Generation and analysis of highly hydrated ions using electrospray ionization mass spectrometry

by

Keri Jean McQuinn
B.Sc., University of Guelph, 2006

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

in the Department of Chemistry

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University of Victoria

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Supervisory Committee

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Dr. George R. Agnes, External Member
(Simon Fraser University, Department of Chemistry)
Abstract

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A variety of highly hydrated ions were generated and studied using electrospray ionization mass spectrometry (ESI-MS) including proton, a series of triply charged lanthanide ions, the doubly charged lead ion and various methylated guanidinium ions. In each case large hydrated water clusters were mass selected and fragmented through collision induced dissociation (CID) to investigate their properties. The fragmentation of protonated water clusters highlighted the stability of the “magic” water cluster \([\text{H(H}_2\text{O)}_{21}]^+\). Typically unstable triply charge lanthanide water clusters and the previously unobserved doubly charged lead water clusters were generated. Fragmentation studies indicated that both the charge density and the geometry of the clusters affect their stability. The charge reduction of triply charged lanthanide clusters led to the direct observation of ion evaporation. Finally, the dehydration of various methylated guanidinium ions indicated a structural basis for differences in their ability to hydrogen bond.
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**List of Abbreviations**

<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CID</td>
<td>Collision Induced Dissociation</td>
</tr>
<tr>
<td>CRM</td>
<td>Charge Residue Model</td>
</tr>
<tr>
<td>DFT</td>
<td>Density Functional Theory</td>
</tr>
<tr>
<td>aDMA</td>
<td>Asymmetric Dimethylarginine</td>
</tr>
<tr>
<td>sDMA</td>
<td>Symmetric Dimethylarginine</td>
</tr>
<tr>
<td>EDESI</td>
<td>Energy Dependent Electrospray Ionization</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray Ionization</td>
</tr>
<tr>
<td>HF</td>
<td>Hartree Fock Perturbation Theory</td>
</tr>
<tr>
<td>IE</td>
<td>Ionization Energy</td>
</tr>
<tr>
<td>IEM</td>
<td>Ion Evaporation Model</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass-to-Charge Ration</td>
</tr>
<tr>
<td>MM</td>
<td>Molecular Mechanics</td>
</tr>
<tr>
<td>MP</td>
<td>Møller Plesset Perturbation Theory</td>
</tr>
<tr>
<td>MRE11</td>
<td>Meiotic Recombination 11</td>
</tr>
<tr>
<td>MS/MS</td>
<td>Tandem Mass Spectrometry</td>
</tr>
<tr>
<td>$n_{\text{min}}$</td>
<td>Minimum Number of Water Molecules Necessary to Stabilize an Ion</td>
</tr>
<tr>
<td>NBS1</td>
<td>Nijmegen Breakage Syndrome 1</td>
</tr>
<tr>
<td>PRMT</td>
<td>Protein Arginine Methyltransferase</td>
</tr>
<tr>
<td>RAD50</td>
<td>RAD50 Homolog (S. Cerevisiae)</td>
</tr>
<tr>
<td>ToF</td>
<td>Time-of-Flight Mass Analyzer</td>
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Chapter 1: Introduction

1.1 Electrospray ionization mass spectrometry

Mass spectrometry is an instrumental technique used to determine the composition of a sample through the identification and quantification of the ions generated in the ion source via their mass-to-charge ratios \((m/z)\). The instrument consists of multiple components including: the source, where the gas-phase ions are generated; the mass analyzer, where the ions are separated by their mass-to-charge ratio using an electric field; and a detector that measures the number of ions present. Electrospray is one example of an ionization source that is extremely popular and allows for the analysis of complex mixtures at low concentrations.\(^{[1-3]}\)

Electrospray ionization mass spectrometry (ESI-MS)\(^{[4]}\) was first introduced by Dole and coworkers in the late 1960s\(^{[5]}\) and was developed further by Fenn and coworkers.\(^{[6, 7]}\) Fenn was awarded a Nobel prize in 2002 for his efforts in advancing this technology and using it to analyze biological macromolecules.\(^{[8]}\) ESI-MS is a soft ionization technique, meaning there is little or no fragmentation of the ions produced. It involves the generation of desolvated ions from the continuous injection of a solution. The sample consists of a volatile solvent with a low concentration of the analyte of interest. The analyte is either already charged in solution, polar or acidic/basic so that it can become charged readily. The solution passes through a metal capillary which typically has an applied potential difference of approximately 3 kV. Under these conditions, a “Taylor cone” is generated from which emerges an aerosol of highly
solvated ions into a chamber at atmospheric pressure. These ions are desolvated as they pass through a stream of nitrogen gas to generate naked gas-phase ions. There are two proposed mechanisms for the generation of individual, completely desolvated ions; the Charge Residue Model (CRM) also known as Coulomb explosion, and the Ion Evaporation Model (IEM). These will be discussed later in the chapter.\cite{1, 2}

![Figure 1.1. Schematic of an electrospray ionization source.](image)

Once the gas-phase ions are generated they are drawn towards the sample cone and then to a skimmer cone, a small orifice which guides the charged species into the rest of the instrument (Figure 1.1). The ions move into various differentially pumped compartments, until a sufficiently high vacuum is attained so that the ions can be focused into the mass analyzer. Here the ions are separated based on their mass-to-charge ratio using a combination of electric (and/or magnetic) fields. The number of ions that hit the
detector are counted and using mass spectrometric software ion arrival times are converted to m/z values and a mass spectrum is produced.\textsuperscript{[1, 2]}

### 1.2 Process of generating gas phase ions by electrospray

For more than three decades there has been significant debate over the correct mechanism to describe the generation of gas-phase ions in the ESI process. Two mechanisms (see Figure 1.2), the Charge Residue Model (CRM) and the Ion Evaporation Model (IEM) have been proposed.

CRM was first described by Dole et al.\textsuperscript{[5]} This model states that as desolvation occurs, the charge density of the ion increases causing Coulombic repulsion between like charges in the droplet to increase until the Raleigh limit is reached which renders the droplet unstable. The Raleigh limit is simply the maximum charge a droplet of a certain radius can have before the repulsion between like charges overcomes the surface tension of the droplet. This value depends on the pressure due to the curvature of the droplet, the surface tension of the droplet, and the electrostatic pressure of the droplet.\textsuperscript{[9]} At this critical Raleigh limit the droplet “explodes” into multiple smaller droplets, all of which will have similar charge densities. The most recent studies suggest that in most cases this mechanism does not describe the ionization process for low-mass ions;\textsuperscript{[10-12]} however, this model does seem to be favoured in the gas-phase generation of large, globular proteins. In a careful ESI-MS study of globular proteins, De la Mora\textsuperscript{[13]} observed that the number of charges on the desolvated protein was only slightly smaller than the Raleigh limit for water droplets of similar size. Because these charged proteins represent the species immediately after the final stages of solvent evaporation, the similarity between the charges on the protein and the Raleigh limit would suggest that CRM is occurring.
Similarly, it has been suggested that the gas-phase generation of non-covalent complexes can also be described by the CRM.\textsuperscript{14}

Iribarne and Thomson\textsuperscript{15} proposed the alternate Ion Evaporation Model for the gas-phase generation of small ions. As desolvation occurs and the charge density increases, the ion is ejected (evaporates) from the surface of a droplet before the Raleigh limit is reached and the droplet has become unstable. This occurs when the electric field on the surface of the droplet becomes large enough to overcome solvation forces. In this model a single charge with a few solvent molecules leaves the surface of a much larger droplet such that the evaporated ion leaves with a significant proportion of the charge but only a small amount of the mass from the original droplet. The number of charges in a droplet that undergoes ion evaporation (IE) is lower than the number of charges in a droplet that undergoes a Coulomb explosion.\textsuperscript{9} In the case of IE there is a relationship between the number of charges and the radius of the droplet.\textsuperscript{14} Because neither CRM or IEM have been directly observed, this relationship has been exploited in attempts to prove the validity of IEM. A variety of experiments have been carried out to determine both the sizes and the charges of solid charged species which have undergone ESI. The results suggest that IEM is a valid mechanism and is more likely to occur for small ions than the CRM mechanism.\textsuperscript{10-12}
Figure 1.2. The desolvation process in ESI. Two mechanisms: the Charge Residue Model (Coulomb explosion) and the Ion Evaporation Model are used to describe the production of individual gas phase ions.

1.3 Tandem mass spectrometry

It is often necessary to obtain more detailed structural information on an unknown peak observed in a mass spectrum generated using a soft ionization technique (such as ESI). This additional data can be obtained by fragmenting the species of interest and analyzing the product ions through tandem mass spectrometry experiments. There are a variety of commercial instruments available with tandem mass spectrometry (MS/MS) capabilities. Our instrument, a Quadrupole-Time-of-Flight instrument (QToF), allows for MS/MS experiments to be carried out due to a second mass analyzer. In these experiments the first mass analyzer, a quadrupole,\textsuperscript{[4]} is used to mass select an precursor ion of interest. The quadrupole is made up of four parallel rods and acts as a mass filter. An alternating RF and a fixed DC voltage are applied to each rod so that opposing rods
have the same polarity while adjacent rods have opposite polarities. As the RF potentials are scanned and the polarity of the rods switch, the ions travelling down the quadrupole follow a very complicated trajectory. Only ions of a specific $m/z$ ratio are able to make it through the quadrupole. All other ions are neutralized through collisions with the rods.

Once the ions of interest pass through the quadrupole, they pass through a gas-filled hexapole where collision-induced dissociation$^{[16-18]}$ (CID) occurs. The ions are accelerated into this collision cell where they collide with inert gas molecules (usually nitrogen, helium, argon or xenon). A fraction of the translational energy of each ion is converted into internal energy as the energy is distributed to the bonds within the ion. When this energy becomes greater than the threshold for fragmentation the parent ion dissociates into smaller product ions and neutral fragments. The fragmentation that occurs depends on the pressure of the gas in the collision cell (as collisions will occur more frequently), the collision energy, and the mass of the inert gas (higher mass means more fragmentation). The product ions are accelerated to the second mass analyzer where they are again separated by their mass-to-charge ratio and subsequently detected. The second mass analyzer is typically another quadrupole or a Time-of-Flight$^{[19]}$ (ToF) mass analyzer. Our system utilizes a ToF mass analyzer. At the beginning of the flight tube in a ToF analyzer the ions are pulsed by an electric field and all ions of the same charge are given the same amount of kinetic energy, $KE = zeV$, where $z$ is the charge of the ion, $e$ is the Coulombic charge of an electron, and $V$ is the strength of the electric field. The kinetic energy is also related to the mass, $m$, and velocity, $v$, of the ion by the relationship $KE = \frac{1}{2} mv^2$. These two equations can be combined to give the relationship $m/z = 2eV/v^2$, i.e. the velocity of the ion is inversely proportional to the square root of the
mass-to-charge ratio; therefore, ions with smaller \( m/z \) values travel down the flight tube faster and reach the detector first, while ions with larger \( m/z \) values move more slowly and will reach the detector later. This type of mass analyzer often includes a reflectron which is an ion mirror used to improve resolution. Although the quadrupole and ToF are the only mass analyzers discussed, there are a variety of other instruments that allow for not only MS/MS but also MS\(^n\) experiments, however these techniques were not used for this work and will not be discussed.

**Figure 1.3.** Schematic of the MS/MS process. A species is mass selected based on its mass-to-charge ratio in MS1. The species is then accelerated into the collision cell where it undergoes collisions with an inert gas and fragmentation occurs. Product ions are then scanned by the MS2 and detected. In our system, MS1 is a quadrupole mass analyzer and MS2 is a Time-of-Flight mass analyzer.
1.4 Energy-Dependent Electrospray Ionization Tandem Mass Spectrometry (EDESI-MS/MS)

In order to obtain maximum information from the CID experiments, the collision energy can be increased sequentially (from 0 to 200 V) to induce more and more fragmentation. Each spectrum collected at a different collision voltage is a snapshot of the fragmentation process, however this produces a lot of information that needs to be presented in a compact manner. Energy-Dependent Electrospray Ionization (Tandem) Mass Spectrometry\textsuperscript{[20, 21]} (EDESI-\{MS/\}MS) solves this problem. This approach to data analysis was developed to condense large amounts of CID information so it can be presented more compactly. Mass spectra are collected at successive collision voltages and the data is then combined to generate a two-dimensional contour map, where the horizontal axis represents the mass-to-charge ratio, the vertical axis represents the collision voltage, and the contours are generated from ion intensity information. This map allows for the observation of fragmented species at each collision voltage at the same time (Figure 1.4 bottom). A summation plot is presented above the two-dimensional contour map and it constitutes the summation of intensities at each collision voltage for each mass-to-charge ratio (Figure 1.4 top). It allows for the comparison of relative intensities of each species. Shown together, these plots provide all the information necessary to analyze the entire fragmentation process.
Figure 1.4. EDESI-MS/MS of $[\text{PtBr}_6]^{2-}$. The fragmentation energy increases vertically on the contour map. The top plot is a summation of all 81 mass spectra used to generate the contour map.\textsuperscript{[22]}

1.5 Advantages and drawbacks of ESI-MS

Since the development of electrospray ionization, its versatility has been proven by its use in inorganic, organometallic, organic, biological and analytical chemistry. Its extensive use in these fields is due to the multiple advantages associated with this technique.\textsuperscript{[23]} ESI-MS allows for the facile detection of cations and anions (in positive mode or negative mode) directly from solution. Spectra are typically very clean and
relatively simple to assign directly from their mass-to-charge ratio and isotope patterns and, as mentioned previously, because ESI is a soft ionization technique which makes resulting spectra less convoluted. However, if fragmentation is desired, tandem MS studies can be performed easily to help identify unknown species. ESI-MS is also advantageous because of its low (nmol) detection limits, which makes it particularly useful for environmental and biological samples.

Despite its many advantages, ESI-MS is sometimes criticized as there are some problems that arise upon application of this technique to solution equilibria, all of which have been previously reviewed.\textsuperscript{[23]} One of the main concerns is that upon ionization, the equilibrium conditions are changed and the composition of the solution is altered. Possible equilibrium perturbations include volume changes,\textsuperscript{[24]} temperature changes,\textsuperscript{[25]} as well as pH changes\textsuperscript{[24]} each of which could affect the observed stoichiometry, number of species in solution, and/or the relative abundances of species in solution. Gas-phase reactions are also a large concern. Often times species that are stable in solution are no longer stable in the gas phase due to the removal of solvent.\textsuperscript{[23]} For both of these reasons the mass spectrum may not always accurately depict exactly what was in solution.

Solvent molecules and ion adducts can also be problematic as they can make spectra more complicated. Solvent molecules often coordinate to the metal centre of the molecule of interest during the ESI process or are removed from a coordinating site in solution through desolvation making it more difficult to differentiate vacant coordination sites from orthometalation. It is also common to observe the formation of ion adducts with ions like Cl\textsuperscript{−}, Na\textsuperscript{+}, K\textsuperscript{+} etc. These adducts are often not found in solution but are generated by the ESI process.
ESI is known to be a gentle ionization technique however, fragmentation and polymerization do still occur.\textsuperscript{[23]} These phenomena can be observed for a variety of bound and unbound ligand molecules but are most often observed in the case of large or aliphatic molecules. The more fragmentation or polymerization that occurs, the more complicated and difficult the spectrum becomes.

Another drawback of ESI-MS is that it cannot determine the number of acidic protons on the molecule of interest because the speed of electron transfer exceeds that of the ESI process. Usually only the species with a single charge (positive or negative) will be observed. Although not typical, species with a different number of protons have been observed and quantified.\textsuperscript{[26, 27]}

The species generated in ESI and their relative intensities are influenced by the source conditions under which the experiment is run. Depending on the sensitivity of the species in solution, the capillary and cone voltages and temperatures can affect the observed spectra again making quantification difficult. Also making quantification difficult is the fact that some species are ionized more efficiently than others. The method of internal standards has been developed to deal with this.\textsuperscript{[28]} Other methods including the direct calculation of ionization efficiency\textsuperscript{[29, 30]} or specific to host-guest interactions, using known binding constants\textsuperscript{[31, 32]} have also been used.

Lastly, ESI ionization is limited by the strength of any electrolytes in solution and of the solvent itself. Some organic solvent should be added to aqueous solutions since pure water is often difficult to spray and can lead to poor signal intensities. Also, concentrations of any supporting electrolyte in the solution must be restricted as high concentrations of electrolyte will dominate the spectrum. This is problematic for
biological or environmental samples as they are typically aqueous with high concentrations of electrolytes. The restricted sample conditions required for ESI-MS also makes comparing results to other analytical techniques more difficult since the solvents are different. Although this should affect stability constants, it should not affect the types of species that are observed.\textsuperscript{[23]}

Despite the disadvantages in the use of ESI-MS for solution equilibria, these drawbacks are often not significant and ESI is a useful tool for studying many different systems.

\section*{1.6 Generation of gas-phase water clusters}

Mass spectrometry has been successfully used to study solvated ions in the gas phase in order to provide insight into the behaviour of these ions in solution. A variety of techniques have been used to generate and ionize protonated water clusters in the gas phase. They include supersonic expansion,\textsuperscript{[33-35]} liquid ionization,\textsuperscript{[36, 37]} adiabatic expansion of a liquid jet,\textsuperscript{[38, 39]} electron impact ionization,\textsuperscript{[40]} chemical ionization,\textsuperscript{[41]} femtosecond photoionization,\textsuperscript{[42]} and electrospray ionization (ESI).\textsuperscript{[43-47]} In all cases, with the exception of liquid ionization and electrospray ionization, neutral water clusters are generated through supersonic or adiabatic expansion. These processes involve the successive addition of the ligand (a water molecule) to the analyte through condensation. Supersonic expansion\textsuperscript{[33-35]} is the expansion of the sample gas at high pressure through a small orifice into a vacuum. As collisions occur the cluster grows in size.\textsuperscript{[48]} This technique is often used to generate clusters because as the gas molecules undergo supersonic expansion multiple collisions the molecules lose vibrational and rotational
energy and cool down therefore, clusters do not immediately decompose upon collisions. Adiabatic expansion is similar to supersonic expansion, but instead, a liquid jet of the sample of interest passes through a nozzle into a vacuum and droplets are generated.[36]

Neutral clusters generated by supersonic or adiabatic expansion must then be ionized. In liquid ionization experiments the liquid sample is supplied to a sample holder, usually a needle, and the sample is ionized through collisions with argon at atmospheric pressure.[36] Neutral clusters can also be ionized using electron impact. Here a liquid jet, having already undergone adiabatic expansion, is ionized by a pulsed electron beam. Clusters are highly excited and therefore dissociation occurs quickly making this method suitable for studying the more stable clusters but less ideal if fragmentation of parent clusters is unwanted.[40] Photoionization is a more gentle way to ionize water clusters. Neutral clusters are pulsed with a soft x-ray laser that provides enough energy to induce ionization.[42] The technique of chemical ionization uses supersonic or adiabatic expansion to create neutral species (water clusters) in some reagent gas. A reagent gas commonly used is methyl iodide vapour in an inert carrier gas. The reagent gas is excited and interacts with the molecules of interest (water clusters) to form ions.[41] These are all adequate methods for generating singly charged hydrated species, however the condensation of a solvent to a multiply charged species can be troublesome.

Previously, only singly charged species were studied because clusters were generated by the successive addition of the ligand (water molecules) onto bare or minimally solvated ions. Multiply charged species would often undergo charge reduction[49-51] (see Eq. 1.1) at small cluster sizes and stable multiply charged species
could not be observed. The development of electrospray ionization has led to the ability to generate multiply charged ion clusters since the ligands stay coordinated to the ion throughout the ionization process and large droplets are generated which then decrease in size through evaporation to generate clusters of a variety of sizes. In recent years ESI-MS has be used to study a variety of doubly and triply charged hydrated water clusters with metal centres including Mg\textsuperscript{2+},\textsuperscript{[51]} Ca\textsuperscript{2+},\textsuperscript{[51]} Ln\textsuperscript{3+},\textsuperscript{[52]} Cu\textsuperscript{2+},\textsuperscript{[53, 54]} Zn\textsuperscript{2+},\textsuperscript{[55]} Mn\textsuperscript{2+},\textsuperscript{[55]} and Sr\textsuperscript{2+}\textsuperscript{[51]} among others.

\[ [\text{M(H}_2\text{O)}_n]^{m+} \rightarrow [\text{M(OH)(H}_2\text{O)}_{n-1}]^{(m-1)+} + \text{H}_3\text{O}^+ \quad m = 2, 3 \quad (1.1) \]

Alterning the conditions under which typical electrospray experiments are run allows for the generation of highly solvated clusters. Samples are usually injected into the electrospray source at a rate of 5-20 µL/min with the source and desolvation gas at temperatures well above the boiling point of the solvent. To generate hydrated clusters in the gas phase using the electrospray technique it is necessary to use “cold flooding” conditions which involves increasing the flow rate significantly to approximately 100 µL/min and decreasing the source and desolvation gas to almost ambient temperatures. These conditions ensure that the ions will be highly solvated upon exit from the capillary and will be only partially desolvated by the desolvation gas, producing a large distribution of cluster sizes.
1.7 Weak interactions

Interactions between atoms fall into two categories: covalent interactions and non-covalent interactions. Covalent interactions are chemical bonds where the atoms involved share electrons. There are a variety of non-covalent interactions; these include van der Waals forces, electrostatic interactions (ionic bonds), cation-π interactions, and dipolar interactions. These are all considered weak interactions and vary in strength.\textsuperscript{[56, 57]}

Water clusters, protonated water clusters in particular, are held together by one of these weak interactions: hydrogen bonds. A hydrogen bond is a weak attractive force caused by dipole-dipole interactions between an electronegative atom, which will have a partial negative charge, and a hydrogen atom attached to another electronegative atom, which induces a partial positive charge on the hydrogen atom. Hydrogen bonds are fairly strong dipole-dipole interactions. Their strengths depend on the electronegativity of the atoms involved; for example, a more electronegative atom will interact more strongly with the hydrogen atom. The strength of a hydrogen bond is determined from the Coulombic interaction between the lone pair electrons on the electronegative atom and the proton. Hydrogen bond strengths range from 0.2 to 40 kcal/mol. In water, the hydrogen bond network is particularly strong because each water molecule can form four hydrogen bonds with the surrounding water molecules. Hydrogen bond strengths are also increased in the presence of an ion and the strength of hydration is affected by the number of hydrogen bonds that can form.

Hydrogen bonds are important in a vast number of chemical and biological systems. They determine the structure and properties of bulk water.\textsuperscript{[58]} they are partially
responsible for determining the structure and therefore function of proteins and nucleic
acids;[59] and they can be important in molecular recognition and binding.

Mass spectrometry coupled with a soft ionization technique, like ESI, can be used
to study hydrogen-bonded systems. In the gas phase, solvated ions are free from
interference of the bulk solution and the properties of the ion itself can be studied. By
comparing the gas-phase results and properties of the ion in solution, the effects of
solvation can be analyzed.[60] We use CID on highly solvated ions to look at solvation
beyond the first solvation sphere as well as relative strengths of hydrogen bonds within
the first solvation shell.

1.8 Conclusions

ESI mass spectrometry is an extremely versatile technique and can be used to
study a variety of charged species. The ability to generate singly and multiply charged
hydrated species in the gas phase provides a more realistic view of solution than
completely desolvated ions in the gas phase. Although triply charged metal ions are
stable in solution, until recently they could not be observed in the gas phase. ESI is
pivotal to generation of these species. CID has also been crucial in the investigation of
the properties of both singly and multiply charged clusters.

In the following chapters the dehydration of ions, via CID, to probe various
phenomena will be discussed. It will be seen that the sequential loss of water molecules
from large protonated water clusters can be used to investigate the stability of magic
water clusters (those with particularly stable structures). Water molecules can be stripped
from triply charged water molecules and the charge reduction process can be investigated
and used to probe the ESI mechanism. The triply charged lanthanides, along with never before observed doubly charged lead hydrated species, can be used to examine the physical properties responsible for the charge separation phenomenon. Finally, the effect of arginine methylation on hydrogen bonding will be investigated through the dehydration of various methylated guanidinium species, which are analogues to the amino acid, arginine.

1.9 References


Chapter 2: Protonated Water Clusters

2.1 Introduction

There is significant interest in solvent cluster research as a link between the gas phase and the solution phase. Water clusters are of particular interest because of the unique hydrogen bonding capabilities of water and its importance in chemistry and biology. Protonated water clusters have been studied extensively for many years and are routinely used for calibration purposes in mass spectrometry as \([H(H_2O)_n]^+\) are produced readily and cover a large \(m/z\) range, as clusters are produced where \(n > 100\).

One of the main focuses of water cluster research has been on the “magic” \([H(H_2O)_{21}]^+\) cluster, first identified in 1973 by Lin, who observed through mass spectrometry experiments that water cluster intensities decreased as the number of water molecules increased with the exception of the 21-mer and the 22-mer where a discontinuity occurred. The \([H(H_2O)_{21}]^+\) ion had a larger intensity than expected while the \([H(H_2O)_{22}]^+\) cluster had a smaller intensity than expected. This \([H(H_2O)_{21}]^+\) cluster has been described as “magic” due to this anomalous intensity observed in many different mass spectrometry experiments, indicating that it has a particularly stable structure. The \([H(H_2O)_{28}]^+\) cluster has also been observed to have special stability as a weak maximum is seen for the 28-mer in \([H(H_2O)_n]^+\) mass spectra. The behaviour and existence of these “magic” number clusters are independent of the source of ionization. Many different techniques have been successful in generating these ensembles of water clusters as mentioned in Chapter 1.
Figure 2.1. Electrospray ionization mass spectrum of protonated water clusters. The $[\text{H(}\text{H}_2\text{O})_{21}]^+$ cluster is “magic” due to its unusually high stability, also true but to a markedly lesser extent for $[\text{H(}\text{H}_2\text{O})_{28}]^+$.

Searcy and Fenn suggested that the $[\text{H(}\text{H}_2\text{O})_{21}]^+$ cluster had a pentagonal dodecahedral cage structure to account for its high stability. The structure of a range of protonated water clusters has been probed through vibrational predissociation spectroscopy. In these experiments $[\text{H(}\text{H}_2\text{O})_n]^+$ are generated in an ion source, mass selected in a tandem mass spectrometer and excited using an infrared laser. The spectrum produced was dependant on the size and structure of the water cluster being investigated. Shin and co-workers reported the O-H stretching vibrational spectra of $[\text{H(}\text{H}_2\text{O})_n]^+$ clusters with $6 \leq n \leq 27$. The spectra of the $[\text{H(}\text{H}_2\text{O})_{21}]^+$ and $[\text{H(}\text{H}_2\text{O})_{22}]^+$ differ from their neighbouring clusters as they only showed a single peak instead of a doublet, implying that all of the dangling OH groups on the $[\text{H(}\text{H}_2\text{O})_{21}]^+$ cluster are due to water molecules that are bound in a similar way, providing support for the pentagonal dodecahedral cage structure.

The structure of $[\text{H(}\text{H}_2\text{O})_{21}]^+$ has also been modeled by a variety of computational methods including ab initio calculations, Monte Carlo simulations and
molecular dynamics simulations.\cite{23} These models also suggest that the stability of the $[\text{H(H}_2\text{O)}_{21}]^+$ cluster is due to a distorted pentagonal dodecahedral cage structure (Figure 2.2). This structure is especially stable because each water molecule is hydrogen bonded to three other water molecules within the cage. There has been much debate as to what is found in the centre of the cage: a single water molecule or a hydronium ion.\cite{24} Many Monte Carlo simulations and \textit{ab initio} calculations have suggested that the hydronium ion is located on the surface of the cage\cite{16,25,26} while others suggest that the hydronium ion is found in the centre of the cage.\cite{18,21,27,28} More recent computational studies suggest that there is in fact a water molecule within the cage and the hydronium ion is found on the surface of the cage.\cite{16,17} A similar clathrate-like structure is proposed for the $[\text{H(H}_2\text{O)}_{28}]^+$ cluster.\cite{6}

**Figure 2.2.** An energy minimized model of the $[\text{H(H}_2\text{O)}_{21}]^+$ ion,\cite{12} a distorted dodecahedral cage in which each edge is a hydrogen bond. Note the 10 "dangling" O-H bonds (the dodecahedron has 20 vertices and 30 edges). The interior of the cage is occupied by a water molecule, clathrate-fashion; one of the 21 water molecules is protonated to form a hydronium ion.
Wu et al.\textsuperscript{[6]} studied the stability of $[\text{H(H}_2\text{O)}_{21}]^+$ under a variety of conditions. The authors used a continuous corona-discharge ion source to generate the clusters and performed the experiments in a vibrational predissociation ion trap tandem mass spectrometer with a pulsed infrared laser. Mass spectra showed that even at different backing pressures, anomalous intensities between the $n = 21$ and $n = 22$ clusters were observed. At higher backing pressures (340 and 200 Torr) the $[\text{H(H}_2\text{O)}_{21}]^+$ cluster was the most intense peak in the spectrum and when compared to the dissociation fraction, it was observed that this cluster had significantly smaller dissociation rates than the $n = 20$ and $n = 22$ clusters. In order to present evidence for a distorted pentagonal dodecahedral cage the authors compared their results from vibrational predissociation spectroscopy with Monte Carlo simulations and density functional theory (DFT) calculations. The vibrational predissociation spectrum of $[\text{H(H}_2\text{O)}_{21}]^+$ showed a single feature suggesting a three-coordinated water cluster. The results supported the distorted pentagonal dodecahedral cage structure. Although these “magic” clusters have been studied at length with mass spectrometric, computational and spectroscopic methods, few have performed MS/MS studies to determine if larger clusters preferentially fragment to these magic clusters. Stace and Moore\textsuperscript{[29]} generated water clusters using a pulsed molecular beam, which was then ionized by electron impact and dissociation fractions were monitored by mass spectrometry. They measured dissociation fractions of $[\text{H(H}_2\text{O)}_n]^+$ and $[\text{D(D}_2\text{O)}_n]^+$, with $n$ ranging from 5 to 26. Although they did not see any anomalies for the $n = 21$ cluster, they did find that the dissociation of $n = 22$ mer was much faster than the other clusters. Echt et al.\textsuperscript{[10]} generated and studied $[\text{H(H}_2\text{O)}_{21}]^+$ clusters using electron impact ionization time-of-flight (ToF) mass spectrometry. The ToF spectra showed that after a
time delay, anomalous intensities for the [H(H₂O)₂₁]⁺ and [H(H₂O)₂₈]⁺ clusters were evident. The authors varied the positive potential barrier applied in front of the detector and monitored the intensity of various cluster ions in order to study the amount of decomposition occurring in the drift tube of the ToF mass analyzer. They determined that while only 27% of the [H(H₂O)₂₁]⁺ cluster decomposed, almost twice as much, about 50%, of the [H(H₂O)₂₂]⁺ ion decomposed under the same conditions. Magnera et al.⁴ calculated the proton hydration energies for clusters with 1 to 28 water molecules. Clusters were generated by fast-atom bombardment of ice and binding energies were subsequently studied using collision induced dissociation (CID) mass spectrometry. It was determined that for cluster sizes n ≤ 9 the binding energy decreased significantly but started to increases slowly for n > 9. [H(H₂O)₂₁]⁺ was found to bind about 2 kcal/mol more strongly than its neighbouring clusters due to its high stability. Schindler et al.⁵ used an FTICR-MS and selected the [H(H₂O)₅₈]⁺ water cluster for fragmentation. They noted that this cluster fragmented to form clusters of n = 57-51 but clusters n = 55 and n = 53 had particularly long lifetimes. While they did not fragment this large cluster further to study the production of smaller clusters, they showed that the n = 21 cluster had a longer lifetime than other clusters. The majority of fragmentation experiments in this field have focussed on determining the dissociation fraction of various clusters and simply show that clusters only slightly larger than the “magic” cluster (n = 21 and n = 23) are less stable which is facilitated by the especially stable “magic” cluster and that the [H(H₂O)₂₁]⁺ cluster decomposes much more slowly than its neighbouring clusters.

Until now, no work has been done to determine if fragmenting large clusters will lead to the preferential formation of the n = 21 cluster. Here EDESI-MS/MS⁶–⁸
analysis of \([H(H_2O)_n]^+\) where \(n = 26 – 76\) is presented as a novel way to observe the stability of the “magic” water clusters. Clusters larger than \([H(H_2O)_{21}]^+\) are mass selected for CID in the argon-filled collision cell of the mass spectrometer. The collision voltage is increased and the intensities and distributions of the product ions are monitored. As the collision energy is increased the sequential loss of water molecules is observed. It is observed that clusters with as many as 76 water molecules attached preferentially fragment to form the \([H(H_2O)_{21}]^+\) cluster.

2.2 Experimental

All experiments were run on an unmodified Micromass Q-Tof micro™ mass spectrometer at a capillary voltage of 2900 V and an ion energy of 1.0 V. Water clusters were generated by injecting 0.05% trifluoroacetic acid into the mass spectrometer at a rate of 50 μL/min under optimized source conditions. The cone voltage was maximized at 200 V and the source and desolvation temperatures were set to 60°C and 20°C respectively. In order to optimize the ion intensity, the cone gas was turned off and the desolvation gas flow rate was 250 L/h. The formation of water clusters was not much affected by other instrumental parameters.

EDESI experiments were carried out by performing MS/MS on a selected peak and increasing the collision voltage in one-volt increments from 2 V until the spectrum was dominated by \([H(H_2O)_3]^+\). This final collision voltage depended on the size of cluster chosen for fragmentation. EDESI-MS/MS experiments were performed on \([H(H_2O)_n]^+\) \((n = 26, 31, 36, 41, 46, 51, 56, 61, 66, 71\) and \(76\)). Spectra were collected for 3 seconds at each collision voltage.
2.3 Results and Discussion

Protonated water clusters can be generated readily with ESI-MS by using high flow rates and low temperatures. As previously reported, the peak corresponding to the \([\text{H(H}_2\text{O)}_{21}]^+\) cluster is observed to have much higher intensity than its neighbouring peaks. Not only does it have the greatest intensity but here, it is observed that the overall distribution peaks around this cluster size (Figure 2.1). This distribution may well have more to do with the instrument and the experimental conditions than anything else as other studies show somewhat different overall distributions of water clusters.\textsuperscript{[3, 5, 6]} It has been reported that this distribution changes depending on the temperature of the solution, the temperature in the gas phase and the humidity in the air.\textsuperscript{4} In all studies, the \(n = 21\) peak is significantly larger than its immediate neighbours. The disruption in distribution is observed for a range of clusters. The \(n = 21\) cluster has an anomalously large intensity while the intensities of its nearby clusters, \(n = 22, 23\), and possibly 24, are smaller and facilitate the formation of the \(n = 21\) cluster. The “weak” maximum of the \(n = 28\) peak can also be observed in Figure 2.1. This peak is emphasized by the particularly low intensity of the neighbouring \(n = 29\) cluster which dissociates much faster to generate the \(n = 28\) cluster.

Highly solvated protonated water clusters (\(n = 26\)-76) were mass selected for EDESI-MS/MS experiments. In each experiment, the selected peak preferentially fragmented to the \([\text{H(H}_2\text{O)}_{21}]^+\) cluster, as can be seen in Figure 2.3 for the example of \([\text{H(H}_2\text{O)}_{66}]^+\). The contour plot shows the successive loss of water molecules as the collision voltage is increased. A summation plot is presented above the contour map. This plot is generated by summing all the product ion data generated from the EDESI-
MS/MS experiment. The most intense peak in this summed plot corresponds to 
\([H(H_2O)_{21}]^+\). Again this peak is significantly larger than its neighbouring peaks and a decrease in intensity of clusters with \(n = 22 - 23\) is observed. This decrease could be explained by the instability of these clusters previously observed.\(^{[10, 29]}\) Although the \([H(H_2O)_{28}]^+\) ion does not appear to have a particularly special intensity in the summation plot, it does have a significantly larger intensity than the \([H(H_2O)_{29}]^+\) cluster.

![Figure 2.3. EDESI-MS/MS plot of \([H(H_2O)_{66}]^+\). The fragmentation energy increases vertically on the contour plot. The top spectrum is a summation of all 139 spectra (collision voltage = 2-140) used to generate the contour plot.](image-url)
A more dramatic depiction of the special intensity of the [H(H₂O)₂₁]⁺ cluster ion in this experiment can be obtained by representing the intensity data as a 3D surface (Figure 2.4). In this 3D plot, a fairly steady intensity of water clusters is observed with the exception of the anomalously strong peak at m/z 379.24 corresponding to the [H(H₂O)₂₁]⁺ cluster. What appears as a fairly uniform “wave” of ions is interrupted by a distinct spike in intensity.

![3D plot of water clusters](image)

**Figure 2.4.** EDESI-MS/MS 3-D plot of [H(H₂O)₆₆]⁺. The stability of the [H(H₂O)₂₁]⁺ is also observed through CID as larger clusters preferentially fragment to this species.

Note also, that this peak is broader than all other peaks in the plot indicating that this cluster is a predominant feature at multiple collision voltages. In fact, the [H(H₂O)₂₁]⁺ cluster is observed to dominate the MS/MS spectrum at lower collision voltages than expected and maintains its strong intensity for a larger range of collision voltages than other clusters. Typically a single cluster will be the base peak in the
MS/MS spectra for only 1 or 2 different values of the collision voltage. The magic \([\text{H(H}_2\text{O)}_{21}]^+\) cluster dominates the MS/MS spectra (is the base peak) for more than 15 consecutive collision voltage settings, where the voltage ranged from 49 to 64 V. As seen in Figure 2.3, the intensities of clusters generated at higher collision voltages decreases steadily as the collision voltage increases, with the exception of \([\text{H(H}_2\text{O)}_{21}]^+\). The general decrease is accounted for by the fact that the ion intensity of the originally selected ion, \([\text{H(H}_2\text{O)}_n]^+\), is now distributed across a large number of product ions.

A peak that is slightly higher than its neighboring clusters can also be seen for the \([\text{H(H}_2\text{O)}_{28}]^+\) cluster and an anomalously small peak is observed for the \([\text{H(H}_2\text{O)}_{29}]^+\) cluster indicating the preferential formation of the magic \([\text{H(H}_2\text{O)}_{28}]^+\) cluster. This species however did not have a prolonged existence over a large range of collision voltages.

EDESI-MS/MS data from the fragmentation of clusters containing 26 to 76 water molecules each show the \([\text{H(H}_2\text{O)}_{21}]^+\) cluster having an anomalous intensity (see Appendix A). In all cases, fragmentation of a larger water cluster leads to the preferential generation of the \([\text{H(H}_2\text{O)}_{21}]^+\) magic water cluster.

To demonstrate the large range of water clusters that fragment preferentially to generate the \([\text{H(H}_2\text{O)}_{21}]^+\) cluster, an EDESI-MS/MS plot of the much smaller \([\text{H(H}_2\text{O)}_{36}]^+\) cluster is presented in Figure 2.5. Again, the most intense peak in the summation plot is the \([\text{H(H}_2\text{O)}_{21}]^+\) cluster which has a much larger intensity than its neighbouring clusters. Similarly, the \([\text{H(H}_2\text{O)}_{28}]^+\) cluster is not especially intense, but is much more abundant than the \([\text{H(H}_2\text{O)}_{29}]^+\) cluster.
Figure 2.5. EDESI-MS/MS plot of \([\text{H(H}_2\text{O)}_{30}]^+\). The fragmentation energy increases vertically on the contour plot. The top spectrum is a summation of all 92 spectra (collision voltage = 2.94) used to generate the contour plot.

One unexpected feature of all the EDESI-MS/MS spectra is a modulation of broad intensity maxima located near the 13-mer, 25-mer and 36-mer clusters, and a distinct minimum at the 32-mer. These broad maxima are different from the single intense peaks observed for the “magic” water clusters; the increased intensity is not at the expense of the neighbouring peaks and is not as dramatic. This modulation appears to occur every 12-13 water molecules, and although less exaggerated, it seems to extend to larger clusters. Figure 2.6 shows a composite spectrum, compiled from the summation plots for every experiment conducted.
Figure 2.6. Composite of summation plots of each experiment completed.

These features are extremely consistent for each EDESI-MS/MS spectrum, regardless of the initial size of the mass selected cluster. For this reason it is most likely that these features are real and not some strange artefact of the CID experiment. If inspected closely this same pattern can be seen in the original spectrum of clusters (Figure 2.1). The significance of this observation is unclear, as theoretical studies determining the energies of global minima of protonated water clusters (to the 20-mer) show that the change in energy with the addition of each water molecule is consistent, with no notable discontinuities.\[^{20}\] Interestingly, experimental and theoretical studies of some rare gas clusters indicate particularly stable icosahedral structures for the 13-mer among others.\[^{35, 36, 37}\] The mass spectra of these species however are typical of magic clusters where sharp peaks are observed, not the broad maxima seen here. It is unlikely that the fragmentation pattern observed in Figure 2.6 is due to the same icosahedral structure responsible for the stability of the rare gas clusters.
2.4 Conclusions

These EDESI-MS/MS experiments demonstrate a novel approach to show that [H(H₂O)₂₁]⁺ does indeed have much higher stability than not only its neighbouring clusters, but all other protonated water clusters less than [H(H₂O)₇₆]⁺. The existence of some new, subtle stability trends in protonated water clusters that appear as broad maxima every 12-13 water molecules is revealed, though no explanation is provided as yet for this phenomenon.

2.5 References


Chapter 3: Triply Charged Lanthanide Water Clusters

3.1 Introduction

ESI-MS is a powerful tool for studying singly charged ions, however, it is often difficult to generate triply charged, $M^{3+}$, trications in the gas phase due to their high charge density. Most often these $M^{3+}$ species are observed to be accompanied by anionic species to generate a monocation in the gas phase, e.g. $[MO]^+$. Charge reduction is another common event that occurs to stabilize multiply charged species; this reaction involves the deprotonation of a coordinating solvent molecule (Eq 3.1 and 3.2). Typical spectra of triply charged ions at low cone voltages show the species slightly solvated and coordinated to anionic species. At slightly higher cone voltages, the solvent molecules are stripped away but the ion is still coordinated to the anion(s). At high cone voltages bare ions are observed, however they are typically reduced to a lower oxidation state.\[1\] Until recently $M^{3+}$ species have not been observed in the gas phase solvated by water.\[2, 3\]

Singly and doubly charged hydrated ions have been studied extensively in the gas phase using mass spectrometry as they provide insight into processes that occur in bulk solution. Singly charged species have been generated by a variety of methods, however only electrospray ionization (ESI),\[3-11\] thermospray ionization,\[12\] and a “pick up” technique\[6, 13, 14\] have been able to generate multiply charged species and avoid the immediate charge reduction which occurs during supersonic expansion processes. Triply charged water clusters have proven to be even more difficult to generate. Until recently there have been many attempts to generate triply charged water clusters without success. Although triply charged species in acetonitrile,\[15\] dichloromethane,\[16\] and
diacetonealcohol\textsuperscript{[17]} clusters have been generated, only the doubly and singly charged, charge-reduced water cluster species were observed (Eq 3.1 an 3.2).

\[
[M(H_2O)_n]^{3+} \rightarrow [M(OH)(H_2O)_{n-2}]^{2+} + [H(H_2O)]^+ \quad (3.1)
\]

\[
[M(OH)(H_2O)_n]^{2+} \rightarrow [M(OH)_2(H_2O)_{n-2}]^+ + [H(H_2O)]^+ \quad (3.2)
\]

Of late there have been two reports of the generation of triply charged lanthanide water clusters. Bush et al.\textsuperscript{[2]} were the first to generate \([\text{Ln}(H_2O)_n]^{3+}\) (Ln = La, Ce, Eu) species using a home-built source that incorporated a resistively heated copper block. Presented in this chapter is the facile generation of triply charged lanthanide water clusters using a commercial mass spectrometer and the same modified source conditions employed to generate the protonated water clusters discussed in Chapter 2 (high flow rates and ambient temperatures).\textsuperscript{[3]} EDESI-MS/MS experiments were performed on \(\text{La}^{3+}, \text{Pr}^{3+}, \text{Eu}^{3+}, \text{Tb}^{3+}, \text{Ho}^{3+}, \text{Tm}^{3+}\) and \(\text{Lu}^{3+}\) clusters for an extensive look at the lanthanide series.

Upon dissociation of a multiply charged cluster through collision induced dissociation (CID) the cluster can shrink either by the loss of a neutral water molecule or through charge separation. At large cluster sizes the droplet shrinks through the loss of a neutral water molecule until some critical cluster size where the charge separation reaction begins to compete with the loss of a water molecule. Below some minimum number of water molecules \((n_{\text{min}})\) the multiply charged droplet is no longer stable and only charged reduced species are observed. EDESI-MS/MS can be used to directly observe this charge separation reaction and can therefore be used to investigate and
explain not only why this process occurs in the first place but also provide insight into the debated ESI mechanism.

As mentioned in Chapter 1, there are two mechanisms that are currently used to describe the production of desolvated ions via ESI; CRM and IE. The most recent indirect evidence seems to suggest that under most conditions IE is the appropriate explanation.\textsuperscript{[18]} For an instant in the charge separation process there exists two charged species within the same droplet and this process can therefore be used to directly probe the ESI mechanism.

Comparing the minimum triply charged cluster sizes observed for the lanthanide series the very reason charge separation occurs can be investigated. It is often reported that this charge reduction occurs because the third ionization energies of the lanthanides are larger than the first ionization energy of water rendering the small clusters unstable.\textsuperscript{[9, 14]} Williams and coworkers suggest that this relationship however is only indirect and charge reduction is due to the charge density of the ion.\textsuperscript{[19]} Stace and coworkers have even suggested pK\textsubscript{a} might effect the ability to generate multiply charge species.\textsuperscript{[20, 21]} Along with probing the ESI mechanism the effect of the third ionization potential, charge density, enthalpy of hydration and pK\textsubscript{a} on the generation and stability of the $[\text{Ln(H}_{2}\text{O})]^{3+}$ clusters will be investigated.

\section*{3.2 Experimental}

All experiments were run on an unmodified Micromass Q-ToF \textit{microTM} mass spectrometer in positive ion mode. Lanthanide solutions (5 mM LnCl\textsubscript{3} in water) were injected into the instrument with the capillary voltage set to 2900 V and the ion energy at
1.0 V. Maximizing the cone voltage (200 V) and setting the source and desolvation temperatures to 60 °C and 20 °C respectively was necessary to observe the highly solvated species. As the Ln$^{3+}$ cluster intensity varied, the solvent flow rate was changed between 20 and 50 µL min$^{-1}$. In order to optimize [Ln(H$_2$O)$_3$]$^{3+}$ formation, the cone gas was turned off and the desolvation gas was set to 100 L h$^{-1}$.

Concentration studies were first performed to optimize Ln$^{3+}$ cluster formation; 3 mM, 4 mM, 5 mM and 10 mM solutions of TbCl$_3$ were injected into the mass spectrometer. Triply charged species were observed for the three lowest concentrations, however they were not generated from the 10 mM solution. The 5 mM solution generated clusters with sufficient intensity and therefore this concentration was used for the investigation of all Ln$^{3+}$ water clusters.

EDES-MS/MS experiments were carried out on a selected peak and increasing the collision voltage from 2 to 75 V in one-volt increments. Spectra were collected for times ranging from 30 seconds to 2 minutes depending on cluster intensities. Spectra of Pr, Eu, Tb, Ho, and Tm were collected with unit resolution while slightly higher than unit resolution was necessary for La and Lu species. Automation of the mass spectrometer software (MassLynx®) to carry out the EDES-MS experiments (ramping of the collision voltage) was achieved using the program Autohotkey (freely available from http://www.autohotkey.com).

Selected lanthanide water cluster structures were optimized in MOPAC using the semi-empirical SPARKLE/AM1 method.$^{[22]}$ Sparkle is a parameter set specifically intended for lanthanide calculations. Protonated water cluster calculations were completed using the AM1 method in MOPAC and the HF 6-31+G* basis set in
Spartan.\textsuperscript{[23]} There was little difference between the calculated charge densities and therefore, the AM1 calculations are presented for the protonated clusters. Various orientations of water molecules in the outer coordination sphere of the lanthanide clusters were minimized and all resulted in similar charge densities. The positions and orientations of the nine water molecules in the first coordination sphere were taken from the crystal structure of [Er(H$_2$O)$_9$][CF$_3$SO$_3$].\textsuperscript{[24]} These water molecules were held fixed with respect to the lanthanide core while the system was minimized. The charge densities were determined using average volumes of 2-4 energy-minimized structures.

3.3 Results and Discussion

In the past doubly and triply charged metal-ion water clusters have been difficult to generate as they undergo charge separation reactions. In order to observe these clusters they must be solvated by a large number of water molecules and there is a minimum number of water molecules ($n_{\text{min}}$) necessary to stabilize the charge. Below this value the doubly or triply charged species are unstable in the gas phase and are not observed. Triply charged lanthanide water clusters can be easily generated using the “cold flooding” conditions described above. A typical spectra contains a series of [H(H$_2$O)$_n$]$^+$ clusters as well as an extensive series of [Ln(H$_2$O)$_n$]$^{3+}$ and the charge reduced species [Ln(OH)(H$_2$O)$_n$]$^{2+}$ (Figure 3.1). For an extensive look at the lanthanide series La$^{3+}$, Pr$^{3+}$, Eu$^{3+}$, Tb$^{3+}$, Ho$^{3+}$, Tm$^{3+}$ and Lu$^{3+}$ were studied as they are mono- or di-isotopic allowing for maximum spectral intensity and a less convoluted spectrum.
Figure 3.1. ESI mass spectrum of an aqueous solution of PrCl$_3$. The spectrum is dominated by [Pr(H$_2$O)$_n$]$^{3+}$ (*), but [Pr(OH)(H$_2$O)$_n$]$^{2+}$ (▪) and [H(H$_2$O)$_n$]$^+$ (•) are also present. Singly, doubly, and triply charge clusters differ in m/z spacing as they are separated by 18, 9, and 6 m/z respectively.

No clusters were observed below $n_{min}$, which depended on the size of the lanthanide ion. Lanthanum required the fewest water molecules, only 15, to stabilize the 3+ charge as this is the largest lanthanide (La$^{3+}$, r = 130 pm) and therefore has the smallest charge density. Moving across the lanthanide series the number of water molecules necessary to stabilize the charge increases as seen in Table 3.1.
Table 3.1. Minimum cluster size needed to stabilize the 3+ charge of the lanthanide ion.

<table>
<thead>
<tr>
<th>Ionic Radii (pm)</th>
<th>Minimum number of water molecules</th>
</tr>
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<tbody>
<tr>
<td>La</td>
<td>130</td>
</tr>
<tr>
<td>Pr</td>
<td>127</td>
</tr>
<tr>
<td>Eu</td>
<td>121</td>
</tr>
<tr>
<td>Tb</td>
<td>118</td>
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<tr>
<td>Ho</td>
<td>115</td>
</tr>
<tr>
<td>Tm</td>
<td>113</td>
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<tr>
<td>Lu</td>
<td>112</td>
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</tbody>
</table>

MS/MS experiments were carried out to investigate the desolvation and fragmentation of these triply charged species. The \([\text{La(H}_2\text{O)}]^{3+}\), \([\text{Pr(H}_2\text{O)}]^{3+}\), \([\text{Eu(H}_2\text{O)}]^{3+}\), \([\text{Tb(H}_2\text{O)}]^{3+}\), \([\text{Ho(H}_2\text{O)}]^{3+}\), \([\text{Tm(H}_2\text{O)}]^{3+}\), and \([\text{Lu(H}_2\text{O)}]^{3+}\) were mass selected, fragmented via collision induced dissociation and EDESI-MS/MS plots were generated. The EDESI-MS/MS of \([\text{Pr(H}_2\text{O)}]^{3+}\) is shown in Figure 3.2. As the collision voltage is increased between 2V and approximately 14 V the sequential loss of water molecules is observed. At a collision voltage of 15 V the droplet is small enough that the charge density within the droplet has made it less stable and charge reduction begins to compete with solvent loss and \([\text{Pr(H}_2\text{O)}]^{3+}\) (shown in black) fragments into \([\text{Pr(OH)}(\text{H}_2\text{O})]^{2+}\) (shown in blue) and \([\text{H(H}_2\text{O)}]^{+}\) (shown in red). The doubly charged species can be distinguished from the triply charged clusters as they are separated by 9 \(m/z\) units instead of 6 \(m/z\) units. In the case of \(\text{Pr}^{3+}\), the smallest triply charged water cluster observed is that with 16 water molecules. Below this size, the cluster is no longer stable.
3.3.1 The ESI Mechanism

The charge separation process is directly observed and it can be used to probe the ESI mechanism. When a water ligand dissociates into a hydroxide ligand and a proton, [Ln(OH)]^{2+} and [H]^+ simultaneously exist in the same water droplet for only an instant. As seen in the EDES1 contour maps, this droplet dissociates into [Ln(OH)(H_2O)_n]^{2+} and primarily [H_3O(H_2O)_3]^+, the “Eigen cation”. It is also interesting to note that a proton
leaving the droplet never leaves with more than four water molecules. This process is depicted below in Figure 3.3.

![Diagram](image)

**Figure 3.3.** Cartoon depiction of the solvent/ion evaporation process. A) the mass-selected water cluster. B) solvent evaporation through CID removes water from the droplet. C) the instant following charge reduction; both $[\text{La(OH)(H}_2\text{O)}_n]^{2+}$ and $[\text{H(H}_2\text{O)}_n]^+$ simultaneously exist within the droplet. D) the $[\text{H}_3\text{O(H}_2\text{O)}_3]^+$ ion evaporates from the surface of the droplet. The dark grey circles are the same size in each case and approximate the size of the inner coordination sphere; the pale grey circles inscribe the outer solvation sphere.

As far as we are aware, this observation represents the first example of the direct observation of a fully solvated ion departing from a charged droplet.

Charge density calculations were carried out on the precursor and the product ions after dissociation in order to determine whether the ESI mechanism observed can be represented by the ion evaporation model or the charge residue model. At each collision voltage a distribution of 3+, 2+, and 1+ ions were observed making it impossible to determine which 3+ ion charge-reduces to produce which 2+ and 1+ ions. However, it is
still interesting to compare the charge densities of potential precursor and product ions observed at the same collision voltage.

<table>
<thead>
<tr>
<th>Precursor ion</th>
<th>Charge density (Å⁻³)</th>
<th>Product ion</th>
<th>Charge density (Å⁻³)</th>
<th>Product ion</th>
<th>Charge density (Å⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[La(H₂O)₁₈]³⁺</td>
<td>0.00897</td>
<td>[La(OH)(H₂O)₁₃]²⁺</td>
<td>0.00768</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Tb(H₂O)₁₈]³⁺</td>
<td>0.00923</td>
<td>[Tb(OH)(H₂O)₁₃]²⁺</td>
<td>0.00789</td>
<td>[H(H₂O)₃]⁺</td>
<td>0.01360</td>
</tr>
<tr>
<td>[Lu(H₂O)₂₀]³⁺</td>
<td>0.00841</td>
<td>[Lu(OH)(H₂O)₁₃]²⁺</td>
<td>0.00695</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Upon charge separation, it is important to note that of the two product ions, the [Ln(OH)(H₂O)ₙ]²⁺ species has significantly more water than necessary to fill the first coordination sphere while the largest protonated water cluster, [H₃O(H₂O)₃]⁺ does not, suggesting the ion evaporation model best describes this process. Further evidences are the charge densities which show that the [H₃O(H₂O)₃]⁺ has a charge density which is 50-100% larger than the charge density of the 2⁺ product ion. This protonated water droplet comes off the surface of the cluster and takes with it 33% of the charge but only 15.8% of the mass (see Table 3.2); this is indicative of the ion evaporation process.

3.3.2 Lanthanide properties that affect charge reduction

Looking more closely across the entire series of lanthanides, it is interesting to compare the physical properties of these lanthanides to their ability to form stable triply
charged water clusters. Here a direct relationship is seen between the charge density of the lanthanide and the size of the smallest \([\text{Ln}(\text{H}_2\text{O})]^{3+}\) species. As the size of the lanthanide ion decreases and its charge density increases, the minimum number of water molecules necessary to stabilize the charge increases (Table 3.1).

Many reports suggest however that this charge reduction reaction occurs due to the ionization energies of the metal ion and the ligands involved. In the case of doubly charged metals, charge reduction (Eq 3.3) is observed when the second ionization potential, IE(\(M^\ddagger\)), of the metal is larger than the first ionization potential of the ligand, in this case, water.\[^{[9, 14, 25, 26]}\]

\[
[M(\text{H}_2\text{O})_n]^{2+} \rightarrow [M(\text{OH})(\text{H}_2\text{O})_{n-a-b}]^+ + [\text{H}(\text{H}_2\text{O})_a]^+ + b\text{H}_2\text{O}
\]  \quad (3.3)

Kebarle et al.\[^{[9]}\] observe a trend among alkaline earth metals that as IE(\(M^\ddagger\)) increases the cluster size at which charge reduction occurs also increases. The same trend was also observed for the first row transition metals with the exception of Fe\(^{2+}\). The following thermodynamic cycle (Eq 3.4) was subsequently used to estimate the hydration energies of the first solvation shell.

\[
\Delta H(3.3) = \Delta H_{r,0}(M^{2+}) - \text{IE}(M^\ddagger) - D(M^\ddagger\cdot\text{OH}) - \Delta H_{r,2,0}(\text{MOH}^\ddagger) + \Delta H(2\text{H}_2\text{O} \rightarrow \text{H}_3\text{O}^\ddagger + \text{OH} + e^-) + E_{\text{cou}}
\]  \quad (3.4)

Here \(\Delta H_{r,0}\) is the energy of dissociation of \(r(\text{H}_2\text{O})\) from \(M^{2+}(\text{H}_2\text{O})_r\), \(D\) is the dissociation bond energy and \(E_{\text{cou}}\) is the energy released upon separation of the product ions. From this equation Kebarle noted that although IE(\(M^\ddagger\)) is not the only factor (the
bond dissociation energy also plays a role) it is important in determining at what hydration number charge reduction will occur. With this same argument, in the case of triply charged metals, charge reduction would occur when the third ionization potential of the metal ion is greater than the first ionization potential of water.

Agreeing that Equation 3.4 is correct, Beyer et al.[19] present the same equation in a more intuitive form. Upon charge separation the metal cation maintains its 2+ charge such that the charged complex is \( \text{M}^{2+}\text{OH}^- \) rather than \( \text{M}^+\text{OH}^- \). The authors suggest that it is therefore more realistic to include the bond dissociation energy \( D'(\text{M}^{2+}\text{OH}^-) \) instead of \( \text{IE(M}^+) \) and \( \text{D(M}^+\text{OH}) \), a state which does not actually occur. \( D'(\text{M}^{2+}\text{OH}^-) \) can be written as:

\[
D'(\text{M}^{2+}\text{OH}^-) = \text{IE(M}^+) + D'(\text{M}^+\text{OH}) + \text{EA(OH)}
\]

Equation 3.4 then becomes:

\[
\Delta H(3.3) = \Delta H_{r,0}(\text{M}^{2+}) - D'(\text{M}^{2+}\text{OH}^-) - \Delta H_{r,-2,0}(\text{MOH}^+) + \Delta H(2\text{H}_2\text{O} \rightarrow \text{H}_3\text{O}^+ + \text{OH}^-) + E_{cou}
\]

Although Eq 3.6 is the same as Eq 3.4, it is a more realistic representation of what is actually observed as it shows a proton transfer reaction instead of an electron transfer process. Beyer and coworkers[19] suggest that the relationship between charge separation and \( \text{IE(M}^+) \) is only indirect and the charge reduction is more directly related to the charge density of the metal ion.[19, 27] They performed DFT calculations on \([\text{M(H}_2\text{O})_2]^{2+}\) species where \( \text{M} = \text{Be, Mg, Ca, Sr, and Ba} \) to investigate the dissociation pathways of these clusters. Both proton transfer and loss of a neutral water molecule were observed. Electron transfer did not occur. Beyer et al. observed a correlation between \( D'(\text{M}^{2+}\text{OH}^-) \)
and ionic radius: as the ionic radius of the doubly charged metal cation increased $D'(M^{2+}-OH^−)$ decreased. They did not observe a correlation with $D(M^+-OH)$, proving their interpretation of Equation 3.4 to be more realistic. The computational results show a salt bridge mechanism facilitates proton transfer and the relative energies of the processes was determined by the ionic radius of the metal and the relationship to $IE(M^+)$ was only indirect.

Other properties have also been suggested in attempt to rationalize the charge separation process. Most doubly charged metal ions have been observed to generate $[M(H_2O)_n]^{2+}$ species, with the exception of lead. Lead(II) has the same ionic radius (133 pm) and therefore charge density as strontium(II) (132 pm) and a lower second ionization potential (1450.5 kJ mol$^{-1}$) than copper(II) (1957.9 kJ mol$^{-1}$), but both $[Sr(H_2O)_n]^{2+}$ [4] and $[Cu(H_2O)_n]^{2+}$ [5, 6] have been observed experimentally. Stace et al. have suggested that the anomalous acidity of not only Pb$^{2+}$ but also Sn$^{2+}$ and Hg$^{2+}$ in the gas phase might explain why these doubly charged water clusters cannot be generated. [20] For many doubly charged metal ions, their pK$_a$ can be related to their charge density, however Pb$^{2+}$, Sn$^{2+}$, and Hg$^{2+}$ do not follow the same trend. They each have a pK$_a$ value lower than expected from their charge density. Stace et al. used ab initio calculations to investigate the energetics and structures of $[M(H_2O)_2]^{2+}$ and $[M(H_2O)_4]^{2+}$ where $M = Sn$, Pb, Hg. When compared to Ca$^{2+}$ the doubly charged species Pb$^{2+}$, Sn$^{2+}$, and Hg$^{2+}$ were observed to be more acidic. For these species, it was observed that the addition of water molecules in the gas phase lowers the energy barrier to hydrolysis. The calculated geometries of clusters containing Pb$^{2+}$, Sn$^{2+}$, and Hg$^{2+}$ also suggest high acidities. Both Pb$^{2+}$ and Sn$^{2+}$ have inert lone pair electrons which affect their coordination geometries. A high electron
density on one side of the metal cation will facilitate proton transfer. In the case of Hg$^{2+}$, its high degree of covalent character allows it to take electron density away from coordinating water molecules again making proton transfer easier.

In a different study Stace et al. studied the effect of solvent on Pb(II) solvent cluster formation.\textsuperscript{[21]} They were unable to generate doubly charged lead clusters with water, methanol, or ethanol. However, as they increased the softness of the base, the solvent, to match the softness of lead (moderate/soft) they found that they were able to generate doubly charged clusters, for example [Pb(propan-1-ol)$_n$]$^{2+}$ and [Pb(butan-1-ol)$_n$]$^{2+}$. Again, this lends credence to the idea that the acidity of the metal might affect its ability to form stable multiply charged hydrated species.

Figure 4 shows the trends in ionization energy, ionic radius, enthalpy of hydration, and pK$_a$ across the lanthanide series.
Figure 3.4. Properties of the Ln$^{3+}$ ions: a) ionic radii; b) third ionization energy; c) pK$_{a}$; d) hydration enthalpy.

As expected, the third ionization energy generally increases across the lanthanide series, though there are significant deviations. The large discontinuities between Eu and Gd and Yb and Lu are due to their differences in exchange energy (removing a third electron from Eu or Yb involves a large decrease in the number of pairs of parallel spins) and the more subtle variations of Pr, Pm, Dy and Er are due to one quarter and three quarter shell effects. The minimum number of water molecules necessary to stabilize the
[Ln(H₂O)ₙ]³⁺ does not follow this trend and a direct relationship between ionization energy and charge separation is not observed.

The ionic radius decreases, i.e. the charge density increases, steadily across the lanthanide series and correlates well with the trend that the number of water molecules necessary to stabilize the 3+ charge increases as you move from La to Lu on the periodic table. A similar trend is noted for the enthalpy of hydration,[28] typically ions with a high charge density have more negative enthalpies of hydration. Moving across the lanthanides, the enthalpy of hydration becomes more and more negative and this too correlates with the trend in increasing $n_{\text{min}}$ from La to Lu.

Although missing the pKₐ data for Ce and Pm, in general, pKₐ decreases across the lanthanide series.[29, 30] There is a slight discontinuity at Gd, where the pKₐ is higher than both its neighbouring lanthanides. Unfortunately the resolution of the experimental results do not allow this to be exploited further to determine if pKₐ has a greater affect on $n_{\text{min}}$ than charge density and enthalpy of hydration. In general a correlation between the minimum number of water molecules necessary to stabilize the 3+ charge on the lanthanide is observed with charge density, the enthalpy of hydration, and pKₐ. Further studies into [Pb(H₂O)ₙ]²⁺ might shed more light onto the relationship between acidity, coordination geometry and water cluster stability.

3.4 Conclusion

It has been demonstrated that highly hydrated M³⁺ species can be generated quite easily in the gas-phase using the same “cold flooding” conditions used to generate singly charged water clusters. The ability to observe these species has led to the investigation of
various phenomena. It has allowed for the direct observation of the separation of two charged species within a charged droplet, best described as an ion evaporation process. Due to the unique physical properties of the lanthanide series the generation of these triply charged water clusters has also shed some light onto the main factors contributing to the charge reduction phenomenon. The evidence suggests that the charge separation process is not directly related to the ionization energy of the ion but rather more closely linked to its charge density.

3.5 References


[22] MOPAC2007, Colorado Springs, CO, USA, **2007**.

[23] Spartan'06, Wavefunction, Inc., Irvine, CA, USA, **2006**.


Chapter 4: Doubly Charged Lead Water Clusters

4.1 Introduction

As already recounted in Chapters 2 and 3, electrospray ionization has been used successfully to generate singly and multiply charged hydrated species in the gas phase. Doubly charged hydrated species including \([\text{Mg(H}_2\text{O)}_n]^{2+}\), \([\text{Mn(H}_2\text{O)}_n]^{2+}\), \([\text{Cu(H}_2\text{O)}_n]^{2+}\), \([\text{Ca(H}_2\text{O)}_n]^{2+}\), \([\text{Zn(H}_2\text{O)}_n]^{2+}\), \([\text{Sr(H}_2\text{O)}_n]^{2+}\) among others, have been successfully generated using both ESI and a “pick up” technique.\(^1\) This latter method involves passing a beam of neutral solvent clusters through a vapour of the metal of interest. The clusters are then ionized using electron impact and the species are analyzed using a mass spectrometer.

Still, the problem of charge reduction occurs for some doubly charged ions. Both electron transfer\(^1,7\) (Eq 4.1) and proton transfer (Eq 4.2) have been observed in the reduction of these 2+ species; however, proton transfer via a salt-bridge mechanism is much more likely as it is energetically favoured upon collision induced dissociation.\(^2\)

\[
\begin{align*}
[\text{M(H}_2\text{O)}_n]^{2+} & \rightarrow [\text{M(H}_2\text{O)}_{n-1}]^+ + [\text{H}_2\text{O}]^+ \quad (4.1) \\
[\text{M(H}_2\text{O)}_n]^{2+} & \rightarrow [\text{M(OH)}(\text{H}_2\text{O})_{n-2}]^+ + [\text{H(H}_2\text{O)}]^+ \quad (4.2)
\end{align*}
\]

Even if hydrated doubly charged clusters can be generated they can still undergo charge reduction at small cluster sizes as there is a minimum number, \(n_{\text{min}}\), of water molecules necessary to stabilize the charge. As discussed in Chapter 3, it is often reported that this charge reduction occurs because the second ionization potential of the
metal is larger than the first ionization potential of the water ligand.\textsuperscript{[8, 9]} However results of the previous chapter coincide with work by Williams and coworkers that suggests that the relationship between $\text{IE}(\text{M}^+)$ and $n_{\text{min}}$ is only indirect and the more dominant influence is the charge density.\textsuperscript{[10, 11]} There are some ions however that do not seem to follow these same trends.

To date, no method has been successful in generating doubly charged, hydrated lead water clusters, $[\text{Pb}(\text{H}_2\text{O})_n]^{2+}$. Instead, the charge-reduced $[\text{Pb(OH)}(\text{H}_2\text{O})_n]^+$ species is typically observed. This outcome is somewhat surprising as Pb(II) has the same ionic radius as Sr(II) and a smaller $\text{IE}(\text{M}^+)$ than Cu(II), and both $[\text{Sr(H}_2\text{O})_n]^{2+}$ and $[\text{Cu(H}_2\text{O})_n]^{2+}$ have been observed in the gas phase. Many other doubly charged, ligated lead clusters have been generated successfully and observed in the mass spectrometer including $[\text{Pb}($\text{d-glucose}$)_n]^{2+}$, \textsuperscript{[12]} $[\text{Pb(DMSO)}_n]^{2+}$, \textsuperscript{[13]} and $[\text{Pb(CH}_3\text{CN})_n]^{2+}$.\textsuperscript{[14]} Discussed in greater detail in Chapter 3, it has been suggested that $[\text{Pb(H}_2\text{O})_n]^{2+}$ species may not be observed due to the anomalous acidity of Pb$^{2+}$ in the gas phase.\textsuperscript{[15]} Although unable to generate $[\text{Pb(H}_2\text{O})_n]^{2+}$ clusters using the “pick-up” technique, Stace and coworkers were able to generate $[\text{Pb(ROH)}_n]^{2+}$ where ROH was propan-1-ol or butan-1-ol.\textsuperscript{[16]} This difference in reactivity is explained using the hard-soft acid-base theory; however, both Pb(II) and Cu(II) are considered borderline soft acids so this explanation does not completely account for why $[\text{Pb(H}_2\text{O})_n]^{2+}$ species have not been observed in the gas phase. Stace and coworkers implicate the coordination of the first solvation shell. Cu(II) coordinates four water molecules in a square planar geometry in the first solvation shell,\textsuperscript{[17]} while their density functional calculations of $[\text{Pb(H}_2\text{O})_4]^{2+}$ showed that only three water molecules were found in the first solvation shell as seen in Figure 4.1 B. The lone pair on the Pb(II)
ion takes up a significant amount of space and forces the three coordinated water molecules to crowd together.[16] This geometry could influence the generation of [Pb(OH)(H₂O)ₙ]⁺ species, though other computational studies do not show this same coordination but rather suggest a coordination number of seven[18] or nine water molecules[19, 20] in the first solvation shell.

![Figure 4.1](image.png)

Figure 4.1. Optimized geometries of Pb(H₂O)₄. Two stable structures were found A) a butterfly geometry and B) a 3+1 geometry.

One report has even gone as far as to suggest that no matter how many water molecules are attached [Pb(H₂O)ₙ]²⁺ will never be stable in the gas phase.[21] More recently the doubly charged lead monohydrate, [Pb(H₂O)]²⁺, has been shown to be stable as it was observed in the gas phase as a product of ligand exchange between [Pb(CH₃CN)]²⁺ and H₂O, or alternatively, [Pb(N₂)ₙ]²⁺ and H₂O upon collisional activation.[14, 22] Below, the first observation of the generation of a series of [Pb(H₂O)ₙ]²⁺ species using ESI-MS is discussed. For a better understanding of the stability of these clusters EDESI-MS/MS studies were carried out on these species as well as [Ca(H₂O)ₙ]²⁺, [Sr(H₂O)ₙ]²⁺, [Ba(H₂O)ₙ]²⁺, and [Cu(H₂O)ₙ]²⁺ for comparison. Energy
minimization calculations were also performed to shed light on the structural influence of
the smallest [Pb(H$_2$O)$_n$]$^{2+}$ cluster observed.

4.2 Experimental

All experiments were run on an unmodified Micromass Q-ToF micro™ mass
spectrometer in positive-ion mode. Aqueous solutions of 5 mM BaCl$_2$, SrCl$_2$, CaCl$_2$,
Cu(NO$_3$)$_2$, and Pb(NO$_3$)$_2$ were injected into the instrument with the capillary voltage set
to 2900 V and the ion energy at 1.0 V. Maximizing the cone voltage (200 V) and setting
the source and desolvation temperatures to 60 °C and 20 °C respectively was necessary
for observing the highly solvated species. As the cluster intensity varied the solvent flow
rate was adjusted between 20 µL min$^{-1}$ and 50 µL min$^{-1}$. In order to optimize water
cluster formation, the cone gas was turned off and the desolvation gas was set to
100 L h$^{-1}$.

EDESI-MS/MS experiments were carried out by performing MS/MS on a
selected peak and increasing the collision voltage in one-volt increments from 2 to 85 V
in the case of Sr$^{2+}$, Cu$^{2+}$, Ca$^{2+}$, and Ba$^{2+}$ and from 2 to 43 V in the case of Pb$^{2+}$. Pb$^{2+}$
clusters were not fragmented further as cluster intensities were low. Spectra were
collected for times ranging from 30 seconds to 2 minutes depending on cluster intensities.
Spectra of Ba$^{2+}$ and Ca$^{2+}$ were collected with unit resolution while slightly higher than
unit resolution was necessary for Sr$^{2+}$, Cu$^{2+}$ and Pb$^{2+}$ species. Automation of the mass
spectrometer software (MassLynx®) to carry out the EDESI experiments (ramping of the
collision voltage) was achieved using the program AutoHotkey (freely available from
Density Functional B3LYP 6-31G* equilibrium geometry calculations were carried out in Spartan\textsuperscript{[23]} using the LANL2DZ pseudopotential. The convergence criterion was lowered to $10^{-5}$ to ensure convergence of all structures. The lowest energy geometries were identified by manual searching of a limited number of possible starting geometries.

4.3 Results and Discussion

Highly hydrated, doubly charged species including Ca\textsuperscript{2+}, Sr\textsuperscript{2+}, Ba\textsuperscript{2+}, Cu\textsuperscript{2+} and Pb\textsuperscript{2+} were easily generated using ESI-MS. Ca\textsuperscript{2+}, Sr\textsuperscript{2+}, Ba\textsuperscript{2+}, and Cu\textsuperscript{2+} water clusters have all been generated previously by others; however, lead monohydrate, [Pb(H\textsubscript{2}O)]\textsuperscript{2+}, is the only hydrated lead species that has been observed in the gas phase. Here, a series of [Pb(H\textsubscript{2}O)\textsubscript{n}]\textsuperscript{2+} hydrated species were readily generated by injecting an aqueous solution of Pb(NO\textsubscript{3})\textsubscript{2} into the mass spectrometer using the conditions discussed above. The mass spectrum (Figure 4.2) is dominated by two large peaks corresponding to [Pb(OH)(H\textsubscript{2}O)\textsubscript{2}]\textsuperscript{+} and [Pb(NO\textsubscript{3})(H\textsubscript{2}O)\textsubscript{2}]\textsuperscript{+}, but there is also an extensive series of the never-before-observed [Pb(H\textsubscript{2}O)\textsubscript{n}]\textsuperscript{2+} clusters where \(n\) extends as low as eight water molecules. A series of [Pb(NO\textsubscript{3})(H\textsubscript{2}O)\textsubscript{n}]\textsuperscript{+} and [Pb(OH)(H\textsubscript{2}O)\textsubscript{n}]\textsuperscript{+} clusters are also observed in the mass spectrum. The reduced species Pb\textsuperscript{+} was also observed, however the series [Pb(H\textsubscript{2}O)\textsubscript{n}]\textsuperscript{+} was not seen. It is interesting that the two largest peaks in the mass spectrum of Pb(II) are three-coordinate species. This would suggest that this is a very stable structure and supports the calculations mentioned previously for [Pb(H\textsubscript{2}O)\textsubscript{4}]\textsuperscript{2+} that show three water molecules in the first hydration sphere (Figure 4.1).
Mass spectra of CaCl$_2$, SrCl$_2$, BaCl$_2$ and Cu(NO$_3$)$_2$ also show series of [M(H$_2$O)$_n$]$^{2+}$. Although not observed in the spectra of Sr and Ca, it is likely that the monohydrates and the bare ions of each of these species are generated but their masses are outside the detection range of the instrument. The bare ion and slightly solvated species of Cu$^{2+}$ are not observed as charge reduction does occur at low hydration numbers.

![Figure 4.2. ESI-MS of Pb(NO$_3$)$_2$ solution in water. Three discrete series of ions are observed (see inset).](image)

EDESI-MS/MS experiments were carried out on each doubly charged species. EDESI-MS/MS spectra not shown here can be found in Appendix C. For Ca$^{2+}$, Sr$^{2+}$ and Ba$^{2+}$ the [M(H$_2$O)$_{30}$]$^{2+}$ clusters were mass selected and fragmented. No charge reduction occurred. [Ba(H$_2$O)$_{30}$]$^{2+}$ was completely fragmented down to the bare ion. Again, fully
fragmented species of Sr$^{2+}$ and Ca$^{2+}$ could not be observed due to the detection range of the instrument. The smallest observable clusters for these species were $[\text{Sr(H}_2\text{O)}]^{2+}$ (Figure 4.3) and $[\text{Ca(H}_2\text{O)}]^{2+}$ (Appendix C). Because no charge-reduced species were observed (they would be observed at larger mass-to-charge values within the range of the instrument) it can be speculated that the monohydrated species of Sr$^{2+}$ and Ca$^{2+}$ are stable.

![Figure 4.3. EDESI-MS/MS of $[\text{Sr(H}_2\text{O)}]^{30}$]. Fragmentation energy increases vertically in the contour plot. The top spectrum is a summation of all 84 (collision voltage = 2-85) spectra used to generate the contour plot.

Larger clusters of copper and lead, $[\text{Cu(H}_2\text{O)}]^{49}$ and $[\text{Pb(H}_2\text{O)}]^{48}$, were mass-selected and fragmented to ensure the entire charge reduction process was observed.
Upon collisional activation of \([\text{Cu(H}_2\text{O)}_{49}]^{2+}\), the sequential loss of water is observed at large hydration numbers, but charge reduction does occur as \(n\) decreases (see Figure 4.4). The EDESI-MS/MS contour plot shows that the smallest doubly charged copper species observed is \([\text{Cu(H}_2\text{O)}_{4}]^{2+}\). Previous reports show the existence of smaller hydrated \(\text{Cu}^{2+}\) clusters. Stace and coworkers performed fragmentation experiments on \([\text{Cu(H}_2\text{O)}_{4}]^{2+}\) and observed the species with \(n = 3\) but not clusters with \(n = 1\) or 2.\(^{24}\) The same experiment by Stone et al. revealed the existence of stable \([\text{Cu(H}_2\text{O)}_{n}]^{2+}\) species with \(n\) as low as one.\(^{25}\) Computational work by Nahas et al. also suggests that this monohydrated species should be stable in the gas phase.\(^{26}\) It is possible that the smallest cluster observed in the present data, \([\text{Cu(H}_2\text{O)}_{4}]^{2+}\), is due to a particular stability of this cluster. Multiple mass spectrometry results show a particularly intense peak for the doubly charged copper water cluster with eight water molecules indicating a more stable structure. The \([\text{Cu(H}_2\text{O)}_{4}]^{2+}\) cluster, although not as intense as the \([\text{Cu(H}_2\text{O)}_{8}]^{2+}\) cluster, did appear to be more stable than its neighbouring clusters.\(^{5, 27}\) DFT calculations performed by Bérces et al.\(^{17}\) results in the global minimum of the \([\text{Cu(H}_2\text{O)}_{8}]^{2+}\) cluster having a rather surprising structure: the first four water molecules in a square planar geometry and the four secondary water molecules hydrogen bonded to two adjacent water molecules in the primary shell (Figure 4.5). This structure would explain the particular stability of the \([\text{Cu(H}_2\text{O)}_{8}]^{2+}\) and \([\text{Cu(H}_2\text{O)}_{4}]^{2+}\) clusters observed in Stace’s experiments and might also explain the smallest doubly charged copper cluster observed in these CID experiments. This lends credence to the idea that structural geometry might be important in determining the minimum number of water molecules necessary to stabilize a multiply charged species. Although clusters with less than four water molecules have been
observed to be stable, the data presented shows that upon fragmentation of large clusters the smallest doubly charged species we observed is [Cu(H₂O)₄]²⁺, indicating it is more stable than the less hydrated species. Also interesting to note is the most intense peak in the summation plot (Figure 4.4 top) is the [Cu(H₂O)₈]²⁺ cluster, again corresponding to the stability of this slightly larger cluster.

Figure 4.4. EDESI-MS/MS of [Cu(H₂O)₄]²⁺. Three different series can be seen: [Cu(H₂O)ₙ]²⁺ (black), [Cu(OH)(H₂O)ₙ]⁺ (blue), and [H(H₂O)ₙ]⁺ (red).
Figure 4.6 shows the EDESI-MS/MS summation and contour plots of [Pb(H$_2$O)$_{48}$]$^{2+}$. At low collision voltages the major pathway by which the cluster shrinks is through the sequential loss of neutral water molecules. As the collision voltage increases charge reduction begins to compete with the loss of a neutral water cluster and [Pb(OH)(H$_2$O)$_n$]$^+$ clusters are observed at a collision voltage of 29 V. At very low collision voltages a peak corresponding to [Pb(OH)(H$_2$O)$_2$]$^+$ is observed. This would suggest a third pathway for fragmentation: the loss of a small, hydrated lead species from a large, neutral droplet indicating for at least some clusters the lead ion is not centred in the droplet. It is interesting also that a lead species coordinated to three water molecules leaves the droplet. This would again support Stace and coworkers’ computational results that only three water molecules directly coordinate to the Pb(II) metal centre (Figure 4.1 B).
Figure 4.6. EDESI-MS/MS of \([\text{Pb}(\text{H}_2\text{O})_{48}]^{2+}\). Three different series can be seen: \([\text{Pb}(\text{H}_2\text{O})_{n}]^{2+}\) (black), \([\text{Pb}(\text{OH})(\text{H}_2\text{O})_{n}]^{+}\) (blue), and \([\text{H}(\text{H}_2\text{O})_{n}]^{+}\) (red).

The smallest doubly charged lead cluster observed is that with eight water molecules; below this cluster size, the droplet is unstable and charge reduction is the only pathway for further fragmentation. A small signal for \([\text{H}(\text{H}_2\text{O})_4]^{+}\) is also observed as a product of this charge reduction reaction. These protonated water clusters had very low intensities and their presence is more evident in the summation plot presented above the contour map. Unlike the EDESI summation plot of copper, the summation plot for lead has no distinct features indicating any structural preferences.

Comparing these results to those of \(\text{Cu}^{2+}\), this could possibly suggest that \(\text{Pb}^{2+}\) has a hydration coordination number of eight. The coordination number of hydrated \(\text{Pb}^{2+}\) is
thus far ill defined. Molecular dynamics studies by Gourlaouen et al.\textsuperscript{[18]} on the hydration of Pb\textsuperscript{2+} suggest that lead is heptacoordinated. MM and HF/MM calculations on the other hand suggest that Pb\textsuperscript{2+} has a coordination number of 9.\textsuperscript{[19, 20]} Bock et al.\textsuperscript{[28]} used \textit{ab initio} calculations with the LANL2DZ basis set to model Pb(II) species with 2 to 10 ligands. They found that Pb(II) complexes had a preferred coordination of 4 or 6. The structure obtained for [Pb(H\textsubscript{2}O\textsubscript{4})\textsuperscript{2+}] was hemidirected with each water molecule directly coordinating to the metal centre. Further analysis of the structure of [Pb(H\textsubscript{2}O\textsubscript{4})\textsuperscript{2+}] by Stace and coworkers\textsuperscript{[16]} using the LANL2DZ basis set showed three possible structures for this species: a butterfly structure, a tetrahedral type structure, and a 3+1 structure where three water molecules were directly coordinating to Pb(II) and one water molecule was found farther away coordinating to two other water molecules in the first coordination shell. The 3+1 structure was found to have the lowest energy. Note that Bock et al. did not investigate this geometry and therefore no comparison can be made.

In order to explain the minimum cluster size observed in the EDESI spectrum, DFT calculations were carried out on [Pb(H\textsubscript{2}O\textsubscript{8})\textsuperscript{2+}]. To ensure the validity of the method and basis set used, the three [Pb(H\textsubscript{2}O\textsubscript{4})\textsuperscript{2+}] structures calculated by Stace et al.\textsuperscript{[16]} were calculated here using DFT B3LYP with the LANL2DZ basis set for comparison. Similar structures and relative energies were obtained for the butterfly and 3+1 structures (Figure 4.1) and again, although there were only small differences in energy, the 3+1 structure was found to be lowest in energy.

A variety of starting geometries were optimized using this basis set to find the global minimum structure of [Pb(H\textsubscript{2}O\textsubscript{8})\textsuperscript{2+}]. The resulting minimized structures all had very similar energies. All structures were slight variations on those shown in Figure 4.7
and varied in energy by 10 kcal/mol. Structure C had the lowest energy and clearly depicts why [Pb(H$_2$O)$_8$]$^{2+}$ is a particularly stable structure. It shows four water molecules directed towards the metal centre and the remaining four water molecules each forming two hydrogen bond contacts with the water molecules coordinating to the Pb(II) nicely depicting a structure with full coordination. The other two structures D and E show only three water molecules directly coordinating to the Pb(II). The remaining water molecules form either one or two hydrogen bond contacts with those in the inner shell. Interestingly, structure E even has one water molecule in what appears to be the beginning of a third shell where it is hydrogen bonded to one water molecule that is not directly coordinated to the metal and one that is. In each structure all hydrogen bonding sites of the water molecules in the inner shell are bound and the structures are hemidirected due to the lone pair on Pb(II).
Figure 4.7. Minimized geometries of [Pb(H₂O)₆]^{2+} with relative energies of C 0 kcal/mol D +7 kcal/mol and E +10 kcal/mol. Solid grey lines represent coordination to Pb(II) while dotted blue lines represent hydrogen bonds.
It is not surprising that there are a variety of \([\text{Pb}(\text{H}_2\text{O})_8]^{2+}\) structures with similar energies as previous calculations have shown hydrated Pb(II) to have a flexible coordination shell that can easily rearrange.\textsuperscript{118, 19} Taken together, structure A and the results of the EDESI-MS/MS experiment suggest that eight water molecules are enough to stabilize Pb\(^{2+}\) due to the distribution of charge to a first coordination shell of 3-4 water molecules and an additional 4-5 hydrogen bonded water molecules in a second coordination shell.

### 4.4 Conclusions

For the first time, an extensive series of \([\text{Pb}(\text{H}_2\text{O})_n]^{2+}\) clusters have been generated in the gas phase. To explain the previous difficulty in generating these species CID dissociation experiments were compared with other 2+ species. Charge density and ionization energy were not enough to explain this difficulty since Sr(II) has the same charge density as Pb(II) and hydrated species of Sr(II) are easily generated and do not undergo charge reduction, also Cu(II), which has a higher second ionization energy, formed doubly charged hydrated species requiring fewer stabilizing water molecules. In the data presented eight water molecules are necessary to stabilize Pb\(^{2+}\), calculations suggest that this hydrated ion has a symmetric coordination shell reminiscent of the \([\text{Cu}(\text{H}_2\text{O})_8]^{2+}\) ion, but with a stereochemically active lone pair distorting the structure from square planar to square pyramidal. The four water molecules in the second coordination shell all form two hydrogen bonds to the water molecules directly bound to the lead(II) ion.
4.5 References


Chapter 5: Guanidinium Water Clusters

5.1 Introduction

The post-translational methylation of arginine is important in mediating various biochemical pathways. Methylation of arginine is controlled by protein arginine methyltransferases (PRMTs). Type I PRMTs produce asymmetric dimethylarginine (aDMA) while type II PRMTs produce symmetric dimethylarginine (sDMA) (Figure 5.1b and 5.1c).\(^1\) Monomethylarginine is produced as an intermediate in the synthesis of each form of dimethylarginine, but no selective monomethylase has been identified.\(^2\) Arginines are often found at protein-RNA interfaces, and methylation of arginine can disrupt protein-RNA interactions through steric hindrance or by interrupting hydrogen bonding between the arginine and RNA.\(^3\) Methylation also causes arginine to be more hydrophobic and this aspect of the alteration has been proposed to promote binding with RNA.\(^3\)

![Figure 5.1. Biologically relevant arginine side chains: a) arginine; b) asymmetric dimethylarginine (aDMA); c) symmetric dimethylarginine (sDMA).](image-url)
Arginine methylation is also known to affect protein-protein interactions, and a growing list of reports on arginine demethylation\textsuperscript{[4-6]} have shown that this process is reversible and is used for signalling in a variety of ways.\textsuperscript{[7]} The T cell receptor, cytokine receptor, and the nerve growth factor receptor all use arginine methylation for signal transduction.\textsuperscript{[1]} Transcription is also affected by arginine methylation; methyltransferases have been shown to methylate histones and coactivators involved in transcription.\textsuperscript{[1]}

Dimethylated arginine is also important in DNA repair. It has been suggest that MRE11, a protein in the MRE11/RAD50/NBS1 complex which is involved in DNA repair, has an asymmetrically dimethylated arginine necessary for MRE11’s role in regulating DNA damage response.\textsuperscript{[8]}

We are interested in the binding changes brought about by arginine methylation. Symmetric and asymmetric dimethylated arginines differ in the way they interact with other proteins and complexes. For example antibodies SYM10 and SYM11 are specific for sDMA but not aDMA\textsuperscript{[9]} due to shape selectivity and the Tudor domains of the spinal muscular atrophy gene product SMN bind sDMA.\textsuperscript{[10]} In contrast to this differential binding, it has also been found that arginine dimethylation increases the stability of arginine-π interactions of sDMA and aDMA equally for both isomers.\textsuperscript{[11]} Upon folding of β-hairpin model peptides, the incorporation of either aDMA or sDMA led to an increased interaction between Arg-Trp residues. This increased cation-π interaction was found to be entropically driven due to hydration and was independent of the shape of the dimethylarginine.

This study of β-hairpin folding suggests that the increased strength in the cation-π interaction is due to the hydrophobic effect and may trigger various protein-protein
interactions. Methylation of arginine’s guanidinium side chain reduces its hydrogen bonding ability, decreases its charge density, and can force structural changes of the guanidinium core. Each of these factors may interfere with the side chain’s hydration, and therefore its recognition properties. Guanidinium is a common denaturant and Mountain and Thirumalai as well as Mason et al. suggest that this property is due to its weak hydration, its ability to interact with hydrophobic species, and its ability to form hydrogen bonds preferentially to proteins instead of water.\cite{12,13}

EDES\-MS/MS\cite{14-17} is used to investigate the effect of arginine methylation on hydration and hydrogen bond strength. This technique involves mass selecting a large ion-centred water cluster and sequentially stripping off water molecules via collision induced dissociation.\cite{18,19} It is the charged guanidino functional group at the terminus of the arginine side chain that is important in protein-protein and cation-\textpi interactions. For this reason we use various methylated guanidinium ions as a model of arginine and its post-translationally methylated species (Figure 5.1). The hydration of arginine in the gas-phase has been previously reported,\cite{20-24} but to the best of our knowledge this is the first report on the effect of arginine methylation on hydration.
Figure 5.2. The guanidinium ions studied

5.2 Experimental

\(N,N,N'-\text{Trimethylguanidine} \ (N,N,N'-\text{Me}_3\text{Guan}^+)\) was synthesized from \(S,N\)-dimethylithiouronium iodide (Sigma-Aldrich) and aqueous dimethylamine according to a literature method.\(^{[25]}\) Similarly, \(N,N'-\text{dimethylguanidine} \ (N,N'-\text{Me}_2\text{Guan}^+)\) was synthesized using aqueous methylamine. Guanidine (Guan\(^+\)) hydrochloride, methylguanidine (MeGuan\(^+\)) hydrochloride, \(N,N\text{-dimethylguanidine} \ (N,N'\text{-Me}_2\text{Guan}^+)\) sulphate, and \(N,N,N',N'\text{-tetramethylguanidine} \ (N,N,N',N'\text{-Me}_4\text{Guan}^+)\) were purchased from Sigma-Aldrich. Hexamethylguanidine (Me\(_6\)Guan\(^+\)) chloride was purchased from Acros Organics and \(N,N',N''\text{-trimethylguanidine} \ (N,N',N''\text{-Me}_3\text{Guan}^+)\) was purchased from the Florida Center for Heterocyclic Compounds.

All experiments were run on a Micromass Q-Tof micro\(^{TM}\) mass spectrometer in positive ion mode with a capillary voltage of 2900 V, and an ion energy of 1.0 V. Methylated guanidinium solutions ranging in concentration from 5 mM to 10 mM were injected into the mass spectrometer with the cone voltage optimized at 200 V and the source and desolvation temperatures set to 60 °C and 20 °C respectively. Solvent flow rates ranged between 50 and 100 μL min\(^{-1}\) as cluster intensities varied. In order to optimize the formation of methylated guanidinium clusters, the cone gas was turned off and the desolvation gas flow rate set to 100 L/h.

EDESI experiments were carried out by performing MS/MS on the \([\text{Me}_n\text{Guan}(\text{H}_2\text{O})_{21}]^+\) peak and increasing the collision voltage from 2 V to 50 V in one-volt increments. The \([\text{M}(\text{H}_2\text{O})_{21}]^+\) cluster of one species, \(N\text{-methylguanidinium} \ ([\text{MeGuan}]^+)\), overlaps with the signals arising from the protonated water cluster.
[H(H$_2$O)$_{26}$]$^+$, and therefore [MeGuan]$^+$ was run in D$_2$O and the [MeGuan(D$_2$O)$_{21}$]$^+$ cluster was mass selected for fragmentation. No significant differences in the resulting EDESI spectra were observed to arise from this isotope substitution. Spectra were collected for 30 s or 1 min (depending on cluster intensities) at each collision energy. For [Guan]$^+$, [N,N,N',N':Me$_4$Guan]$^+$, [N,N'-Me$_2$Guan]$^+$, [N,N'-Me$_3$Guan]$^+$, [N,N',N':Me$_3$Guan]$^+$, [N,N',N':Me$_3$Guan]$^+$, and [Me$_6$Guan]$^+$ solutions, the data was collected with unit resolution while slightly higher than unit resolution was necessary for [MeGuan]$^+$ experiments to ensure only the peak of interest was mass selected. Automation of the mass spectrometer software (MassLynx®) to carry out EDESI experiments (ramping of the collision voltage) was achieved using the program AutoHotkey (freely available from www.http://www.autohotkey.com). EDESI summation spectra were generated by summing the intensities of all 49 spectra (from collision voltages 2 V to 50 V). To eliminate noise, EDESI contour plots were generated such that contours lines appear only when the peak intensity had 4 % of the intensity of the largest peak in a given spectrum.

Hartree Fock 6-31+G*, Density Functional B3LYP 6-31G**, B3LYP 6-311+G**, and B3LYP 6-311++G** and Møller Plesset 6-311++G** equilibrium geometry calculations of each guanidinium ion, each guanidinium ion with one water molecule and a lone water molecule were carried out.$^{[26]}$ The water affinity for each guanidinium ion was determined by subtracting the sum of the energies of the individual water molecule and the guanidinium ion from the energy of the complex. Geometries were identified as global minima by manual searching of the limited number of different possible starting geometries.
5.3 Results and Discussion

The study of gas-phase water clusters of biologically relevant molecules provide insight into the role of hydration in biomolecular behaviours. Water clusters of arginine have been generated using IR-laser ablation techniques and nanoelectrospray ionization. These studies primarily looked at the hydration of these ions. Here highly solvated guanidinium water clusters were easily generated by modifying standard ESI-MS source conditions and instead, using “cold flooding” conditions (high flow rates and ambient temperatures). Other highly solvated species have been observed under these conditions including H$_3$O$^+$, Ln$^{3+}$, various substituted anilines, Cu$^{2+}$, and Mg$^{2+}$.

Similar to work presented in the previous chapters in which water molecules were sequentially removed from various ion-centred water clusters, the same EDESI-MS/MS technique was used to mass select a large methylated guanidinium water cluster and sequentially removed water molecules to investigate the hydration of theses large nanodroplets as well as hydrogen bonding within the first hydration sphere. The [Me$_n$Guan(H$_2$O)$_{21}$]$^+$ cluster of each methylated guanidinium ion (Figure 5.3) was selected for all EDESI-MS/MS experiments because these clusters were sufficiently large to observe the sequential loss of water outside of the first hydration sphere.
Figure 5.3. EDESI of a) [Guan(H₂O)ₙ⁺]⁺ and b) [Me₆-Guan(H₂O)₂₁⁺]. The bottom of each figure shows the EDESI contour map with fragmentation energy increasing vertically and ion intensity in the third dimension (out of page). At the top of each data set is a 2-D summation plot generated by adding all 49 spectra (collision voltages from 2 to 50 V) used to generate the contour map.

The numbers on the plot represent the number of water molecules in that cluster.

There are two possible pathways for the fragmentation upon CID: the loss of a water molecule (Eq 5.1) or the loss of the guanidinium ion (Eq 5.2). The loss of a water molecule is the most likely fragmentation pathway. As seen in Figures 5.3 and 5.4, at low collision voltages little or no bare ion is observed.

\[
\begin{align*}
[Guan(H₂O)_n]⁺ & \rightarrow [Guan(H₂O)_{n-1}]⁺ + H₂O \quad (5.1) \\
[Guan(H₂O)_d]⁺ & \rightarrow [Guan]⁺ + (H₂O)_n \quad (5.2)
\end{align*}
\]

As the methylation increases the probability of the ion being found on the surface of the water cluster increases because the strength of hydration decreases. Figures 5.4c
and 5.4d show the bare ion of \([N,N',N''-\text{Me}_3\text{Guan}]^+\) and \([N,N,N'\text{-Me}_3\text{Guan}]^+\) to be more prevalent than the less methylated species (Figures 5.3a, 5.4a, and 5.4b). For these species, fragmentation via Eq 5.2 appears to be especially significant. This however is somewhat misleading due to the nature of the contour plot. When plotted such that only species with 10% of the intensity of the largest peak are shown the bare ions of \([N,N',N''-\text{Me}_3\text{Guan}]^+\) and \([N,N,N'\text{-Me}_3\text{Guan}]^+\) are not observed at low collision voltages, again indicating that Eq 5.1 best describes the fragmentation pathway.

Although all the methylated guanidinium species studied are not analogous to biologically observed methylated arginine species, the variety of species studied extensively looks at the effect of methylation on hydration. Figure 5.3 shows the EDESIMS/MS plots of the most extreme species \([\text{Guan}(\text{H}_2\text{O})_n]^+\) and \([\text{Me}_6\text{Guan}(\text{H}_2\text{O})_n]^+\). The similar slopes of fragmentation pattern of each species at high hydration numbers (beyond the first hydration sphere) indicate that water molecules are lost from the droplet at approximately the same rate. This is expected for the step-wise dehydration of these species beyond the first hydration shell since each water molecule is only hydrogen bonded to other water molecules. At high collision voltages and low hydration numbers, a significant difference is noted between the hydration of these two species. The contour map of \([\text{Guan}(\text{H}_2\text{O})_n]^+\) (Figure 5.3a) shows that dehydration becomes much more difficult at lower hydration numbers as this ion holds onto the last few water molecules very strongly. In the case of \([\text{Me}_6\text{Guan}(\text{H}_2\text{O})_n]^+\) (Figure 5.3b) it is relatively easy to remove water molecules even at low hydration numbers.

Summation plots are presented above the contour maps in Figures 5.3 and 5.4. The plots are a summation of all 49 spectra (collision voltage = 2-50 V) used to generate
the contour maps. The most intense peaks represent species that are particularly stable. These species persist for a larger range of collision voltages compared to species with lower intensities. Comparing these summation plots, differences in hydration can be observed between the methylated species. In Figure 5.3, again comparing the extreme cases, the bare ion peak in the EDESı-MS/MS summation plot of \([\text{Guan}(\text{H}_2\text{O})_{21}]^+\) has a low intensity indicating that \([\text{Guan}]^+\) interacts strongly with the water molecules within the first hydration sphere. Interestingly, the most intense peak in the spectrum is \([\text{Guan}(\text{H}_2\text{O})_3]^+\) suggesting this is the most stable structure. The summation plot of \([\text{Me}_6\text{Guan}(\text{H}_2\text{O})_{21}]^+\) (Figure 5.3b top) shows the bare ion peak to be the dominant peak indicating that water molecules are easily removed from the species. This result is as expected, because \([\text{Me}_6\text{Guan}]^+\) does not have significant hydrogen bonding ability.
Figure 5.4. EDESI-MS/MS spectra (from collision voltages 2 to 50 V) for a) [Guan(D$_2$O)$_{21}$]$^+$, b) [N,N$'$-Me$_2$Guan(H$_2$O)$_{21}$]$^+$, c) [N,N$'$,$N''$-Me$_3$Guan(H$_2$O)$_{21}$]$^+$, and d) [N,N$'$,$N''$-Me$_3$Guan(H$_2$O)$_{21}$]$^+$
Figure 5.4 shows the EDESI summation plots of the rest of the guanidinium ions studied. It is clear that across the whole series, with increasing methylation comes increasing representation of the bare $M^+$ ion in the summed EDESI data. The $[\text{MeGuan}]^+$ and both $[\text{Me}_3\text{Guan}]^+$ ions ($[N,N',N''-\text{Me}_3\text{Guan}]^+$ and $[N,N,N'-\text{Me}_3\text{Guan}]^+$) are most relevant as they model the unmethylated arginine and the dimethylated arginine side chains sDMA and aDMA respectively. The summation plot of the arginine model is similar to that of the unmethylated guanidinium. The bare ion peak has a fairly low intensity compared to the intensity of the other hydrated species. The two aDMA and sDMA (Figures 5.4c and 5.4d) models however look similar to the $[\text{Me}_6\text{Guan}]^+$ (Figure 5.3b) species which was found to be weakly hydrated. These species all show very intense peaks for the bare ion. In fact, not only is the bare ion the base peak in the summation plots, it has at least twice the intensity of all the other species. The data suggests that the presence of three or more methyl groups significantly weakens the hydration of a guanidinium ion. This in turn suggests that dimethylation of arginine dramatically decreases the strength of hydrogen bonding to water.

The more intense peaks in the summation plots indicate a more stable structure. Hydrogen bonding to the guanidinium species was therefore investigated using HF, DFT, and MP2 calculations. Figure 5.5 shows the global minimum structures of each guanidinium species interacting with one water molecule. Note that a variety of starting geometries were optimized to find these global minima. Calculations at each level of theory show that as methylation increases the affinity of each guanidinium ion for a single water molecule decreases – not surprising, as methylation increases the charge density of the ion decreases therefore reducing its ability to hydrogen bond.
Figure 5.5. Optimized hydrogen bonding geometries of each methylated guanidinium using Hartree Fock 6-31+G*. Hydrogen bonds are represented by the dotted lines. Energies are given in Table 5.1.

As methylation increases hydrogen bonding ability decreases due to decreased charge density and the loss of hydrogen bond donor sites. Minimized structures in Figure 5.5 suggest that the structure of the guanidinium ion may also play a role in its ability to hydrogen bond. Soetens et al. performed calculations on the binding of a single water molecule to guanidinium. They found that the most stable structure was that with the water molecule bound in the “pocket” between the two NH groups so that the water molecules was able to make two hydrogen bond contacts. Our calculations show similar results for the less methylated species. [Guan]^+, [MeGuan]^+, and the two [Me₂Guan]^+ species have three, two and one pockets respectively between adjacent NH groups allowing room for a single water molecule to form two hydrogen bonds (Figures 5.5a-d). The more methylated species, [Me₃Guan]^+, [Me₄Guan]^+, and [Me₆Guan]^+
(Figures 5.5e-h) do not have this open pocket available for hydrogen bonding, instead the water molecule is only able to form a single hydrogen-bond contact.

Table 5.1. Calculated water affinities of guanidinium ions

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<td>DFT B3LYP 6-311++G**</td>
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<td>-7.3</td>
</tr>
</tbody>
</table>

* did not converge.

As mentioned previously, the summation plots of [Guan]⁺, [MeGuan]⁺, and both [Me₂Guan]⁺ isomers (see Appendix D) have particularly intense peaks for the species with three, two, and one water molecules respectively, indicating they hold onto these remaining water molecules very strongly. Interestingly this corresponds to the number of available pockets each of these species has. The species with more than three methyl groups do not have these pockets and their summation plots show the dominant species to be that of the bare ion.

Quantitative information on binding energies can be determined using threshold CID experiments. Previous studies have investigated the binding energies of solvated ions, however this is experimentally quite complex. From our relatively simple experiments we have obtained purely qualitative information to gain insight into the relative energies required to dehydrate the various methylated guanidinium species. Figure 5.6 shows a plot of the appearance potentials of the completely desolvated ion; i.e.
the intensity of the bare ion peak at a particular collision voltage. As methylation increases, the bare ion first appears at successively lower collision energies. We also see the separation of the data into five groups. The groups can be identified by the number of pockets available in the guanidinium species. The group that appears at the highest collision voltages is that of [Guan]$^+$ corresponding to three pockets, the group with [MeGuan]$^+$ has two pockets, the group with both [Me$_2$Guan]$^+$ isomers corresponds to one pocket, the group with overlapping [Me$_3$Guan]$^+$ (both isomers) and [Me$_4$Guan]$^+$ plots corresponds to zero pockets, and finally [Me$_6$Guan]$^+$ has its own group corresponding to no pockets and no available NH sites for hydrogen bonding.

![Figure 5.6](image)

**Figure 5.6.** Plot of appearance potential (collision energy corrected for center-of-mass) for the completely desolvated ions versus the intensity of these ions taken from the EDESI-MS/MS experiments. In each case the precursor ion for the EDESI-MS/MS experiments was the [Me$_n$Guan(H$_2$O)$_{21}$]$^+$ cluster. To account for the differences in intensity between each experiment, the ion intensity is plotted as a percentage of the total ion current for all points.
The EDESI-MS/MS data, calculations and the appearance potentials all show the expected result that methylating arginine reduces its hydration and hydrogen bonding ability. We see the same result for both sDMA and aDMA similar to previous binding studies,\textsuperscript{[11]} indicating that they behave identically when shape recognition is not a factor. Unexpected however, is that the data suggest that arginine methylation has the structural ability to modulate hydration. In biological systems arginine undergoes post-translational methylation to generate sDMA and aDMA only; no other more highly methylated species are observed even though the chemistry to generate these species could be possible as is the case for other amino acids. Lysine, for example, can be fully methylated to produce trimethyllysine.\textsuperscript{[43, 44]} sDMA and aDMA seem to be sufficient to reduce arginine hydration by removing the pocket like structures. Because further methylated arginine species do not appear to significantly improve this affect it begs the question: “Is dimethylation special? Has dimethylated arginine evolved to be the only relevant post-translational methylation because it is enough to reduce arginine’s ability to hydrogen bond?”.

### 5.4 Conclusion

Highly hydrated methylated guanidinium ions were easily generated in the gas phase using ESI-MS and fragmented through CID. The data presented provides insight into the hydration of these ions and their analogous arginine side chains. Increasing methylation leads to a decrease in hydrogen bonding ability through decreased charge density but also through a structural capacity of removing available “binding pockets”. It was determined that the only two naturally occurring methylated modifications of
arginine, sDMA and aDMA were sufficient in reducing arginine’s ability to hydrogen bond.

5.5 References


[26] Spartan'06, Wavefunction, Inc., Irvine, CA, USA, **2006**.


[38] The difference in height between the contours is simply due to the manner in which the plots are generated. Only data which has 4% of the intensity of the largest peak is displayed and because the intensity of the Me$_6$Guan$^+$ peak is so large relative to the other peaks the contour lines at other hydration numbers appear to extend over smaller collision voltage ranges.


Appendix A

Figure A 1. EDESI-MS/MS plot of a) [H(H$_2$O)$_{26}$]$^+$ b) [H(H$_2$O)$_{31}$]$^+$ c) [H(H$_2$O)$_{41}$]$^+$ d) [H(H$_2$O)$_{46}$]$^+$. The fragmentation energy increases vertically on the contour plot. The top spectrum is a summation of all spectra used to generate the contour plot.
Figure A 2. EDESI-MS/MS plot of e) [H(H₂O)₂₁]⁺ f) [H(H₂O)₂₆]⁺ g) [H(H₂O)₆₁]⁺ h) [H(H₂O)₇₁]⁺. The fragmentation energy increases vertically on the contour plot. The top spectrum is a summation of all spectra used to generate the contour plot.
Figure A 3. EDESI-MS/MS plot of i) $[\text{H}(_2\text{O})_{76}]^+$. The fragmentation energy increases vertically on the contour plot. The top spectrum is a summation of all spectra used to generate the contour plot.
Appendix B

Figure B 1. ESI-MS LaCl₃ solution in water. Three discrete series of ions are observed

Figure B 2. EDESI-MS/MS of [La(H₂O)₄₈]³⁺. Four different series can be seen: [La(H₂O)₆]³⁺ (black), [La(OH)(H₂O)₆]²⁺ (blue), [La(OH)₂(H₂O)₆]⁺ (green) and [H(H₂O)₆]⁺ (red).
Figure B 3. ESI-MS EuCl$_3$ solution in water. Two discrete series of ions are observed.

Figure B 4. EDESI-MS/MS of [Eu(H$_2$O)$_{49}$]$^{3+}$. Four different series can be seen: [Eu(H$_2$O)$_n$]$^{3+}$ (black), [Eu(OH)(H$_2$O)$_n$]$^{2+}$ (blue), [Eu(OH)$_2$(H$_2$O)$_n$]$^{+}$ (green) and [H(H$_2$O)$_n$]$^{+}$ (red). Grey species are fragments from a contaminating ion with the same m/z as the mass selected Eu$^{3+}$ cluster.
Figure B 5. ESI-MS TbCl$_3$ solution in water. Three discrete series of ions are observed.

Figure B 6. EDESI-MS/MS [Tb(H$_2$O)$_{47}$]$^{3+}$. Four different series can be seen: [Tb(H$_2$O)$_n$]$^{3+}$ (black), [Tb(OH)(H$_2$O)$_n$]$^{2+}$ (blue), [Tb(OH)$_2$(H$_2$O)$_n$]$^+$ (green) and [H(H$_2$O)$_n$]$^+$ (red).
Figure B 7. ESI-MS HoCl₃ solution in water. One discrete series of ions are observed.

Figure B 8. EDESI-MS/MS [Ho(H₂O)₄]³⁺. Four different series can be seen: [Ho(H₂O)ₙ]³⁺ (black), [Ho(OH)(H₂O)ₙ]²⁺ (blue), [Ho(OH)₂(H₂O)ₙ]⁺ (green) and [H(H₂O)ₙ]⁺ (red).
Figure B 9. ESI-MS EuCl$_3$ solution in water. Two discrete series of ions are observed.

Figure B 10. EDESI-MS/MS [Tm(H$_2$O)$_{50}$]$^{3+}$. Four different series can be seen: [Tm(H$_2$O)$_n$]$^{3+}$ (black), [Tm(OH)(H$_2$O)$_n$]$^{2+}$ (blue), [Tm(OH)$_2$(H$_2$O)$_n$]$^{+}$ (green) and [H(H$_2$O)$_n$]$^{+}$ (red). Grey species are fragments from a contaminating ion with the same m/z as the mass selected Tm$^{3+}$ cluster.
Figure B 11. ESI-MS LuCl$_3$. Three discrete series of ions are observed.

Figure B 12. EDES1-MS/MS [Lu(H$_2$O)$_{48}$]$^{3+}$. Four different series can be seen: [Lu(H$_2$O)$_{n}$]$^{3+}$ (black), [Lu(OH)(H$_2$O)$_{n}$]$^{2+}$ (blue), [Lu(OH)$_2$(H$_2$O)$_{n}$]$^{+}$ (green) and [H(H$_2$O)$_{n}$]$^{+}$ (red).
Appendix C

Figure C 1. ESI-MS CaCl₂. No protonated water clusters are observed in the background.

Figure C 2. ESI-MS BaCl₂ solution in water. Two discrete series of ions are observed.
Figure C 3. ESI-MS SrCl$_2$ solution in water. Two discrete series of ions are observed.

Figure C 4. ESI-MS Cu(NO$_3$)$_2$ solution in water. Two discrete series of ions are observed.
Figure C 5. EDESI-MS/MS of [Ca(H$_2$O)$_{30}$]$^{2+}$. 
Figure C 6. EDESII-MS/MS of $[\text{Ba(H}_2\text{O)}_{30}]^{2+}$. 
Appendix D

Figure D 1. EDES1 of a) \([N,N\text{-Me}_2\text{Guan(H}_2\text{O})_2]^+\) and b) \([N,N,N',N'\text{-Me}_4\text{Guan(H}_2\text{O})_2]^+\).
Appendix E

Note: where the prefix V is used it denotes the actual voltage applied to the particular optical element (bracket the readback) e.g. Vref = Rf1 + cc + ent whereas Rf1 is a slider value.

Figure E 1. Potential energy diagram for the Micromass QTof micro™ mass spectrometer.