adv. Mass Spectrom.* **10**, 605, 607 (1986).
24. M. L. Aleksandrov and V. P. Andreev, *Fresenius 'Z Anal. Chem.* **335**, 2 (1989).
25. M. Tsuchiya, E. Aoki and H. Huwabara, *Int. J. Mass Spectrom. Ion Processes* **90**, 55 (1989).
26. A. P. Tinke, C. E. M. Heeremans, R. A. M. van der Hoeven, W. M. A. Niessen, J. van der Greef and N. M. M. Nibbering, *Rapid Commun. Mass Spectrom.* **5**, 188 (1991).
27. C. E. M. Heeremans, R. A. M. van Hoeven, W. M. A. Niessen, U. R. Tjaden and J. van der Greef, *Org. Mass Spectrom.* **24**, 109 (1989).
28. S. J. Stout and A. R. daCunha, *Anal. Chem.* **61**, 2126 (1989).
29. S. J. Stout and A. R. daCunha, *Org. Mass Spectrom.* **25**, 187 (1990).
30. J. Saar and M. Haimberg, *Org. Mass Spectrom.* **26**, 660 (1991).
31. P. Thibault, C. Paris and S. Pleasance, *Rapid Commun. Mass Spectrom.* **5**, 484 (1991).
32. I. V. Iribarne and B. A. Thomson, *J. Chem. Phys.* **64**, 2287 (1976).
33. G. Schmelzeisen-Redeker, L. Bütferring and F. W. Röllgen, *Int. J. Mass Spectrom. Ion Processes* **90**, 189 (1989).