

CHAPTER 1

Mass Spectrometry's Beginnings

1.1 A BRIEF HISTORY

1.1.1 Early Pioneers and Cathode Rays

Mass spectrometry had its beginnings in experiments performed over a century ago. Scientists in the late 19th century began conducting experiments within evacuated glass tubes in order to gain some understanding of the nature of electricity.

George Johnstone Stoney was the first to report that electricity has its basis in a particle, or an “atom of electricity” that he referred to as an electron. Stoney measured the charge of the electron in 1894 but it was left to Joseph John (J.J.) Thomson to measure the charge-to-mass ratio (e/m) of the electron and estimate its mass at a thousand times less than that of a hydrogen atom. Thomson had developed an interest in the electron while investigating the passage of electricity through gases in his laboratory in Cambridge. Thomson believed that the stream of rays emitted from a negatively-charged cathode, known as *cathode rays*, consisted of these particles. He also proposed that the particles (which Thomson preferred to call *corpuscles*) were one of the bricks from which all atoms were built – a controversial theory at the time. Thomson went on to describe his case in the book *Corpuscular Theory of Matter* published in 1907.

1.1.2 Positive Rays

Some time earlier Eugen Goldstein, a scientist in Germany who had given the name to cathode rays and studied them for several decades, discovered that the presence of gases in *cathode ray tubes* also gave rise to rays that behaved very differently from cathode rays (Figure 1.1). Wilhelm Wein in 1898 was able to deflect these rays in the opposite direction to cathode rays using magnetic and electrical fields. He

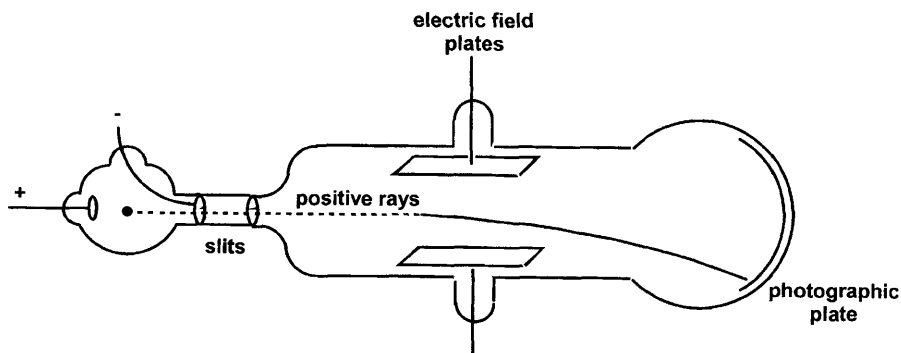


Figure 1.1 Representation of the cathode ray tube showing the deflection of positive rays (ions)

concluded that they were the positive equivalent of the negatively-charged cathode rays and carried “positive electricity”. Wein’s experiments, however, showed that these rays contained particles with masses much larger than an electron and of the order of a hydrogen atom. Fascinated by this positive form of electricity, Thomson improved upon the data of Wein by operating his cathode ray tube at lower pressures and showed also these positive rays could be deflected from a straight line by perpendicular electric fields onto a photographic plate (Figure 1.2).

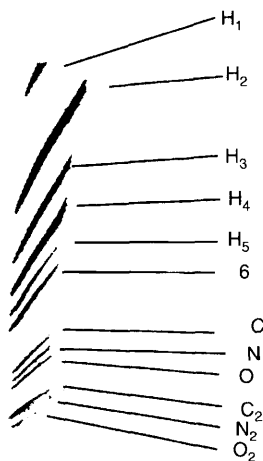


Figure 1.2 Parabolic paths of ionised forms of atoms and small molecules within a cathode ray tube

(Source: Fig. 5, plate 1, J.J. Thomson, *Recollections and Reflections*, G. Bell and Sons Ltd., London, 1936)

1.1.3 The First Mass Spectra

First with hydrogen, and later with other atoms and molecules of carbon, nitrogen and oxygen, Thomson discovered that each charged particle followed its own parabolic path based upon their detection on the photographic plate. He reasoned that no two particles would strike the plate at the same place unless they possessed the same velocity and charge-to-mass ratio. Thomson quickly realised that an inspection of the plate showed at a glance how many kinds of particles there were in the rays and that, by knowing the value of elm for one parabola, the values of elm for all the others could be deduced.

In his recollections, published in 1936, four years before his death, Thomson recounted that the positive rays contained atoms and molecules of all gases, common elements or molecules present, and correctly suggested that the positively charged particles were formed by the loss of an electron. In other words, the positive rays consisted of charged atoms or molecules, known as *ions*. Thomson concluded that the positive ray spectra possessed many advantages over other approaches for chemical analysis. The number of components as well as their atomic or molecular weight could be measured from these spectra. He argued that by using long exposures the approach could be “exceedingly delicate” allowing for the presence of a trace of gas to be detected “too small in amount to be measured by any other spectroscopic method”. Another noted advantage was that the method was not dependent on the purity of gas analysed. Impurities merely appeared as additional parabolas in the spectrum and did not contribute errors to measurements of atomic or molecular weight.

Thus the field of mass spectrometry was born and these important features, recognised by Thomson, remain to this day. Mass spectrometers are able to:

- (i) measure the atomic and molecular weights of charged species in complex sample mixtures,
- (ii) analyse compounds at extremely low sample levels,
- (iii) analyse compounds in a mixture without purification of that mixture.

These advantages, and many others, considerably outweigh the few disadvantages described later such as the loss of the sample once it is analysed. But first the work of Thomson's student Francis Aston is briefly reviewed. Aston's work led to the discovery of isotopes which have important implications for mass analysis.

1.2 ISOTOPES AND THEIR IMPLICATIONS FOR MASS MEASUREMENT

1.2.1 Discovery of Isotopes

Commencing in 1909, Francis Aston accepted an invitation to work as an assistant to Thomson in Cambridge to study positive rays. It was during this period that improvements to Thomson's cathode ray experiments were made and several new "mass spectrographs" were constructed. These instruments, forerunners of the modern mass spectrometer, enabled Aston to separate two isotopes of elemental neon, ^{20}Ne and ^{22}Ne . The principle was extended to other chemical elements and led to the discovery of 212 naturally occurring isotopes. From this work, Aston formulated the so-called "Whole Number Rule" that states when the mass of the oxygen isotope is defined, all other isotopes have masses that are nearly whole numbers or integers. Carbon, rather than oxygen, is now considered the standard isotope mass upon which all other isotopes are measured.

1.2.2 Isotopes and Mass Measurement

Isotopes are atoms of the same element with different numbers of neutrons in the atomic nucleus. These add mass but not charge to the atoms and molecules composed of them. Naturally occurring carbon, for example, is a mixture of two isotopes ^{12}C and ^{13}C that represent 98.9% and 1.1% of all the carbon on Earth. The individual carbon atoms have masses of either 12.00000 (assigned as a standard by the International Union of Pure and Applied Chemistry, IUPAC) or 13.003354 mass units (u, formerly atomic mass units *amu*). The average mass of carbon is calculated as 12.011 u based on these masses and the natural occurrence of each isotope ($98.9/100 \times 12.000 + 1.1/100 \times 13.003 = 12.011$). Isotope masses and relative abundances for all the common elements are provided in Appendix 2.

If a mass measurement is made where the isotopes of each atom are separated, the mass will reflect a *monoisotopic* (or one isotope) *mass*. If the isotopes are unresolved, the mass will reflect an *average mass*. Note that the average mass value is larger than the monoisotopic mass for the lightest isotope since it contains a contribution from the heavier isotopes.

Unlike Thomson's early mass spectra, modern mass spectrometers record both the mass-to-charge ratios of ionised forms of atoms or molecules and also their *relative abundance* in the spectrum. Thus

when the isotopes of an atom are separated or resolved in a mass spectrum, the relative intensities of the ions reflect their relative levels. To see this, consider the mass spectrum of an atom of chlorine. Chlorine has two isotopic forms ^{35}Cl and ^{37}Cl . The mass spectrum for an ionised chlorine atom will therefore appear as shown in Figure 1.3 in a bar graph representation.

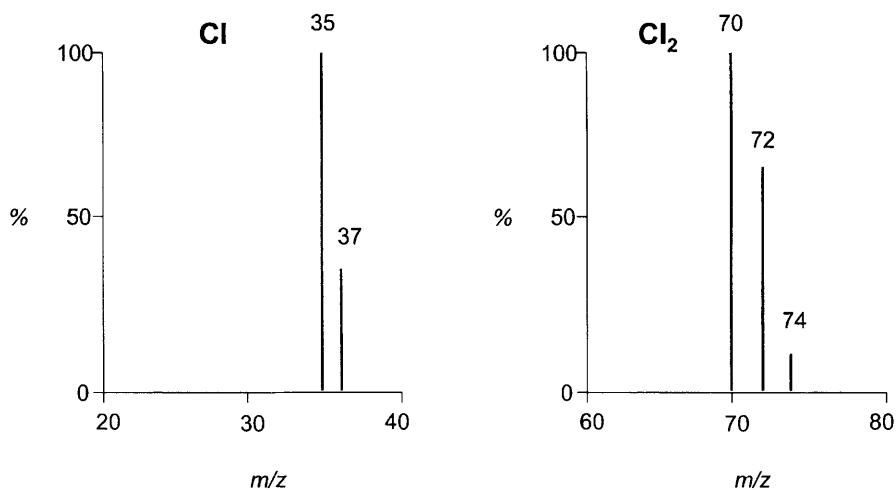


Figure 1.3 Electron ionisation mass spectra of atomic and molecular chlorine

Note that the relative height or abundance of the ^{37}Cl isotope is one third of that of ^{35}Cl . This is consistent with the relative abundances of the two isotopes which are 75.78% and 24.22% for ^{35}Cl and ^{37}Cl respectively (Appendix 2).

Consider a molecule of chlorine gas Cl_2 . A molecule can be formed either from two ^{35}Cl atoms, one ^{35}Cl and one ^{37}Cl atom, or two ^{37}Cl atoms. The mass spectrum of the ionised form of the molecule reflects this and shows three resolved ionic forms (at m/z 70, 72 and 74) with relative abundances of approximately 100, 64 and 10% respectively (Figure 1.2). These values can be derived theoretically by multiplying the relative abundance for each atom and normalising the values to 100. The relative abundance for chlorine molecules comprised of mixed isotopes must be multiplied by two in order to account for the two forms possible; that is $^{35}\text{Cl}^{37}\text{Cl}$ and $^{37}\text{Cl}^{35}\text{Cl}$.

It is a simple matter to calculate the relative height of isotope peaks for any particular molecule. In the case of an isotope of one integer unit of mass higher than the molecule (*i.e.* $M + 1$), the relative height of this peak can be expressed as a percentage given by equation 1.1. In this equation, $\%I$ represents the percentage of naturally occurring isotope one mass

unit greater than the common isotope for each element, E . The number of atoms of that element in the molecule is defined by n .

$$\text{ratio of } (M + 1)/M \text{ peak} = \sum_E (\%I \times nE) \% \quad (1.1)$$

Hence for a molecule of ethanol (C_2H_5O), the ratio of the $(M + 1)/M$ isotope peaks will be equal to $(1.08 \times 2) + (0.015 \times 5) + (0.038 \times 1)$ or 2.27% based upon the percentages of naturally-occurring ^{13}C , 2H and ^{17}O (see Appendix 2).

In general, the contribution of any element with two common isotopes (of a and b percent) to an isotope peak $(M + N)/M$ is given by equation 1.2.

$$\text{ratio of } (M + N)/M \text{ peak} = N!(a)^{n-1}(b)^n/N(a)^n \quad (1.2)$$

Hence the relative peak height of $(M+2)/M$ for a molecule of Cl_2 is calculated to be $2(0.76)^1(0.24)^2/2(0.76)^2 = 0.076$. Note that $N!$ (or N factorial) equals the multiple $N \times (N-1) \times (N-2) \times \dots$ to $\times 1$.

1.3 MOLECULAR WEIGHT

The molecular weight of a compound is the sum of the atomic masses for all atoms in the molecule weighted according to the relative abundances of their isotopes. For example, the molecular weight of a molecule of sucrose or common sugar ($C_{12}H_{22}O_{11}$) is the sum of the atomic masses for 12 carbon atoms, 22 hydrogen atoms and 11 oxygen atoms. The average molecular weight calculated from the atomic weights for these atoms is 342.299. If the isotope peaks for the compound were resolved or separated in a mass spectrum, the expected mass of the monoisotopic ions or ions containing only the lightest isotopes of each element would be 342.116.

Measuring molecular weight is a fundamental activity of modern mass spectrometry, but as we shall see later, mass spectrometers are used for many other purposes including the complete structural characterisation of molecules, the quantitation of components within complex chemical and biological mixtures, fundamental investigations of ion behaviour and reactivity studies of molecular complexes, and even the radiodating of archaeological relics.

1.3.1 Elemental Composition and Mass Accuracy

The manner in which accurate mass measurements are obtained is described later in Chapter 5 in the context of the instrumentation

required, but assume for the moment that the molecular weight of a compound can be measured with a high degree of accuracy. Subject to the accuracy obtained, it may be possible to assign the elemental composition to a compound based solely on the molecular weight measurement. This is because the monoisotopic atomic masses for the elements are not exact integers; rather they have a fractional mass component. Consider for example a small organic molecule whose molecular weight based on a measure of the mass-to-charge ratio of its monoisotopic ions is 46.0419. This molecular weight value is consistent with a molecule of ethanol with an elemental formula of C_2H_6O , but not for instance a molecule of nitrous oxide NO_2 with a molecular weight of 45.9929. The mass accuracy required to distinguish these two molecules can be calculated as one-half of the difference between their molecular weights divided by the molecular weight of one of them. In this example, a mass accuracy of 0.00053 or 530 parts-per-million (ppm) is required. The division of the difference by two arises since if either molecular weight is in error by greater than one-half of the difference, the measurement will not enable the two elemental compositions to be distinguished.

Such determinations are not limited to small molecular weight compounds provided that sufficient mass accuracies can be obtained. It has recently been shown using an ion cyclotron resonance mass spectrometer that two peptides with molecular weights that differ by just 0.00045 Da could be distinguished. This mass difference corresponds to a value less than the mass of a single electron (Figure 1.4).

1.3.2 Nitrogen Rule

The *nitrogen rule* is of use in assigning an elemental composition to a compound based upon its molecular weight. For any compound that contains only C, H, N, O, S, Si or halogen atoms (F, Cl, Br or I), the nitrogen rule states that the *nominal molecular weight* for the compound will be even only if the number of nitrogen atoms is an even number or zero. The nominal molecular weight represents only the integer portion of the value. In the case of ethanol, the nominal molecular weight is 46. This is an even number consistent with the lack of nitrogen atoms in the molecule. The value is inconsistent with the one nitrogen atom present in NO_2 . Thus based on the nitrogen rule, we can assign the correct elemental composition of C_2H_6O even where a mass accuracy of less than 530 ppm is unattained. A compound containing an odd number of nitrogen atoms in addition to atoms of any of those elements detailed above, will have a odd nominal molecular weight. In the case of NO_2 , the nominal molecular weight is seen to be 45.

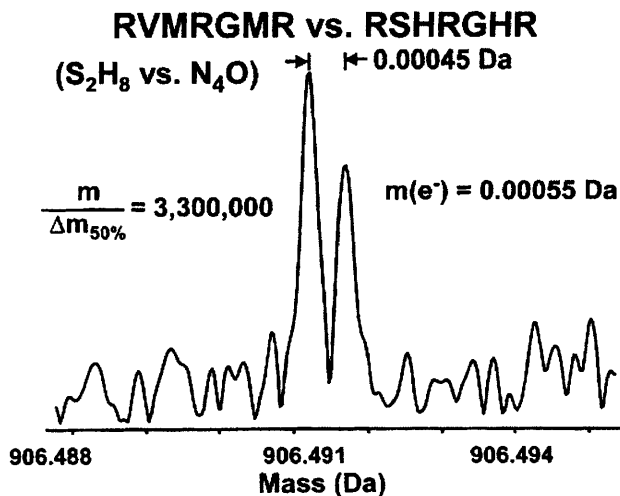


Figure 1.4 ESI FT-ICR mass spectrum of $[M+2H]^{2+}$ ions of two peptides that differ in mass by 0.00045 Da. The mass resolution at FWHM is calculated to be 3.3 million
(Source: Fig. 2, Fei He, Christopher L. Hendrickson and Alan G. Marshall, *Anal. Chem.*, 2001, 73 (3), 647–650)

1.3.3 Double-Bond Equivalents

An additional rule of use in assigning a correct elemental composition is the *double-bond equivalents* (DBE) or number of “double bonds plus rings” rule. Put simply, this rule calculates the number of double bonds or aromatic rings in an organic molecule with the elemental composition $C_xH_yN_zO_n$. The number of DBE in such a molecule is defined by equation 1.3.

$$\text{DBE} = x - y/2 + z/2 + 1 \quad (1.3)$$

In the case of ethanol described above, the DBE calculated based on the elemental composition C_2H_6O equals zero, a value consistent with only single bonds being present in the molecule. For a molecule of benzene with an elemental composition of C_6H_6 the DBE equals 4 [or $6 - (6/2) + 1$]. This is confirmed by its structure since three double bonds are associated with the aromatic ring and a further DBE is associated with the ring itself.

