

Electrospray ionisation – a simple prelude to the understanding of other mass spectrometry techniques

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Introduction

Mass spectrometry (MS) is a powerful analytical technique used in the characterisation of a wide range of chemical and biochemical substances. The technique is extensively used in research, across a wide range of disciplines. One of the main strengths of mass spectrometry is that miniscule amounts of sample are typically needed, in the order of micrograms or less. While the study of spectroscopic techniques has been a part of qualifications such as the International Baccalaureate Diploma for some time,¹ a new Achievement Standard on Spectroscopy has been introduced relatively recently into the New Zealand Chemistry Curriculum, which includes the technique of electron ionisation (EI) mass spectrometry.² This article aims to provide, for those perhaps encountering mass spectrometry for the very first time, a short discussion on electrospray ionisation mass spectrometry as an alternative introduction to this important analytical technique.*

Mass spectrometry

In essence, mass spectrometry separates gas phase ions according to their mass-to-charge ratios (m/z), which is simply the mass of an ion m divided by its charge z . There are two main processes involved in mass spectrometry – ion generation, and ion detection – and this article will focus on the former, of which electrospray ionisation is but one example. Electrospray ionisation is conceptually simple, and its beauty lies in the simple and easily interpreted spectra which are often obtained. The technique can also be used to illustrate the concept of isotopes.

Ionisation techniques

An ionisation technique is the physical process by which a sample of the substance being investigated – the *analyte* – is converted into gas phase ions. Depending on the chemical and physical nature of the sample, the choice of an appropriate ionisation technique is absolutely crucial; an ionisation technique might work beautifully for one type of substance, but be completely useless for another. A good example of this is electron ionisation (EI). In this technique, a sample of the substance is heated under vacuum, causing some of the substance to volatilise. The volatilised substance is then bombarded with high energy electrons; these cause the neutral gas-phase molecules to become positively-charged ions by loss of an electron, and the resulting ions are separated and de-

tected. This technique is ideal for analysis of non-polar but volatile substances such as hydrocarbons, and as an illustrative example, University of Waikato researchers have used electron ionisation mass spectrometry to provide analytical data on oils recovered from the 2011 Rena marine disaster.³ Because electron ionisation typically requires the compound to be volatile (and therefore of low molecular weight, and uncharged) it finds most use in the analysis of small organic molecules. It cannot be used to *directly* analyse polar, involatile substances such as sugars or proteins, which typically degrade rather than volatilising when heated.[†] Until the advent of electrospray ionisation, many types of chemical substances were effectively inaccessible to MS analysis.

Electron ionisation is also rather ‘harsh’, the mass spectrometry equivalent of using a sledgehammer to crack open a walnut; it works, but what is formed can sometimes pay little resemblance to the original nut. The consequence of this is that the *molecular ion* – the original starting molecule which has ionised by losing a single electron – is typically of low intensity, or in some cases unobservable. This is because the high energy of the electron ionisation process imparts excess energy to the ions formed, causing their fragmentation. This results in the formation of various fragment ions. Although this can provide a lot of very useful structural information, to the mass spectrometry beginner it can be somewhat daunting to be faced with an array of peaks. Furthermore, if it is molecular weight information which is desired, a weak or unobservable molecular ion can complicate matters. Electrospray ionisation provides a useful and simple alternative in many cases.

Electrospray ionisation

Today, electrospray ionisation (ESI) mass spectrometry is one of the most important analytical techniques used by chemists, biochemists and materials scientists; its importance was recognised by the co-award of the 2002 Nobel Prize in Chemistry to John Fenn, the inventor of the technique.⁴

Fig. 1 shows a schematic diagram of an electrospray capillary, and a typical modern mass spectrometer is shown in Fig. 2. Electrospray ionisation is conceptually quite simple, and involves forming a very fine spray of droplets of an analyte solution, using an inert nebulising gas (typically nitrogen); this is the spray part of **electrospray**. The solution is sprayed from a fine metal capillary tube,

* This article was originally written for the general readership of *ChemEd NZ*, but with the demise of this publication, is now published in *Chemistry in New Zealand* as a gentle introduction to mass spectrometry (especially electrospray ionisation) for those unfamiliar with the technique.

† Substances such as sugars can, however, be converted into more volatile derivatives by suitably modifying them, typically by converting polar –OH groups into less polar groups such as –OSiMe₃, by reaction with a suitable derivatisation reagent.

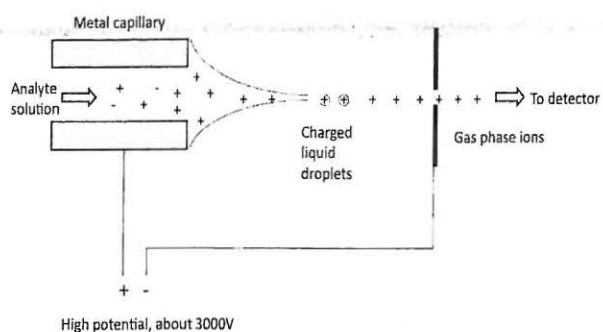


Fig. 1. A schematic diagram of an electrospray capillary, showing the ionisation process

which is held at a high voltage (hence, the electro part of the word **electrospray**). The effect of this is that the droplets which are formed are enriched in positive ions (if the capillary is positive), or negative ions (if the capillary is negative). These droplets are then evaporated using more nitrogen gas, which has the effect of converting the original analyte solution into the gas phase (effectively, it has nowhere else to go!). Solubility in a suitable solvent, rather than volatility, becomes the most important factor in determining the likely success of the technique in generating ions that can be subsequently detected.

How does it work in practice? Imagine the electrospray process applied to the analysis of a solution of sodium chloride in water. Of course, when sodium chloride is dissolved in water, $\text{Na}^+(\text{aq})$ and $\text{Cl}^-(\text{aq})$ ions are formed; spraying this solution through a positively-charged capillary produces droplets that have an excess of $\text{Na}^+(\text{aq})$ ions. Evaporation of these droplets forms $\text{Na}^+(\text{g})$ ions which can then be analysed. If the capillary is negatively-charged, the resulting droplets are enriched in negatively-charged chloride ions, so the electrospray technique can be used to study both the cations and/or the anions of salts very easily. As stated earlier, lack of volatility is not an issue – large cations such as cetylpyridinium (as its chloride salt, used as an antibacterial agent in mouthwash) can be analysed very easily, and produces the mass spectrum shown in Fig. 3.

Applications of electrospray ionisation mass spectrometry are not restricted to salts; neutral compounds can also be characterised, providing that a charge can be attached to the molecule in solution. In practice this means that the molecule should contain an atom which has at least one lone pair of electrons, for example most (but not all) of the common functionalised organic molecules (alcohols, ketones, esters, amines, amides, carbohydrates, proteins...). Protonation of the analyte molecule can commonly occur [from the small concentration of $\text{H}^+(\text{aq})$ ions which arise from the self-dissociation of water through $\text{H}_2\text{O} \rightarrow \text{H}^+(\text{aq}) + \text{OH}^-(\text{aq})$], but other cations such as Na^+ (and K^+) can also form adducts⁵ water stored in glass bottles will invariably contain sufficiently high concentrations of dissolved sodium ions from the glass. A neutral analyte molecule M will therefore typically give a protonated ion $[\text{M} + \text{H}]^+$, and/or a sodium adduct $[\text{M} + \text{Na}]^+$, and it is normally a very simple process to determine the molecular mass of M from the observed ions.

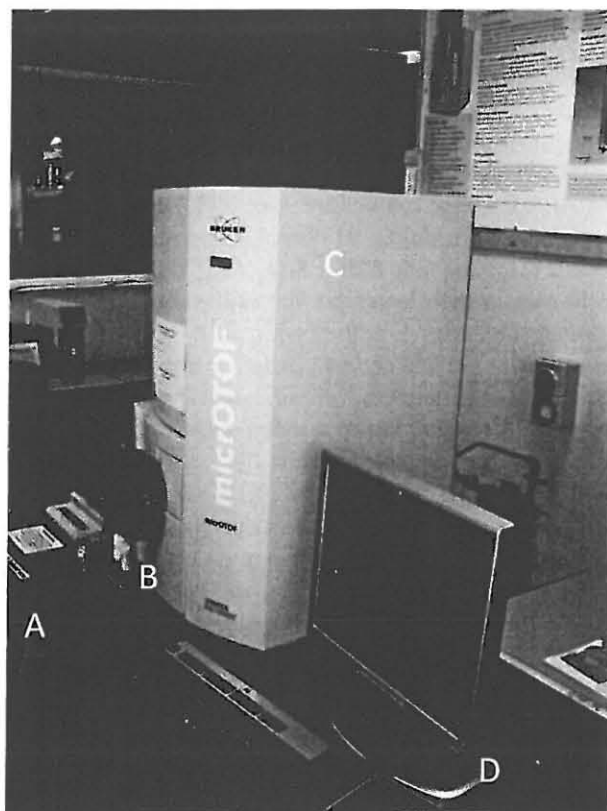


Fig. 2. A typical modern electrospray ionisation mass spectrometer (Bruker MicroTOF); the sample is introduced via a syringe and a syringe pump (A) to the instrument's spray chamber (B). The ion detector in this instrument is a time-of-flight detector (C), which accurately determines the time for ions of differing m/z to travel the length of a long tube under vacuum. Data collection and processing is controlled by a PC (D)

Non-polar organic molecules such as alkanes cannot be analysed by ESI directly because they cannot be protonated; electron ionisation MS remains the technique of choice for these substances.

Obtaining isotope information

Many elements exist as a single stable isotope, but other elements can exist as two or more isotopes.⁶ Electrospray ionisation mass spectrometry can be used to demonstrate isotopic composition very easily – indeed the power of mass spectrometry was realised in the very early days of the technique around 100 years ago, when J.J. Thomson showed that neon existed as two isotopes having atomic masses 20 and 22, in the ratio of 10:1, consistent with the average atomic mass of neon being 20.2. Since then, mass spectrometry has been extensively developed as a way of providing isotopic information, in applications that range from the nuclear industry (the first separation of uranium-235 for an atomic bomb was determined by mass spectrometry), to archaeological and geological dating techniques.

The power of the technique in providing isotope information is best illustrated by an example. Consider the analysis of an aqueous solution of silver nitrate. The ESI process simply transfers $\text{Ag}^+(\text{aq})$ ions to the gas phase, and removes their associated water molecules, giving 'bare' $\text{Ag}^+(\text{g})$ ions. Two peaks are observed, corresponding to the two isotopes of silver (^{107}Ag , 51.8% and ^{109}Ag , 48.2%)

as shown in Fig. 4.

A second example illustrates the ESI MS analysis of the salts KClO_3 (potassium chlorate), KBrO_3 (potassium bromate) and KIO_3 (potassium iodate). The EO_3^- ions are easily observed in the negative-ion mass spectrum. As an approximation we will assume that oxygen exists as a single stable isotope, ^{16}O .⁷ The mass spectrum of a mixture of the three ions $\text{ClO}_3^- + \text{BrO}_3^- + \text{IO}_3^-$ (Fig. 5) shows all three ions at their expected m/z values, but the appearance of the peaks is different for each one. This is due to the different elemental isotopic composition of the three halogens chlorine, bromine and iodine. For IO_3^- , a single peak is seen, because iodine exists as a single isotope (^{127}I), and a single peak at the expected m/z ($127 + 3 \times 16 = 175$) is seen (charge $z = 1$). However, for BrO_3^- two approximately equal intensity peaks are seen, at m/z 127 and 129, which are a consequence of the presence of two isotopes of bromine (^{79}Br , 50.7% and ^{81}Br , 49.3%). For ClO_3^- , two peaks are also seen at m/z 83 and m/z 85, but the intensities are now in a 3:1 ratio because of the two chlorine isotopes in unequal proportions (^{35}Cl , 75.8% and ^{37}Cl , 24.2%).

Fragmentation

Inspection of any electron ionisation mass spectrum will typically show a plethora of fragment ions, which can provide substantial structural information. Ions generated by electrospray ionisation can also be fragmented, to different extents, at will, by causing them to collide with residual nitrogen gas molecules from the ionisation process.⁸ The effect of this can be seen on the negative-ion electrospray mass spectra of BrO_3^- in Fig. 6. Under low fragmentation conditions (Fig. 6a) the parent ion is observed, but under more fragmenting conditions (Fig. 6b) ions formed by loss of oxygen atoms are seen, giving sequentially BrO_2^- (bromite), BrO^- (hypobromite) and finally Br^- (bromide). Unlike electron ionisation, ESI inherently produces relatively 'intact' ions, which can be subjected to fragmentation if this is desired.

Applications of ESI MS to the analysis of polymers and biomolecules

Given that there is no requirement for an analyte to be volatile in order to be analysed by electrospray ionisation mass spectrometry, it is not surprising that the very earliest studies on the technique demonstrated its successful use in obtaining mass spectra of large, involatile and sometimes heat-sensitive molecules such as polymers and proteins. This is important because it allows the molecular weight (or in the case of a polymer, the molecular weight distribution arising from individual polymer molecules having different chain lengths) to be measured. Again, the behaviour can be illustrated by an example, in this case the polyether-amine Jeffamine® D-2000. Polyether-amines find widespread technological applications, including the formation of polymers, and as a fuel additive, for example Techron®.⁹ Jeffamine® D-2000 has the structure shown in Fig. 7; it is based on the $\text{CH}(\text{CH}_3)\text{CH}_2\text{O}$ repeat unit, with NH_2 groups at each end of the chain. These amine groups are basic and provide a site for protonation in electrospray mass spectrometry analy-

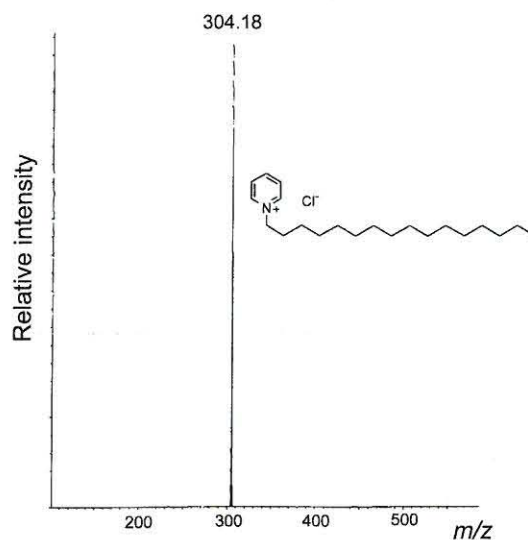


Fig. 3. Positive ion electrospray ionisation mass spectrum of cetylpyridinium chloride (structure shown as inset)

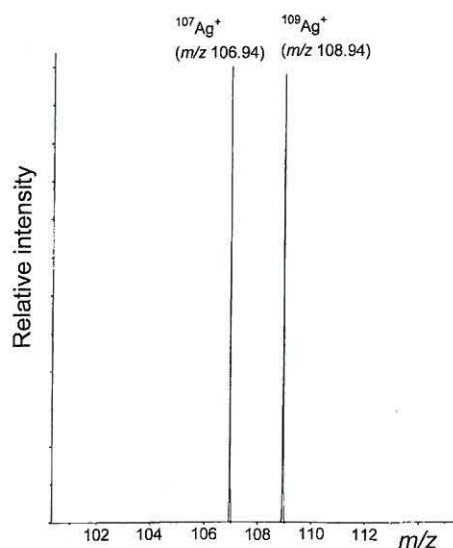


Fig. 4. Positive-ion electrospray mass spectrum of a solution of AgNO_3 showing the two isotopes of silver through the ions $^{107}\text{Ag}^+$ and $^{109}\text{Ag}^+$

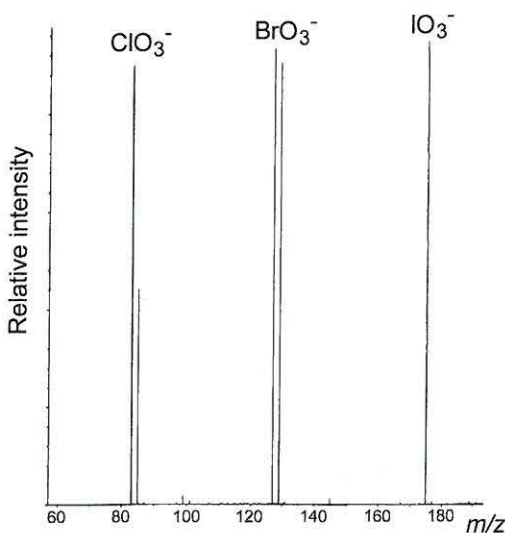


Fig. 5. Negative-ion electrospray mass spectrum of a solution containing a mixture of KClO_3 , KBrO_3 and KIO_3 showing the different appearance of the ClO_3^- , BrO_3^- and IO_3^- ions arising from the elemental isotopic compositions of chlorine, bromine and iodine

sis. The material is not a pure substance, but instead exists as a range of chain lengths, with an average of 33 $\text{CH}(\text{CH}_3)\text{CH}_2\text{O}$ repeat units in the polymer chain.¹⁰ The ESI mass spectrum of Jeffamine® D-2000 is shown in Fig. 7. The spectrum shows two main groups of peaks; the set centred around m/z 1700 are due to the monoprotonated ions $[\text{M} + \text{H}]^+$, where M represents an individual polymer molecule. The separation between adjacent peaks is 59 mass units, corresponding to the mass of a $-\text{CH}(\text{CH}_3)\text{CH}_2\text{O}-$ repeat unit, and the mass of an individual polymer molecule can easily be obtained, as the mass of the ion minus 1 (for the mass of the added proton). The second set of peaks centred around m/z 1054 is due to the doubly protonated ions $[\text{M} + 2\text{H}]^{2+}$. It is easily seen that adjacent peaks in this set are more closely spaced together (by 29.5 m/z units, i.e. half of 59). For these ions the smaller m/z values arise because the charge z on these ions is now 2.

Applications of ESI MS in inorganic chemistry

Although perhaps not immediately obvious, many inorganic substances have features in common with biological molecules such as proteins. For example, inorganic salts, and many coordination compounds are water soluble, ionic, and involatile compounds that decompose (rather than volatilise) on strong heating. Not surprisingly, ESI MS can often be usefully applied to the characterisation of many inorganic compounds, providing of course the substance is soluble in some suitable solvent, and can generate ions (if not already charged). Figs. 5 and 6 (*vide supra*) have already demonstrated the ability of ESI MS to analyse simple salts, and the reader is referred to more comprehensive texts for a full discussion of this topic.¹¹

Conclusions

Electrospray ionisation mass spectrometry (ESI MS) is a powerful analytical technique, which can be applied to almost any compound, providing it is soluble in a suitable solvent, and providing it is either charged, or can become charged by a suitable, gentle, solution-based process (protonation, addition of alkali metal cations such as Na^+ , and others). ESI MS has revolutionised the characterisation of many types of substances that were previously relatively inaccessible to mass spectrometry techniques, in particular polymers, bio-molecules such as proteins, as well as involatile inorganic compounds. The beauty of the technique lies in its gentle ionisation (which causes minimum fragmentation), and the simplicity of the spectra which are often obtained. However, as with any analytical technique, it has its limitations, and electron ionisation mass spectrometry – the technique which has been chosen to represent mass spectrometry in the Chemistry curriculum – retains an important role for the analysis of volatile, and in particular, non-polar organic molecules.

References and notes

1. See for example: Brown, C.; Ford, M.; *Standard Level Chemistry developed specifically for the IB Diploma*, Pearson Education, Harlow, Essex, 2008.
2. Achievement Standard AS91388 Chemistry 3.2: *Demonstrate un-*

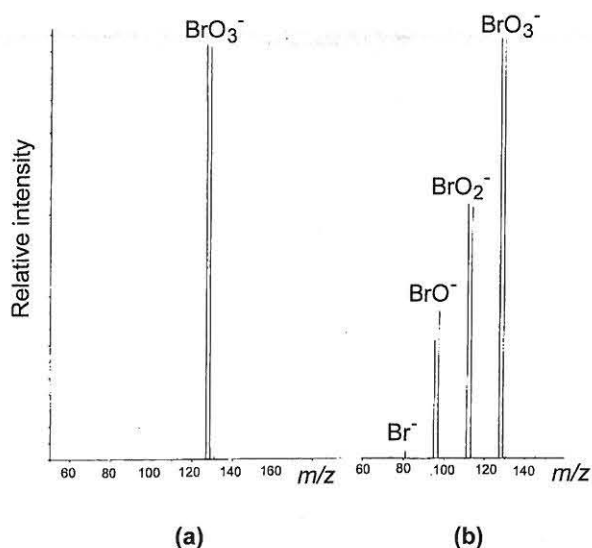


Fig. 6. Negative-ion electrospray mass spectrum of a solution of KBrO_3 at capillary exit voltages of (a) 70 V (non-fragmenting conditions) and (b) 180 V (fragmenting conditions) showing the formation of BrO_2^- , BrO^- and Br^- fragment ions; in each ion the distinctive isotope pattern from ^{79}Br and ^{81}Br isotopes is observed

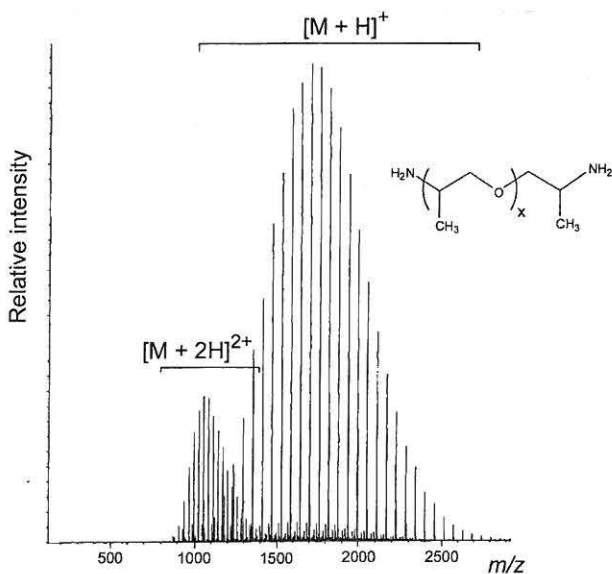


Fig. 7. Positive-ion electrospray mass spectrum of a solution of Jeffamine® D-2000 showing the sets of monoprotonated $[\text{M} + \text{H}]^+$ and diprotonated $[\text{M} + 2\text{H}]^{2+}$ ions, and the structure of Jeffamine® D-2000

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3. See: <http://sci.waikato.ac.nz/research/projects-and-case-studies/rena-research> (accessed 10/03/2015).
4. See: http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2002/illpres/mass.html (accessed 10/03/2015).
5. Sodium and potassium ions are invariably present in low concentrations when solvents such as water and alcohols are stored in glass bottles (glass contains considerable amounts of sodium ions).
6. Information on the isotopic composition of the elements can be obtained from books, for example *Aylward and Findlay's SI Chemical Data*, A. Blackman and L. Gahan, John Wiley & Sons, 7th edition 2013 (or earlier editions). Alternatively, reputable online data sources such as from the Royal Society of Chemistry (<http://www.rsc.org/periodic-table>) and tools such as the University of Sheffield's Chemputer (<http://winter.group.shef.ac.uk/chemputer/isotopes.html>) provide easily-accessed data.

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- Oxygen actually has trace amounts of ¹⁷O (0.038% abundance) and ¹⁸O (0.205% abundance).
- The ions are accelerated by means of a potential difference; the higher the accelerating potential, the greater the acceleration of the ions, producing higher energy impacts resulting in more extensive fragmentation.
- See for example: <http://www.caltex.com/nz/techron-tech/faq/>; <http://en.wikipedia.org/wiki/Techron> (accessed 10/03/2015).
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Corrigendum

The article published in *Chemistry in New Zealand* vol 79, no. 1, Jan 2015, pp36-41 authored by Grant J. McIntosh *et al.* erroneously referred to alumina as an adsorbate in the title and elsewhere in the text. Alumina is in fact the adsorbent.