

# Formation of solvated ions in the atmospheric interface of an electrospray ionization triple-quadrupole mass spectrometer<sup>†</sup>

Gitta Schlosser, Zoltán Takáts and Károly Vékey\*

Chemical Research Center, Hungarian Academy of Sciences, Pusztaszeri út 59–67, H-1025 Budapest, Hungary

A simple method capable of generating and investigating various solvent clusters and solvated ions was developed. The technique opens a door to studying these complexes on commercially available instruments. Formation of the desired solvated ion in the gas phase was achieved by introducing the appropriate volatile solvent vapour into the curtain gas stream. Capabilities of the technique are illustrated by generating alkali, alkaline earth and transition metal cations solvated by various volatile compounds such as water, methanol and acetonitrile. Depending on the ligands and on the experimental conditions, clusters of 2–100 molecules may be observed. Isotope labelling suggests that these are formed by a re-solvation process in the curtain gas region. Copyright © 2003 John Wiley & Sons, Ltd.

**KEYWORDS:** cluster; electrospray; magic number; solvation

## INTRODUCTION

Studying solvation–desolvation mechanisms of various ions in the gas phase has been an issue of interest for more than 30 years. The significance of these studies lies in the analogy between gaseous and condensed-phase systems,<sup>1</sup> but these are also relevant for atmospheric chemistry.<sup>2</sup> Mass spectrometric investigations of solvated ions may provide thermodynamic information about the interaction between ions and solvent molecules and give a better insight into the structure of solution-phase species.<sup>3–6</sup> Hydration of molecules of biological significance (e.g. peptides, proteins or nucleotides) has attracted interest recently.<sup>6–10</sup> Mass spectrometry can provide thermodynamic data on binding and information on the coordination number.<sup>5,11–15</sup> Cluster ions also have various practical applications, e.g. utility as mass calibration standards.<sup>16,17</sup> There are several cluster-forming techniques which can be applied in the investigation of solvated ions.<sup>3,18–24</sup> However, almost all systems used for the generation of solvated ions are laboratory-built, designed for investigating specific processes. The main objective of this paper is to provide a widely available tool for generating such type of ions in a controlled manner using a commercial instrument.

Electrospray ionization (ESI)<sup>25</sup> is a convenient method to generate various supramolecular complexes, including

solvent clusters, solvated metal cations and solvated ions of various organic molecules. The method is often considered as a technique transferring a very small fraction of the solution phase directly into the gas phase. This is a simple picture, and there is growing evidence that the mechanism of cluster formation may be different. Recently, we have described a series of events that occur in ESI following the ion evaporation process.<sup>26</sup> The ions formed in this way are first accelerated in the Coulomb field of the nanodroplet from which they evaporated from. Since this occurs in a relatively high pressure region, a collision cascade will follow. In the collisions, part of the kinetic energy (deriving from the Coulomb field of the nanodroplet and not from potentials in the instrument) is converted to internal energy. This is often sufficient to desolvate the ions completely. Subsequently (but still before the cone–orifice region) there will be collisions at low kinetic energy, resulting in collisional cooling. A typical application of controlled collisional cooling is the stabilization of large protein complexes in the fore-vacuum region of a mass spectrometer.<sup>27–30</sup> It was shown that in the absence of sufficient collisional cooling, large, multiply charged protein complexes will dissociate, and cannot be detected.

A consequence of the proposed scenario is that the curtain gas not only hinders neutral particles from entering the instrument, but also provides a cooling medium for the ions. The large number of collisions occurring in this region were also utilized to provide a medium for H/D exchange, published recently.<sup>31</sup> Based on the same concept, we developed a new method to form solvated ions in the curtain gas–orifice type atmospheric interface of a triple-quadrupole mass spectrometer. The controlled production of cluster ions is based on the addition of complex-forming

\*Correspondence to: Károly Vékey, Chemical Research Center, Hungarian Academy of Sciences, Pusztaszeri út 59–67, H-1025 Budapest, Hungary. E-mail: vekey@chemres.hu

<sup>†</sup>Paper presented at the 21st Informal Meeting on Mass Spectrometry, Antwerp, 11–15 May 2003.

Contract/grant sponsor: Hungarian Research Fund (OTKA); Contract/grant number: T 043538.

Contract/grant sponsor: European Center of Excellence; Contract/grant number: QLK2-CT-2002-90436.

agents to the curtain gas, which will result in the solvation of the electrosprayed ions. This cluster formation phenomenon is described in this paper from the viewpoints of both applications and mechanistic considerations.

## EXPERIMENTAL

In the experiments a PE-SCIEX API 2000 triple-quadrupole mass spectrometer equipped with an electrospray source was used. Spectra were acquired with a 4800 V spray voltage and 30 V orifice voltage; the source housing was kept at room temperature. The nebulizer gas pressure was set to  $3 \times 10^5$  Pa and the curtain gas pressure to  $2 \times 10^5$  Pa. Sample solutions (at 1 mM concentration) were introduced at a flow-rate of  $30 \mu\text{L min}^{-1}$ .

Introducing cluster-forming agents, usually solvents, into the curtain gas flow required only a minor modification on the curtain gas system. Using a stainless-steel T element (Swagelok), a fused-silica capillary (Supelco) was inserted into the curtain gas line just before it enters the mass spectrometer. The cluster-forming agent was introduced through this capillary. The flow-rate was varied in the range  $0\text{--}25 \mu\text{L min}^{-1}$  and was controlled by the built-in syringe pump of the instrument. In typical experiments, if not stated otherwise, solvent infusion into the curtain gas was regulated at  $15 \mu\text{L min}^{-1}$ . An even simpler experimental setup, which yields qualitatively similar results, is to bubble the curtain gas through a solvent reservoir.

Salts and solvents were purchased from Reanal (Budapest, Hungary),  $\text{D}_2\text{O}$  from Merck (Darmstadt, Germany) and fittings and ferrules from Upchurch Scientific (Oak Harbor, WA, USA).

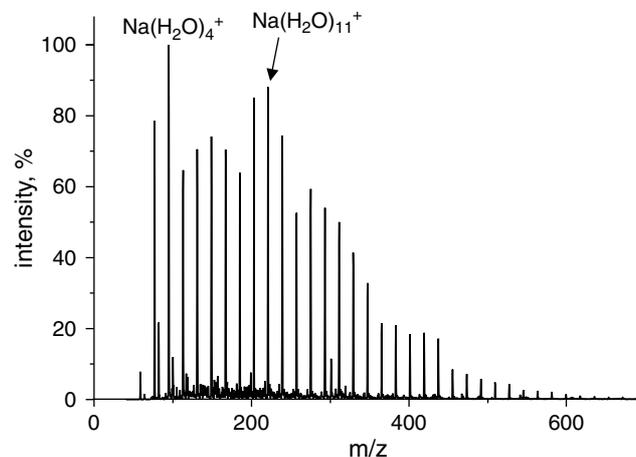
## RESULTS AND DISCUSSION

Clustering with solvent molecules has often been reported in ESI, and it is known to depend on various experimental parameters. The amount of solvent flow, the pressure and type of the curtain gas and the position of the ESI needle all play a significant role in cluster formation.<sup>16,32,33</sup> This suggests that formation of solvent clusters depends on the amount of solvent vapour entering the mass spectrometer. Based on this idea, a simple technique was developed for generating various solvated ions in the atmospheric interface of a mass spectrometer by introducing solvent vapour into the curtain gas in a controlled manner. In this method, following ESI, a volatile compound such as water, acetonitrile or methanol is infused directly into the nitrogen gas line using a simple T element. The presence of these vapours in the curtain gas results in the appearance of abundant cluster ions in a wide mass range. Using this approach, abundant clusters of various composition can be generated on a commercial instrument, which otherwise would require extensive modifications or special, laboratory-built instruments.<sup>3,18–21</sup> Cluster size is influenced mainly by the partial pressure of the volatile compound added to the curtain gas and by the orifice-to-skimmer potential ( $U_{\text{or}}$ ). Clusters formed this way have a composition corresponding to the liquid added to the curtain gas and are independent

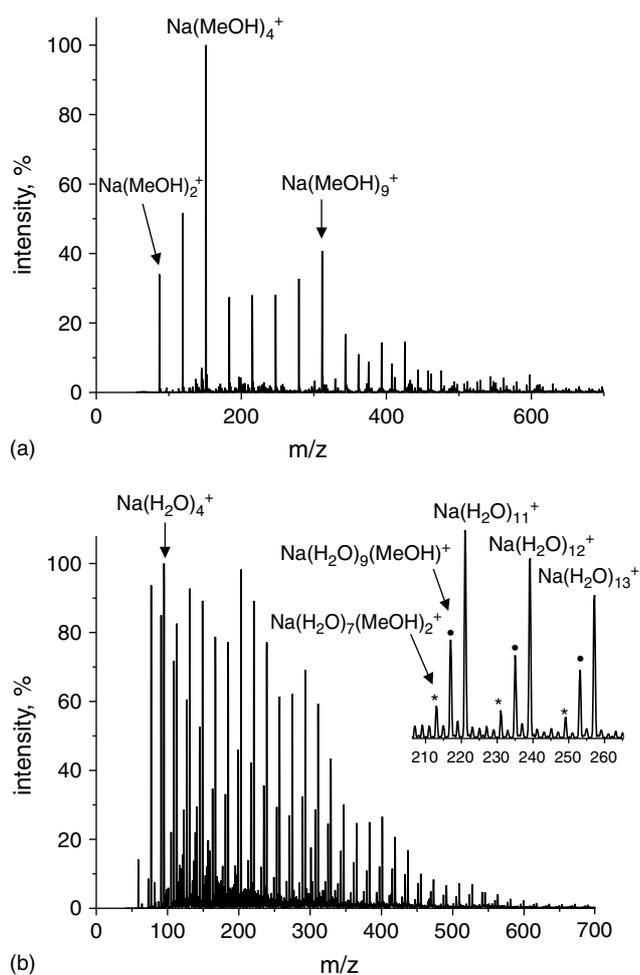
of the solvent used for electrospraying the sample. To avoid confusion between these two liquids, the volatile compound added to the curtain gas will be called 'clustering agent' in the following.

Under typical operating conditions, the curtain gas prevents cluster formation in ESI.<sup>26</sup> Spraying dilute aqueous or methanolic solutions of alkali metal halide salts usually results in the appearance of a noise-like spectrum of low abundance. When a solvent vapour is introduced into the curtain gas flow, the intensity of ion signal is significantly increased, and a series of solvated ions are observed. Figure 1 shows the spectrum obtained in the positive ion mode in the case of spraying dilute aqueous NaCl solution and adding water vapour to the curtain gas. Nearly all ions in the spectrum correspond to  $\text{Na}(\text{H}_2\text{O})_n^+$  clusters, which span a wide range from  $n = 2$  to 50. Increasing the partial pressure of water vapour in the curtain gas, decreasing the orifice voltage and placing the electrospray capillary far from the curtain plate increase the cluster size observed. In this way,  $\text{Na}(\text{H}_2\text{O})_n^+$  clusters with  $n > 100$  can also be observed with 1–5% relative abundance.

To confirm that the observed clusters are indeed formed with the 'clustering agent' added to the curtain gas and not from the solution used for electrospray, two control experiments were performed. First, the 'clustering agent' was changed from water to methanol, while all other parameters were kept constant. The resulting spectrum is shown in Fig. 2(a), showing predominantly  $\text{Na}(\text{CH}_3\text{OH})_n^+$  cluster ions. In addition to these, at large cluster size another cluster series appear, containing one or two water molecules ( $\text{Na}(\text{H}_2\text{O})(\text{CH}_3\text{OH})_n^+$  and  $\text{Na}(\text{H}_2\text{O})_2(\text{CH}_3\text{OH})_n^+$  ions). This is due either to a small partial pressure of  $\text{H}_2\text{O}$  present in the curtain gas region (coming from the spraying solution) or to the initial production of small  $\text{Na}(\text{H}_2\text{O})^+$  or  $\text{Na}(\text{H}_2\text{O})_2^+$  clusters in electrospray, which are subsequently solvated. The appearance of these mixed clusters only at large cluster size is likely to correspond to the stability differences in mixed  $\text{H}_2\text{O}\text{--}\text{CH}_3\text{OH}$  clusters.<sup>34</sup> The other control experiment was a sort of reverse process, spraying methanolic solution of NaCl and adding water to the curtain gas (Fig. 2(b)). In this case, predominantly  $\text{Na}(\text{H}_2\text{O})_n^+$  clusters are formed.



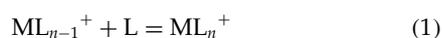
**Figure 1.** ESI spectrum of aqueous NaCl solution after adding water vapour to the curtain gas.



**Figure 2.** ESI spectra of (a) aqueous NaCl solution after adding methanol vapour to the curtain gas and (b) methanolic NaCl solution after adding water to the curtain gas. The inset in (b) shows part of the spectrum on an expanded scale. In the inset,  $\text{Na}(\text{H}_2\text{O})_n(\text{MeOH})^+$  type ions are indicated by full circles and  $\text{Na}(\text{H}_2\text{O})_n(\text{MeOH})_2^+$ -type clusters by asterisks.

In addition to these, there is a ca 2–3 times less abundant series of  $\text{Na}(\text{CH}_3\text{OH})(\text{H}_2\text{O})_n^+$  clusters and also a series of  $\text{Na}(\text{CH}_3\text{OH})_2(\text{H}_2\text{O})_n^+$  clusters of low abundance, but there is clearly no  $\text{Na}(\text{CH}_3\text{OH})_n^+$  cluster series present. The relative abundance of the various mixed  $\text{H}_2\text{O}$ – $\text{CH}_3\text{OH}$  clusters corresponds to an approximately statistical distribution among water and methanol ligands present in a ca 15:1 molar ratio.

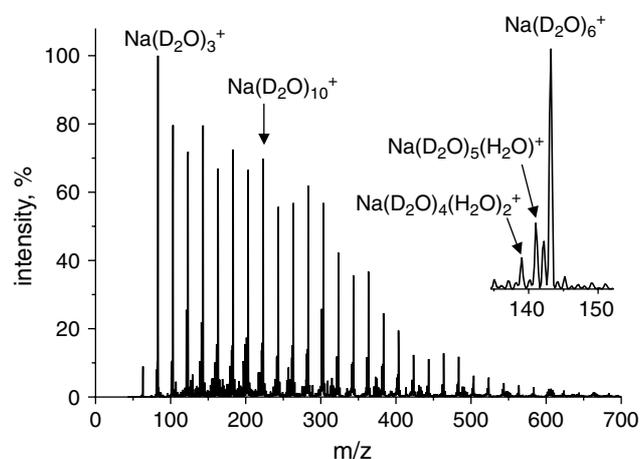
These experiments clearly show that the clusters are formed with molecules present in the curtain gas region and do not reflect composition of the ‘original’ solution which was electrosprayed. A prerequisite of observing clusters depends upon thermochemistry, i.e. the  $\Delta G$  of the reaction



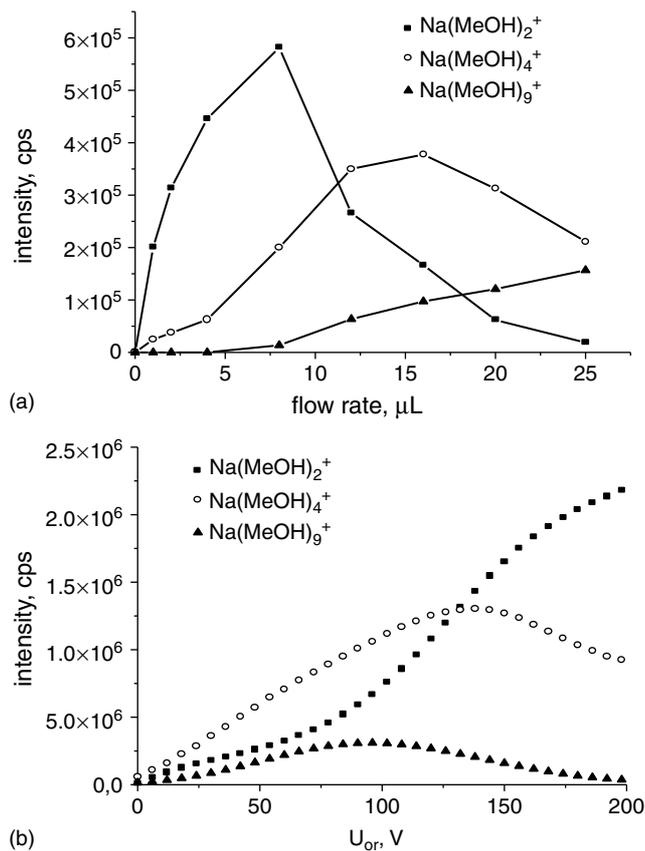
has to be of negative sign to observe an  $\text{ML}_n^+$  cluster with reasonable abundance (where  $\text{M}^+$  is a metal ion and L a ligand). Nitrogen does not form stable clusters, which is the reason why it is an efficient curtain gas to prevent cluster formation.

The mechanism of cluster formation was studied using isotope labelling experiments. Figure 3 shows the result of electrospraying aqueous NaCl solution while adding  $\text{D}_2\text{O}$  vapour to the curtain gas. As can be seen, almost all of the clusters are perdeuterated, confirming the results discussed above that clusters are formed with the ‘clustering agent’ added to the curtain gas and not molecules from the electrosprayed solution. The most interesting results relate, however, to the non-perdeuterated peaks, as shown in the inset in Fig. 3. H/D exchange is known to be very fast in solutions and in long-lived collision complexes and consequently is likely to be very fast also in nanodroplets or clusters. This would produce a statistical distribution of H/D atoms. In contrast, the most intense non-perdeuterated signals correspond to an even number (2 or 4) of residual hydrogens. The most probable explanation is initial formation of naked  $\text{Na}^+$  ions and very small  $\text{Na}(\text{H}_2\text{O})_n^+$  clusters ( $n = 1$  or 2) in the electrospray process. In subsequent collisions these will add  $\text{D}_2\text{O}$  molecules to form larger clusters, producing the cluster pattern shown in Fig. 3. Ligand exchange, either in the gas phase or in a nanodroplet, would result in H/D randomization (i.e. a statistical distribution of  $\text{H}_2\text{O}$ , HDO and  $\text{D}_2\text{O}$  molecules). This scenario should produce a statistical distribution of H/D atoms in the clusters, in clear contrast to the experimental results shown in Fig. 3. Another possibility is that small solvent droplets may be present in the curtain gas flow, and ions may be captured by these droplets, as suggested by a referee. To test this hypothesis, we heated the curtain gas flow by  $40^\circ\text{C}$  (which should facilitate complete evaporation), but this did not change the cluster distribution.

The cluster formation process is significantly influenced by the partial pressure of the cluster-forming agent, which, in turn, is determined by the flow of this agent into the curtain gas. This dependence is illustrated in the case of spraying aqueous NaCl solution and adding methanol to the curtain gas. The result is illustrated in Fig. 4(a), where the abundances of three cluster ions ( $\text{Na}(\text{CH}_3\text{OH})_n^+$ ,  $n = 2, 4$  and 9) are shown as a function of methanol flow-rate, varied in the range  $0$ – $25 \mu\text{l min}^{-1}$ . Increasing the partial pressure (flow-rate) of methanol shifts the cluster distribution to larger



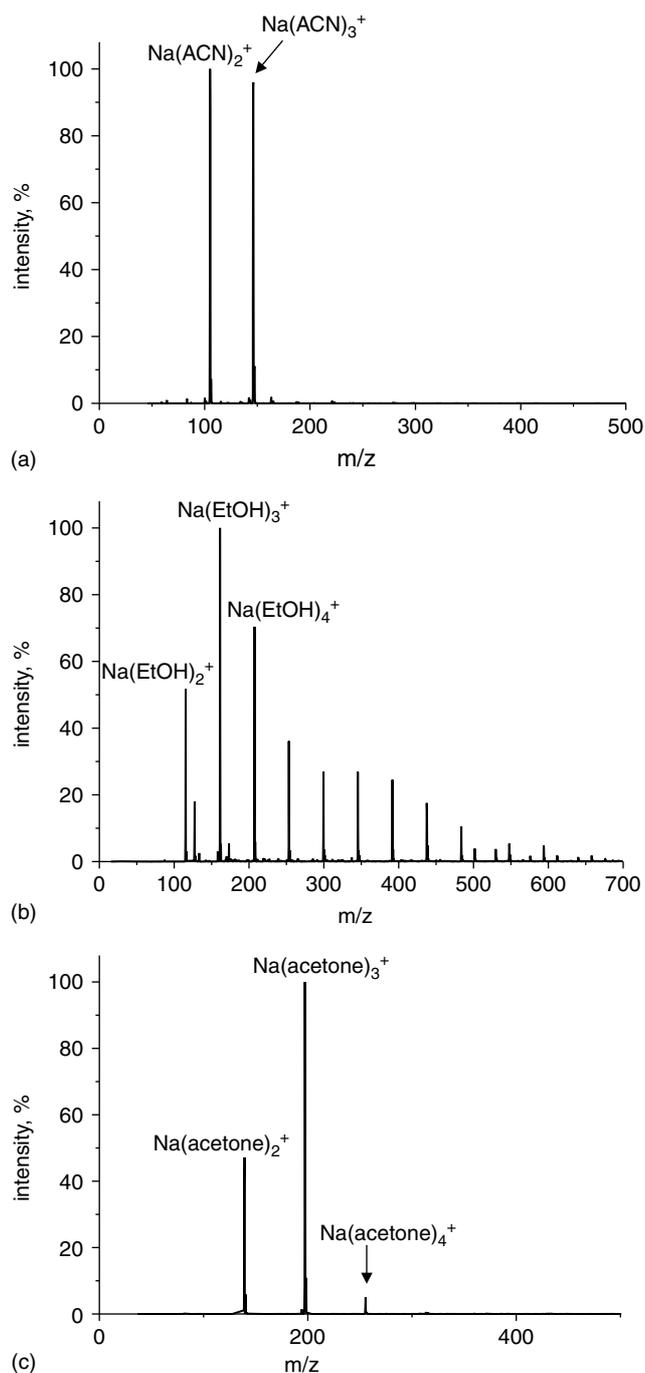
**Figure 3.** ESI spectrum of aqueous NaCl solution after adding  $\text{D}_2\text{O}$  to the curtain gas. The inset shows the  $\text{Na}(\text{D}_2\text{O})_6^+$  cluster on an expanded scale.



**Figure 4.** Effects of increasing (a) the methanol flow-rate and (b) the orifice voltage on the abundance of various  $\text{Na}(\text{MeOH})_n^+$  clusters.

size. Another parameter which has a large influence on cluster distribution is the orifice voltage. Figure 4(b) shows its effect in the same system, illustrated in the case of three  $\text{Na}(\text{CH}_3\text{OH})_n^+$  ions. A high orifice voltage breaks up larger clusters, so the cluster distribution shifts to smaller size. The position of the needle also has an influence on the average cluster size, but only to a small extent: on increasing the distance between the ESI needle and the curtain plate, a slight increase in the average cluster size was observed. The most likely explanation is that cluster formation takes place in the relatively low pressure curtain plate-Q0 region of the mass spectrometer and the spray position influences the number of collisions that the newly formed ions undergo and this, in turn, may influence cluster composition. Analogous results have been observed previously.<sup>31,35</sup>

The formation of various other clusters was also studied. Clustering with acetonitrile is another instructive example (Fig. 5(a), NaCl sprayed from aqueous solution). In contrast to water, the cluster distribution is very narrow, and practically only  $\text{Na}(\text{CH}_3\text{CN})_2^+$  and  $\text{Na}(\text{CH}_3\text{CN})_3^+$  ions can be observed. The cluster with four acetonitrile molecules ( $\text{Na}(\text{CH}_3\text{CN})_4^+$ ) has very low abundance, even at a high acetonitrile flow-rate and low orifice voltage. Analogous behaviour was also observed for other cations, water producing clusters in a very broad range, acetonitrile only few cluster ions, while methanol was in between these two extremes. Cluster formation with ethanol (Fig. 5(b)) and acetone (Fig. 5(c)) was also studied. The results suggest that



**Figure 5.** ESI spectrum of aqueous NaCl solution after adding (a) acetonitrile, (b) ethanol and (c) acetone vapour to the curtain gas.

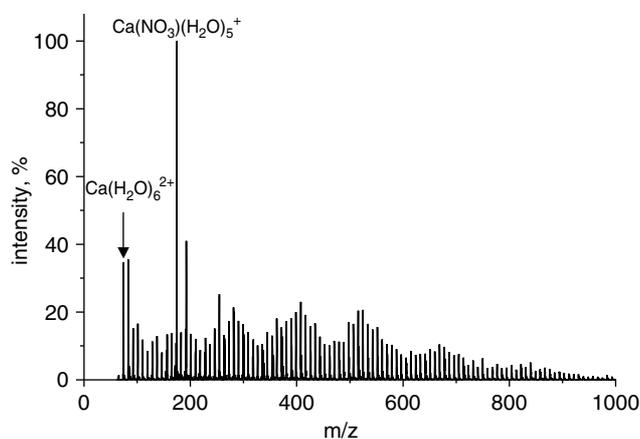
the degree and range of cluster sizes observed depends on H-bonds. Water is known to show a large degree of H-bonded networks, and forms very large clusters with a broad distribution. In alcohols, H-bonded networks are less prominent and the cluster size observed has a smaller range. In those cases where there are no H-bonds, as in acetonitrile and acetone, the cluster size is relatively small and has a very narrow distribution. Maximum cluster size is likely to correspond to filling the first coordination shell. Acetonitrile and acetone molecules bound to the metal atom are unable to establish hydrogen bonds, in contrast to the water. In

consequence, binding additional ligands after saturation of the first coordination sphere is not preferred.

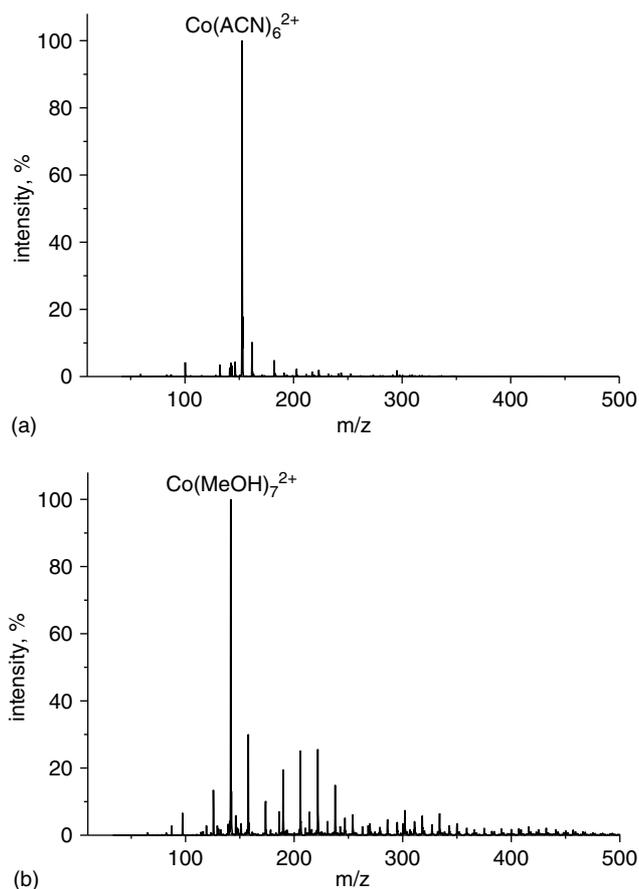
Solvent clusters of other alkali metal ions were also studied, the results being analogous to those discussed above. Divalent alkaline earth metal ions ( $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ ) show a significantly different picture. These were generated by electrospray from dilute  $\text{MgCl}_2$ ,  $\text{Mg}(\text{NO}_3)_2$ ,  $\text{CaCl}_2$ , and  $\text{Ca}(\text{NO}_3)_2$  solutions, adding water to the curtain gas. Analogous clusters were formed in these cases. Predominantly doubly charged ions were formed, as illustrated in the case of  $\text{Ca}^{2+}$ , shown in Fig. 6. In addition to a broad range of  $\text{Ca}(\text{H}_2\text{O})_n^{2+}$ -type clusters ( $n$  starting at 5 and extending to well over 100), singly charged ions containing a counter ion were also observed ( $\text{Ca}(\text{NO}_3)(\text{H}_2\text{O})_n^+$  series,  $n$  spanning a broad range). Using high  $U_{\text{or}}$  settings, smaller doubly charged clusters ( $n = 3$  and 4), and singly charged  $\text{M}(\text{OH})(\text{H}_2\text{O})_n^+$ -type ions were also observed. These are CID products of hydrated alkaline earth metal ions,<sup>36</sup> and could be generated also by tandem mass spectrometry (data not shown).

Studies and applications related to transition metal complexes are among the most exciting research areas.<sup>3,13,37,38</sup> To illustrate the applicability of the present experimental technique, solvation of divalent transition metal ions such as  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  was also studied. Solvation of these ions results in a well-defined, narrow cluster size distribution, in contrast to that of alkali or alkaline earth metals. Acetonitrile and methanol produce abundant doubly charged ions with all transition metals studied.  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  form predominantly  $\text{ML}_6^{2+}$ -type clusters, while with methanol the  $\text{ML}_7^{2+}$  cluster is the most abundant.  $\text{Cu}^{2+}$  forms  $\text{ML}_5^{2+}$ -type cluster with acetonitrile. As examples, spectra of  $\text{Co}^{2+}$  (sprayed from aqueous  $\text{CoCl}_2$  solution) with methanol and acetonitrile are shown in Fig. 7. Interestingly, the reaction with water vapour results in abundant singly charged hydrated ions having an  $\text{OH}^-$  counter ion and the structure  $\text{M}(\text{OH})(\text{H}_2\text{O})_3^+$ ; these may be fragmentation (charge separation) products of larger doubly charged clusters, as discussed above for Ca and Mg.

The cluster size distribution reflects the stability of ions. When narrow size distributions are observed, these are likely to reflect closed solvation shells. When broad



**Figure 6.** ESI spectrum of aqueous  $\text{Ca}(\text{NO}_3)_2$  solution after adding water vapour to the curtain gas.

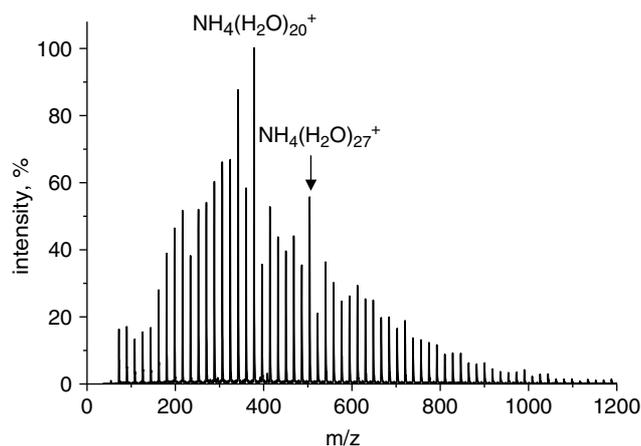


**Figure 7.** ESI spectrum of aqueous  $\text{CoCl}_2$  solution after adding (a) acetonitrile and (b) methanol vapour to the curtain gas.

cluster distributions are observed (Figs 1, 2, 3, 6 and 8), the signal intensity distribution often shows the presence of clusters with higher than average abundance. These ions are referred to in the literature as 'magic number clusters'.<sup>32,39-43</sup> In addition to these abundant clusters, a decrease in the abundance of neighbouring ions is also often observed. The appearance of magic numbers clusters is connected with clusters of special stability, and often can be connected with the saturation of a coordination shell or formation of nanocrystals.<sup>42-47</sup> Well-documented cases are the hydrated  $\text{H}_3\text{O}^+$  and  $\text{NH}_4^+$  ions.<sup>41,46,48-51</sup> Both show well-known magic numbers at  $\text{H}_3\text{O}(\text{H}_2\text{O})_{20}^+$  and  $\text{H}_3\text{O}(\text{H}_2\text{O})_{27}^+$  and at  $\text{NH}_4(\text{H}_2\text{O})_{20}^+$  and  $\text{NH}_4(\text{H}_2\text{O})_{27}^+$ , respectively (Fig. 8). Using the developed technique, solvated  $\text{H}_3\text{O}^+$  and  $\text{NH}_4^+$  clusters were generated by spraying aqueous acetic acid or ammonia solution and introducing water vapour into the curtain gas. In these spectra the well-known magic number clusters at  $n = 20$  and  $27$  can be identified unambiguously (Fig. 8). Note that the high-intensity magic number is accompanied by neighbouring ions of reduced abundance.

## CONCLUSIONS

A simple technique is presented capable of producing abundant cluster ions in ESI using commercially available instruments. The cluster-forming agent is introduced into the curtain gas flow at a few  $\mu\text{l min}^{-1}$ . The instrument does not need any modification, only a T element is inserted into the



**Figure 8.** Water clusters with an  $\text{NH}_4^+$  centre ion. The spectrum was acquired by electrospraying aqueous ammonia solution and adding water to the curtain gas.

curtain gas line just before entering the mass spectrometer, to introduce the cluster-forming agent. The results strongly suggest that in the ESI process first naked ions or very small clusters are formed. This is in accord with the ion evaporation process, commonly accepted<sup>52–55</sup> for small ions. When, as is usually done, a dry curtain gas is used, its main role is to prevent solvent vapours (originating from the spraying solution) from entering the mass spectrometer. The curtain gas (usually  $\text{N}_2$ ) is not a good cluster-forming agent. The naked ions initially formed consequently cannot collide with solvent molecules, so larger clusters cannot be formed. When a solvent (the so-called cluster-forming agent) is introduced into the curtain gas flow, collisions of the initially formed ion with these molecules may result in the formation of a long-lived complex, which adduct may be stabilized. Repetition of this process results in the formation of larger clusters. This mechanism is supported by isotope labelling experiments. The desolvation process seems unaffected by the experimental conditions used, but seems to be an intrinsic feature of the ESI of small ions.

The technique presented is a generally useful procedure to form and study various clusters by adding appropriate vapours to the curtain gas. Cluster ions can be formed in a wide mass range. Production of solvated ions is not limited to the solvent used for electrospraying the sample, but several other cluster-forming agents can be used, even when the sample is normally not soluble in the desired solvent.

A general conclusion of this study is that solvated ions observed in ESI are formed by gas-phase association reactions, and probably should not be considered as a small fraction of the original liquid-phase structure. Ions entering the mass spectrometer may have a significant amount of internal energy,<sup>26,56–58</sup> characterized by a statistical internal energy distribution.<sup>56,57,59</sup> The time necessary to transfer the ions formed in ESI into the analyser region (in the range of 100  $\mu\text{s}$  – 1 ms) is long enough for rearrangements to take place. This suggests that cluster ions studied in the mass spectrometer are likely to be in their ground electronic state and represent either the most stable isomer or possibly a mixture of low-energy isomers.

## Acknowledgements

This work was supported by the Hungarian Research Fund (OTKA), T 043538, and the European Center of Excellence, project CBCH (QLK2-CT-2002-90436).

## REFERENCES

- Speranza M. Gas-phase ion chemistry versus solution chemistry. *Int. J. Mass Spectrom.* 1992; **118**–**119**: 395.
- Seinfeld JH, Pandis SN. *Atmospheric Chemistry and Physics: from Air Pollution to Climate Change*. Wiley-Interscience: New York, 1997.
- Armentrout PB. Guided ion beam studies of transition metal-ligand thermochemistry. *Int. J. Mass Spectrom.* 2003; **227**: 289.
- Blades AT, Kebarle P. Study of the stability and hydration of doubly charged ions in the gas phase:  $\text{SO}_4^{2-}$ ,  $\text{S}_2\text{O}_6^{2-}$ ,  $\text{S}_2\text{O}_8^{2-}$ , and some related species. *J. Am. Chem. Soc.* 1994; **116**: 10761.
- Castleman AW Jr, Bowen KH Jr. Clusters: structure, energetics, and dynamics of intermediate states of matter. *J. Phys. Chem.* 1996; **100**: 12911.
- Rodríguez-Cruz SE, Klassen JS, Williams ER. Hydration of gas-phase gramicidin S ( $M + 2H$ )<sup>2+</sup> ions formed by electrospray: the transition from solution to gas-phase structure. *J. Am. Soc. Mass Spectrom.* 1997; **8**: 565.
- Lee S-W, Freivogel P, Schindler T, Beauchamp JL. Freeze-dried biomolecules: FT-ICR studies of the specific solvation of functional groups and clathrate formation observed by the slow evaporation of water from hydrated peptides and model compounds in the gas phase. *J. Am. Chem. Soc.* 1998; **120**: 11758.
- Jockusch RA, Lemoff AS, Williams ER. Effect of metal ion and water coordination on the structure of a gas-phase amino acid. *J. Am. Chem. Soc.* 2001; **123**: 12255.
- Jockusch RA, Lemoff AS, Williams ER. Hydration of valine-cation complexes in the gas phase: on the number of water molecules necessary to form a zwitterion. *J. Phys. Chem. A* 2001; **105**: 10929.
- Klassen JS, Blades AT, Kebarle P. Determinations of ion-molecule equilibria involving ions produced by electrospray. Hydration of protonated amines, diamines, and some small peptides. *J. Phys. Chem.* 1995; **99**: 15509.
- Amicangelo JC, Armentrout PB. Relative and absolute bond dissociation energies of sodium cation complexes determined using competitive collision-induced dissociation experiments. *Int. J. Mass Spectrom.* 2001; **212**: 301.
- Cunningham AJ, Payzant JD, Kebarle P. A kinetic study of the proton hydrate  $\text{H}^+(\text{H}_2\text{O})_n$  equilibria in the gas phase. *J. Am. Chem. Soc.* 1972; **94**: 7627.
- Rodgers MT, Armentrout PB. Noncovalent metal-ligand bond energies as studied by threshold collision-induced dissociation. *Mass Spectrom. Rev.* 2000; **19**: 215.
- Wang G, Cole RB. Solvation energy and gas-phase stability influences on alkali metal cluster ion formation in electrospray ionization mass spectrometry. *Anal. Chem.* 1998; **70**: 873.
- Bieske EJ, Maier JP. Spectroscopic studies of ionic complexes and clusters. *Chem. Rev.* 1993; **93**: 2603.
- Ledman DW, Fox RO. Water cluster calibration reduces mass error in electrospray ionization mass spectrometry of proteins. *J. Am. Soc. Mass Spectrom.* 1997; **8**: 1158.
- Anacleto JF, Pleasance S, Boyd RK. Calibration of ion spray mass spectra using cluster ions. *Org. Mass Spectrom.* 1992; **27**: 660.
- Zhan D, Rosell J, Fenn JB. Solvation studies of electrospray ions—method and early results. *J. Am. Soc. Mass Spectrom.* 1998; **9**: 1241.
- Fujii T. Alkali-metal ion/molecule association reactions and their applications to mass spectrometry. *Mass Spectrom. Rev.* 2000; **19**: 111.
- Brutschy B. Ion-molecule reactions within molecular clusters. *Chem. Rev.* 1992; **92**: 1567.
- Takashima K, Riveros JM. Gas-phase solvated negative ions. *Mass Spectrom. Rev.* 1998; **17**: 409.

22. Desfrancois C, Carles S, Schermann JP. Weakly bound clusters of biological interest. *Chem. Rev.* 2000; **100**: 3943.
23. Tsuchiya M. Liquid ionization mass spectrometry for studies of hydrogen-bonded clusters and of a mixture that contains non-volatile and less polar organic compounds. *Mass Spectrom. Rev.* 1998; **17**: 51.
24. Campana JE. Cluster ions. I. Methods. *Mass Spectrom. Rev.* 1987; **6**: 395.
25. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. Electrospray ionization for mass-spectrometry of large biomolecules. *Science* 1989; **246**: 64.
26. Takáts Z, Drahos L, Schlosser G, Vékey K. Feasibility of formation of hot ions in electrospray. *Anal. Chem.* 2002; **74**: 6427.
27. Hernández H, Robinson CV. Dynamic protein complexes: insights from mass spectrometry. *J. Biol. Chem.* 2001; **276**: 46 685.
28. Rostom AA, Robinson CV. Detection of the intact GroEL chaperonin assembly by mass spectrometry. *J. Am. Chem. Soc.* 1999; **121**: 4718.
29. Tito MA, Miller J, Walker N, Griffin KF, Williamson ED, Despeyroux-Hill D, Titball RW, Robinson CV. Probing molecular interactions in intact antibody: antigen complexes, an electrospray time-of-flight mass spectrometry approach. *Biophys. J.* 2001; **81**: 3503.
30. Tito MA, Tars K, Valegard K, Hajdu J, Robinson CV. Electrospray time-of-flight mass spectrometry of the intact MS2 virus capsid. *J. Am. Chem. Soc.* 2000; **122**: 3550.
31. Takáts Z, Schlosser G, Vékey K. Hydrogen/deuterium exchange of electrosprayed ions in the atmospheric interface of a commercial triple-quadrupole mass spectrometer. *Int. J. Mass Spectrom.* 2003; **228**: 729.
32. Nguyen VQ, Chen XG, Yergey AL. Observations of magic numbers in gas phase hydrates of alkylammonium ions. *J. Am. Soc. Mass Spectrom.* 1997; **8**: 1175.
33. Hulthe G, Stenhagen G, Wennerström O, Ottosson C-H. Water clusters studied by electrospray mass spectrometry. *J. Chromatogr. A* 1997; **777**: 155.
34. Nielsen SB, Masella M, Kebarle P. Competitive gas-phase solvation of alkali metal ions by water and methanol. *J. Phys. Chem. A* 1999; **103**: 9891.
35. Bienkowski T, Danikiewicz W. Generation and reactions of substituted phenide anions in an electrospray triple quadrupole mass spectrometer. *Rapid Commun. Mass Spectrom.* 2003; **17**: 697.
36. Peschke M, Blades AT, Kebarle P. Formation, acidity and charge reduction of the hydrates of doubly charged ions  $M^{2+}$  ( $Be^{2+}$ ,  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $Zn^{2+}$ ). *Int. J. Mass Spectrom.* 1999; **185/186/187**: 685.
37. Schröder D, Weiske T, Schwarz H. Dissociation behavior of  $Cu(urea)^+$  complexes generated by electrospray ionization. *Int. J. Mass Spectrom.* 2002; **219**: 729.
38. Stace AJ. Metal ion solvation in the gas phase: the quest for higher oxidation states. *J. Phys. Chem. A* 2002; **106**: 7993.
39. Vaidyanathan G, Herron WJ, Garvey JF. Mass spectrometric investigation of 2-methoxyethanol and 2-ethoxyethanol clusters: magic numbers and structural implications. *J. Phys. Chem.* 1993; **97**: 7880.
40. Vaidyanathan G, Garvey JF. Mass spectrometric investigation of dimethoxyethane clusters: complete solvation of a protonated cation. *J. Phys. Chem.* 1994; **98**: 2248.
41. König S, Fales HM. Formation and decomposition of water clusters as observed in a triple quadrupole mass spectrometer. *J. Am. Soc. Mass Spectrom.* 1998; **9**: 814.
42. Herron WI, Coolbaugh MT, Vaidyanathan G, Peifer WR, Garvey JF. Observation of magic numbers for  $(ROH)_nH_3O^+$  heteroclusters ( $R = CH_3, CH_3CH_2, (CH_3)_2CH$ ), and  $(CH_3CH_2CH_2)$ : implication for cluster ion structure. *J. Am. Chem. Soc.* 1992; **114**: 3684.
43. Garvey JF, Herron WJ, Vaidyanathan G. Probing the structure and reactivity of hydrogen-bonded clusters of the type  $\{M\}_n\{H_2O\}H^+$ , via the observation of magic numbers. *Chem. Rev.* 1994; **94**: 1999.
44. Guillaume C, Le Calvé J, Dimicoli I, Mons M. Molecular solvation in van der Waals heteroclusters. Magic numbers in the mass spectra: a dynamical signature of solvent shell saturation in the ion. *J. Phys. Chem.* 1994; **98**: 13 443.
45. El-Shall MS, Daly GM, Gao J, Meot-Ner (Mautner) M, Sieck LW. How sensitive are cluster compositions to energetics? A joint beam expansion/thermochemical study of water-methanol-trimethylamine clusters. *J. Phys. Chem.* 1992; **96**: 507.
46. Wei S, Shi Z, Castleman Jr AW. Mixed cluster ions as a structure probe: experimental evidence for clathrate structure of  $(H_2O)_{20}H^+$  and  $(H_2O)_{21}H^+$ . *J. Chem. Phys.* 1991; **94**: 3268.
47. Shinohara H, Nagashima U, Tanaka H, Nishi N. Magic numbers for water-ammonia binary clusters: enhanced stability of ion clathrate structures. *J. Chem. Phys.* 1985; **83**: 4183.
48. Kelterbaum R, Kochanski E. Behavior and evolution of the first 28 protonated hydrates from Monte Carlo studies. *J. Phys. Chem.* 1995; **99**: 12 493.
49. Nagashima U, Shinohara H, Nishi N, Tanaka H. Enhanced stability of ion-clathrate structures for magic number water clusters. *J. Chem. Phys.* 1986; **84**: 209.
50. Searcy JQ, Fenn JB. Clustering of water on hydrated protons in a supersonic free jet expansion. *J. Chem. Phys.* 1974; **61**: 5282.
51. Yang X, Castleman Jr AW. Large protonated water clusters  $H^+(H_2O)_n$  ( $1 \leq n < 60$ ): The production and reactivity of clathrate-like structures under thermal conditions. *J. Am. Chem. Soc.* 1989; **111**: 6845.
52. Labowsky M, Fenn JB, de la Mora JF. A continuum model for ion evaporation from a drop: effect of curvature and charge on ion solvation energy. *Anal. Chim. Acta* 2000; **406**: 105.
53. Wang G, Cole RB. Charged residue versus ion evaporation for formation of alkali metal halide cluster ions in ESI. *Anal. Chim. Acta* 2000; **406**: 53–65.
54. Kebarle P, Peschke M. On the mechanisms by which the charged droplets produced by electrospray lead to gas phase ions. *Anal. Chim. Acta* 2000; **406**: 11–35.
55. Thomson BA, Iribarne JV. Field induced ion evaporation from liquid surfaces at atmospheric pressure. *J. Chem. Phys.* 1979; **71**: 4451.
56. Collette C, De Pauw E. Calibration of the internal energy distribution of ions produced by electrospray. *Rapid Commun. Mass Spectrom.* 1998; **12**: 165.
57. Collette C, Drahos L, De Pauw E, Vékey K. Comparison of the internal energy distributions of ions produced by different electrospray sources. *Rapid Commun. Mass Spectrom.* 1998; **12**: 1673.
58. Drahos L, Heeren RMA, Collette C, De Pauw E, Vékey K. Thermal energy distribution observed in electrospray ionization. *J. Mass Spectrom.* 1999; **34**: 1373.
59. McLuckey SA, Goeringer DE. Slow heating methods in mass spectrometry. *J. Mass Spectrom.* 1997; **32**: 461.