CHAPTER 2

The Mass Spectrum

2.1 CONCEPT OF CHARGE AND THE MOLECULAR ION

To this point, only the molecular weight of a molecule has been considered to contribute to its appearance in a mass spectrum. However, as recognized by Thomson, the particles detected in mass spectrometry are ions. Thus they have both mass and charge, the latter of which is important to their detection. Mass spectrometers are unable to detect neutral molecules and radicals; a charge must be imparted onto an atom or molecule before it can be studied. The reason for this is that charged molecules can be "handled" or their paths controlled through the use of electric and magnetic fields, while radicals and neutral molecules are unresponsive.

Depending on the nature of the ionisation process, and also the nature of the atoms and molecules themselves, different ion types can be formed. The most common and traditional way in which ions are produced in a mass spectrometer is through the loss of an electron (equation 2.1). This often occurs by the initial collision of a gaseous atom or molecule with an electron in a process known as *electron impact* or *electron ionisation* (EI) (Chapter 3).

$$M + e^- \rightarrow M^{+\bullet} + 2e^- \qquad (2.1)$$

The product ion formed is known as a *radical cation* as it is an odd electron species with a positive charge. Since the product, $M^{+\bullet}$, has the same mass as the molecular weight of the compound M from which it was produced, it is known as a *molecular ion*. In general, the notation $^{+\bullet}$ adjacent to a molecule's structure indicates that the molecule is deficient of an electron without designating the site of the charge.

The charge of the ion is equal to the charge of an electron (e) defined as 1.6×10^{-19} Coulombs (C). Were the ion to possess two charges, perhaps through loss of electrons from two atoms or groups of atoms within the same molecule, the charge on the ion would be $2e$. Thus the

charge of an ion is always some multiple of the charge of an electron or ze, often just denoted z. In a mass spectrum, ions appear at a mass-to-charge ratio defined m/z, where m is the mass of the ion and z is the charge. Since z often (though not always) has a value of one, early references in mass spectrometry refer to an ion's mass-to-charge ratio as m/e.

It is important to note that not all ions are formed by electron loss. Some electronegative atoms or molecules can attract an electron during electron impact (equation 2.2). These ions, denoted $M^{-\bullet}$, are still referred to as molecular ions. The same is true of even electron species formed by the adduction or loss of a charge carrying atom or group. An ion may be formed for instance by the protonation of a molecule. $[M + H]^+$ is also referred to as a molecular ion, or more strictly a *quasi* or *pseudo-molecular ion* since the mass of the ion is now larger than the molecular weight of the molecule by one atom of hydrogen.

$$M + e^- \rightarrow M^{-\bullet} \tag{2.2}$$

In general terms, most ions formed, dissociated and studied in mass spectrometry are positively-charged ones. This is because their production is usually more efficient for most classes of compounds over their negatively-charged counterparts. This is not to say that negative ion mass spectrometry is not important or not used; indeed many important observations and applications are based on these experiments (see for example Chapter 7). The polarity of the lenses used to repel ions from the source is the same as that of the ions themselves. The polarity of the voltage applied to the detector, on the other hand, is opposite to that of the ions in order to attract them to the ion detector. This leads to the detection of either positive or negative ions, but not both simultaneously.

2.2 FRAGMENT IONS

2.2.1 Formation of Fragment Ions

If sufficient energy is deposited into the molecule during ionisation, the molecular ions may dissociate into smaller mass fragments that themselves may be ions. For example, an odd-electron radical cation, $M^{+\bullet}$, may dissociate to form two fragments one of which is an even electron fragment of product ion, and the other is a radical $R^\bullet$ (equation 2.3).

$$M^{+\bullet} \rightarrow F^+ + R^\bullet \tag{2.3}$$

Alternatively, $M^{+\bullet}$ could dissociate to produce a smaller mass fragment and a neutral molecule N (equation 2.4).

$$M^{+\bullet} \rightarrow F^{+\bullet} + N \tag{2.4}$$

Only the ionic products F^+ and $F^{+\bullet}$ are passed through the instrument and detected. Beyond simple bond cleavages, fragment ions can be produced following the rearrangement of atoms if sufficient energy is available to facilitate bond cleavage and formation. Hydrogen atoms and protons, for instance, are frequently transferred from a remote site to the ionic centre prior to cleavage of the molecular ion.

The most abundant ion peak in a mass spectrum, whether it is that of the molecular ion or that of a fragment, is referred to as the *base peak*. Ion abundances are measured relative to the intensity of the base peak, set arbitrarily to 100%, and usually plotted to the top of the *y*-axis (Figure 2.1).

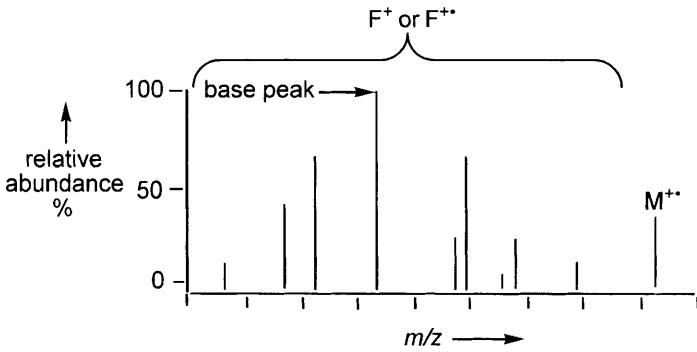


Figure 2.1 *Representation of an EI mass spectrum for molecule M*

2.2.2 Stability of Fragment Ions

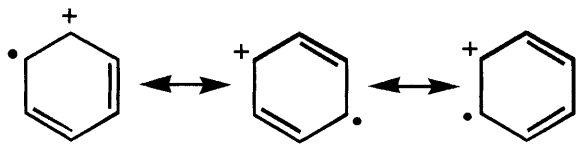
The relative stability of the fragment or product ion is a major factor that influences the appearance of fragment ions in a mass spectrum. For a fragment ion to appear in a mass spectrum, it must be produced from a significant proportion of the precursor ions and be relatively stable to further fragmentation. The most predominant fragmentation pathways are those with the lowest energy barriers to product and with the lowest change in free energy (ΔG, where ΔG is associated with changes in enthalpy, ΔH, and entropy, ΔS, according to $\Delta G = \Delta H - T\Delta S$). Other factors, including the strengths of the bonds broken during the fragmentation process and the time allowed for dissociation, also influence the appearance of fragment ions in the mass spectrum.

A few factors that can stabilise a fragment ion are now considered. Although illustrated for simple organic ions, these effects can be extended to other classes of compound.

2.2.3 Stabilising Effects

One of the most common ways that positive ions can be stabilised is through electron donation from a neighbouring atom or group of atoms. The transfer of electron density toward a positive centre is known as the *inductive effect*. Alkyl groups in hydrocarbons have the ability to donate electrons to a electron-deficient positive ion centre, such that the stability of carbocations follows the order: $(CH_3)_3C^+ > (CH_3)_2CH^+ > CH_3CH_2^+ >> CH_3^+$. Thus the fragmentation of hydrocarbons gives rise to mass spectra in which ions at *m/z* 57 ($(CH_3)_3C^+$) and 43 ($(CH_3)_2CH^+$) are more abundant than those at *m/z* 29 ($CH_3CH_2^+$) and 15 (CH_3^+). In contrast, electron-withdrawing atoms (F, Cl, Br, I) or groups (–OH, $–NO_2$) have a destabilising effect on a neighbouring positive ion centre.

A second stabilising effect is the *mesomeric effect*. Here a positive ion centre is stabilised by its conjugation with multiple (unsaturated) bonds. Hence the ion is stabilised through *delocalisation* of the charge across the molecule or fragment. As an example, the ion $CH_2 = CH–CH_2^+$ (*m/z* 31) can be stabilised by charge delocalisation to the form $^+CH_2–CH = CH_2$. This delocalisation of charge through *bond resonance* also stabilises a phenyl ion (equation 2.5). Ions at *m/z* 77 ($C_6H_5^+$) are a signature of aromatic compounds in EI mass spectra.



(2.5)

2.2.4 Quasi-Equilibrium Theory

The unimolecular decomposition of molecular ions into fragments can be explained by the Quasi-Equilibrium Theory (QET). The QET provides a theoretical description of how these processes take place inside a mass spectrometer. The ionisation of a molecule by electron loss (equation 2.1) or electron capture (equation 2.2) occurs within approximately 10^{-15} s, a time much shorter than that required for a molecular vibration. Hence the ionisation event can be considered to be a "vertical transition" with no change in internuclear distances (Figure 2.2). If the geometry of molecular ion differs from that of the neutral molecule,

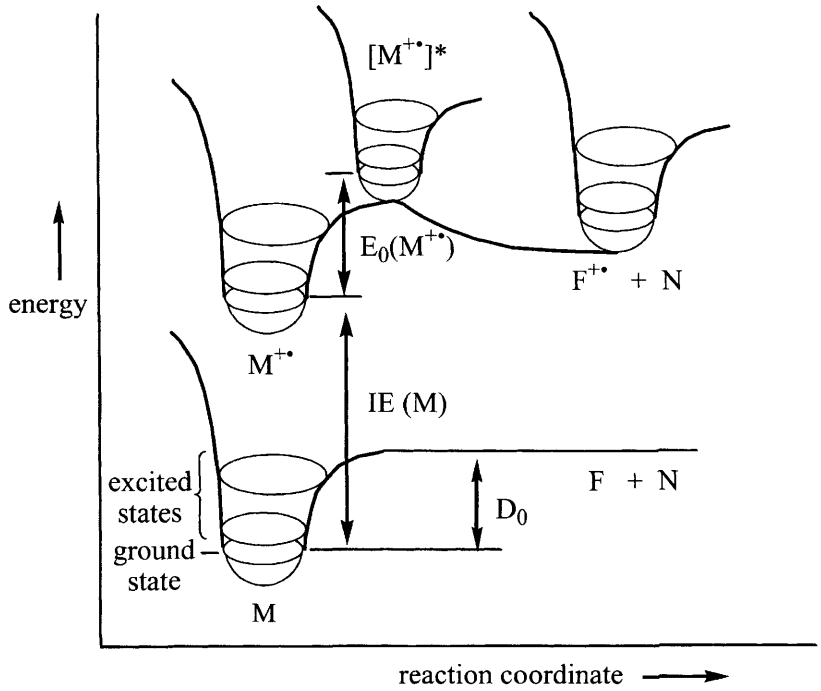


Figure 2.2 *Schematic representation of energy levels associated with the ionisation and dissociation of a polyatomic molecule M along a reaction coordinate*

the states for the latter will appear slightly along the reaction co-ordinate (to products) and the transition is said to be non-vertical or *adiabatic*.

Since pressures within the ion source are typically very low, each ion can be considered to be an isolated system as ions do not collide with each other or background gas molecules. If the excess energy transferred to a molecule during the ionisation process is distributed between all possible excited states in the ion, and these excited states interconvert between one another, a *quasi-equilibrium* is said to exist among these states. Therefore it is postulated that each excited state is of equal probability and that an ion's dissociation pathway depends only on its internal energy and the structure of the molecule, and not upon the initial site of ionisation or the nature of the ionisation process.

Few exceptions have been found to the QET and such dissociations are termed *non-ergodic*.

The *ionisation energy* (IE) is defined as the minimum energy necessary to produce a molecular ion, $M^{+\bullet}$ from its ground neutral state, M. It can be determined by raising the energy of the electrons in an electron impact source until a molecular ion is detected. Since the energy is related

directly to the potential through which the electrons are accelerated, it is often referred to as the *ionisation potential.*

The appearance of a fragment ion in a mass spectrum occurs if there is sufficient energy to ionise the neutral molecule and overcome the activation barrier. This is referred to as the *appearance energy* (E) of the fragment ion. An additional factor, the *kinetic shift*, may contribute to the appearance of a fragment ion and results from the excess energy required in order for the fragmentation process to occur within the time the molecular ion spends in the ion source. The kinetic shift increases with the size of the molecule, the activation energy and the "tightness" of the transition state.

Where there is no activation barrier and the kinetic shift is zero or negligible, the minimum energy required for (M) to dissociate into fragments can be defined as IE. All molecular ions with internal energies less than E_0 do not dissociate regardless of the time available for fragmentation. The appearance energy of a fragment can be defined by equation 2.6.

$$E = IE(\mathrm{M}) + E_0 \tag{2.6}$$

In the case in which two dissociation pathways are possible where $(E_0)_1 \approx (E_0)_2$, the pathway which proceeds through a "loose" transition state, $[\mathrm{M}^{+\cdot}]^*$, will predominate. This is because the energies of the excited states of a transition state in which the components are loosely associated will be similar to one another, over those for a "tightly-activated" transition state, such that they are easier to surmount. Ion dissociations involving the breaking of a single bond (with a bond dissociation energy of D_0) usually proceed *via* a loose transition state complex and are typically favoured over rearrangement reactions that involve tighter transition states. Computer algorithms are available to calculate the energies of such states for low values of $(E - E_0)$. Difficulties, however, arise for a real molecular system in predicting an ion's physical parameters, including its energy for activation.

Both theoretical and experimental results have shown that the rate constant k for a fragmentation process increases proportionately with the internal energy of the ion before reaching a maximum plateau beyond which no rate enhancement is observed. The shape of the curve from a plot of k versus energy E is predominately determined by the geometry of the transition state and the value of E_0. Use of the QET allows the rate of fragmentation of an ion with a given internal energy to be predicted. The maximum rate constant for a simple bond cleavage process is of the order of 10^{14} s^{-1}.

A simplified version of the QET enables the abundances of molecular and fragment ions in mass spectra to be described in a semi-quantitative manner without the aid of a computer. In this version, the rate of fragmentation of an ion (k) is given by equation 2.7 where v is a frequency factor influenced by the entropy of the process and N the number of oscillations (rotations or vibrations) possible.

$$k(E) = v[(E - E_0)/E]^{N-1} \tag{2.7}$$

As explained above, the appearance of fragment ions in a mass spectrum is ultimately influenced by the time allowed for such fragmentation processes. Ions spend approximately 10^{-6}s (or one microsecond) in an electron ionisation source so that only relatively fast fragmentation processes occur in this region of the mass spectrometer. On some mass spectrometers, an entire mass spectrum is recorded in just a few hundred microseconds providing energetically-excited ions with only this amount of total time to fragment.

2.2.5 Metastable Ions

Metastable ions, denoted m^*, are those formed by unimolecular dissociation of molecular ions in the field-free regions of the mass spectrometer anywhere between the ion source and detector. They can be useful in establishing the fragmentation pathways of molecular ions by unequivocally linking a fragment ion with a specific precursor.

Consider a molecular ion of mass m_p that dissociates to a fragment ion plus a radical or neutral molecule (equations 2.3 and 2.4). If a metastable fragment ion is produced in flight from the ion source to the detector it will have less translational energy than a comparable fragment ion generated within the ion source (of mass m_f). This results in the ions appearing in the mass spectrum at an apparent mass (m^*) that is less than that of fragments generated in the source (m_f). On a magnetic sector mass spectrometer, the mass of a metastable ion, m^*, is expressed according to equation 2.8.

$$m^* = m_f^2/m_p \tag{2.8}$$

The widths of the ion signals of these metastable ions of mass m^* are considerably greater than those of other ion fragments. This is because the ions are not energy focused as they leave the ion source and the kinetic energy of the precursor ions is released *isotropically* as they are formed. Thus metastable ions have a broader range of energies over other fragment ions.

As an example, the loss of a methyl radical from ionised propane (of a molecular weight of 44 Da) gives rise to a fragment ion at *m/z* 29 and a metastable ion at *m/z* 19.1 ($29^2/44 = 19.1$) Because the metastable ions appear as broad peaks in the mass spectrum, their mass-to-charge ratios are usually quoted to only a few decimal points at most.

2.3 RELATIVE ION ABUNDANCE

The appearance of an ion in a mass spectrum is the result of an electrical current that is generated and amplified when the ion strikes the detector. A measure of the ion current across all ions in a mass spectrum is referred to as a *total ion current* (TIC). The charge on a singly charged ion is 1.6×10^{-19} coulombs (C). Since one ampere represents one coulomb of charge per second, when one million singly-charged ions strike the detector a current of 1.6×10^{-13} amperes (A) will be produced.

The vertical or *y*-axis of a mass spectrum is usually plotted to display the ion current, the number of ions, or more commonly *relative ion abundances*. Here the ion signal with the highest current is normalized to 100% on the relative abundance scale and all other ions have abundances measured relative to this peak, the *base peak*. The relative abundance of an ion is dependent on a number of factors including the stability of the ion, the stability of the neutral product (in the case of a fragment ion from a unimolecular decomposition), suppression effects, mass resolution, and detector efficiency.

Ion detectors do not detect ions across the *m/z* scale with equal efficiency, and it is common for ions at high *m/z* to be detected less readily than those at low *m/z*. Mass resolution also impacts ion abundance measurements. An ion signal associated with two unresolved ions would have a higher intensity reflecting the contribution of both ion currents to the signal than would be the case if each were mass resolved. Suppression effects have also been observed widely in mass spectrometry. In some cases, a neighbouring ion can completely suppress the signal of another ion such that it is not detected. When ionised separately, both ions are easily detected.

Entropy effects are an important consideration in terms of the intensity or abundance of fragment ions formed by unimolecular dissociation. Entropy considerations favour the production of fragment ions by simple cleavage reactions over rearrangements involving a change in the molecular structure.

2.4 MASS RESOLUTION

The ability to separate two ion signals from one another in a mass spectrum is defined by *mass resolution* (R). Traditionally, this measure has been made based on the resolution of two ion signals above 10% of their height (the so-called 10% valley definition) (Figure 2.3). Mass resolution is defined by equation 2.9 where M_1 represents the *m/z* ratio for the first ion and M_2 represents the *m/z* ratio of the second, and $M_1 > M_2$.

$$R = M / \Delta M = M_1/(M_1 - M_2) \tag{2.9}$$

Therefore in order to resolve ions at *m/z* 1000 and 1001 with a 10% valley, a mass resolution of 1000 is required. The same is true for ions at *m/z* 500 and 500.5. In some cases, a mass resolution is referred to as a ratio, namely 1:1000.

Mass resolutions vary due to a number of factors including the *initial kinetic energy* of the ions as they exit the ion source. Most ions of the same *m/z* are formed with a range of initial energies (often represented by a bell-shape distribution), a result of their proximity to or remoteness from the acceleration lenses to which high potentials are applied. This may be corrected for by the mass analyser by what is known as *energy focusing*. Here only ions of the same *m/z* and energy are allowed to pass to the detector but this is achieved at the expense of ion detection. In cases where this energy spread is not corrected for, the width of the ion signal recorded at the detector will be larger.

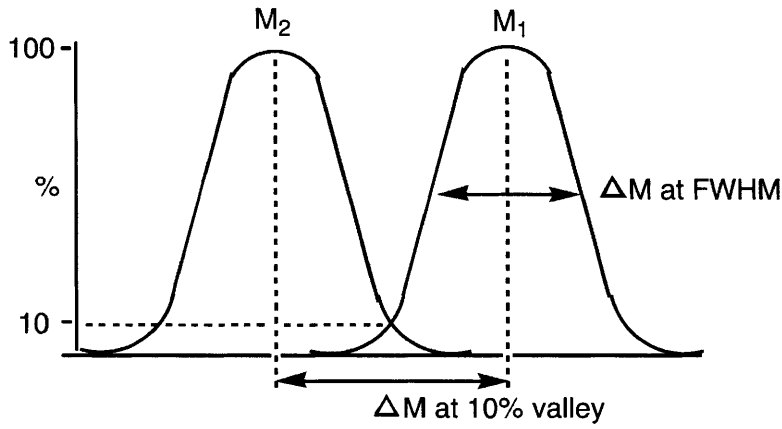


Figure 2.3 *Definition of mass resolution at a 10% valley and full-width at half maximum (FWHM)*

The most important factor, however, that impacts mass resolution is the type of mass analyser used. Magnet-based instruments as a rule achieve superior mass resolution to quadrupole and time-of-flight mass analysers. However, a better understanding of ion motion and optics inside a mass spectrometer has led to improvements in instrument design that have considerably increased the mass resolutions that can be achieved on most instruments. Mass resolutions of the order of 100–500 are considered low, those of the order of 1000–5000 are considered moderate, while those above 10,000 are considered to be high (see Appendix 4). Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometers, described in the next chapter, offer the highest mass resolutions attainable to date, routinely up to 100,000. The ion signals associated with isotopes, even for large macromolecules, can thus be resolved to baseline (or the *x*-axis) on these instruments.

Where isotopes or components in mixtures are not mass resolved, some measurement of mass resolution is often desired. In this case, it is common to measure mass resolution based upon the width of the ion signal at half its height. This measure is referred as *full width at half maximum* (FWHM) (Figure 2.2). For an ion at *m/z* 1000, with a peak width of 2u at half its height, the mass resolution is said to be 500 (or 1000/2).

2.5 MASS MEASUREMENT AND ACCURACY

The capacity of mass spectrometers to resolve ions associated with heavy isotopes (*e.g.* ^{13}C, ^{2}H) from those that contain only the lightest isotopes, enables a mass measurement to be made based on any one or several of these ion signals. For small molecules, the ion signals associated with heavy isotopes are typically of a low relative abundance since such isotopes are rare in nature. As a result, most mass measurements are based on the *peak of lightest isotopes*, and the resulting molecular weight of the compound is referred to as its *monoisotopic mass*. For carbon-based compounds, this is also referred to as the *^{12}C-only mass*, since ion signals at the lowest *m/z* ratios in the isotopic distribution contain no ^{13}C or ^{14}C. Carbon is considered exclusively here since the natural occurrence of heavy isotopes for other common elements in organic compounds are much lower compared with carbon.

Where an ion's isotopes are unresolved, a molecular weight measurement is based upon the *m/z* value at the peak top or a centroid value (where the centre of the peak top is determined from the area above a particular ion intensity level). Such a measurement yields an *average mass*, which contains a contribution to the molecular weight of the

compound from all elemental isotopes. By definition the average molecular weight of a compound is always greater than its monoisotopic value. This difference between the monoisotopic and average value can be quite significant for large molecules. As an example, the monoisotopic molecular weight of the protein ubiquitin, with an elemental formula of $C_{378}H_{629}N_{105}O_{118}S_1$ has a calculated value of 8,559.62 and average value of 8,564.86 Da. Special care must be taken where mass measurements are made based upon the calibration of the mass-to-charge scale using some ion signals with isotopes resolved and others where they are not.

As the molecular weight of a compound becomes large, the probability that any of its ions contain no heavy isotopes becomes exceedingly small. As a result, the peak of lightest isotopes for any of its ions becomes small relative to those ions that contain heavy isotopes. It is necessary in these circumstances to base the molecular weight on the *m/z* value of ions containing some level of heavy isotope. In the case of the electrospray mass spectrum of bovine ubiquitin, a mass measurement based on the ion signal for ions containing five ^{13}C atoms (or some other combination of heavy isotopes of equal mass, *e.g.* $^{13}C_4$ + ^{2}H) at *m/z* 779.613, is 8,564.66 (11 × 779.613 − 11 × 1.0078 to adjust for the 11 protons attached to the protein that gives the ions their charge). This experimental value is within 0.03 u of the theoretical value (8,564.63) at this level of isotope enrichment. Hence a mass accuracy of 0.04/8,564.63 = 3.5×10^{-6} or 3.5 parts-per-million (ppm) has been achieved.

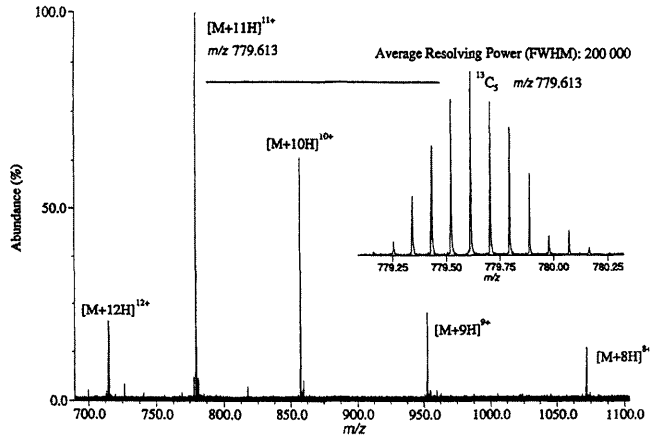


Figure 2.4 *ESI mass spectrum of the protein bovine ubiquitin recorded at high mass resolution (~200,000 at FWHM). Insert shows resolved isotope peaks for the* $[M + 11H]^{11+}$ *ions.*

The difference in mass-to-charge ratio between the isotope peaks for any ion (shown enlarged for the $[M + 11H]^{11+}$ ions to be 0.09 or 1/11 in Figure 2.4) is $1/z$. Thus the charge on any ion can be determined directly from the mass spectrum where its isotopes are mass resolved.

The level of heavy isotope content must be known or predicted in order for the molecular weight of the compound not to be in error. Alternatively, the compound can be constructed in an environment depleted of heavy isotopes such that ions associated with only the lightest isotopes for all elements are detected.

FURTHER READING

F.W. McLafferty and F. Turecek, *Interpretation of Mass Spectra*, 4th edition, University Science Books, 1993.

E. DeHoffmann and J. Charette, *Mass Spectrometry – Principles and Applications*, 2nd edition, John Wiley and Sons, New York, 2001.

H.M. Rosenstock, M.B. Wallenstein, A.L. Wahrhaftig and H. Eyring, Absolute Rate Theory for Isolated Systems and the Mass Spectra of Polyatomic Systems, *Proc. Natl. Acad. Sci. U.S.A.* 1952, **38**, 667–678.

R.G. Cooks, J.H. Beynon, R.M. Caprioli and G.R. Lester, *Metastable Ions*, Elsevier, Amsterdam, 1973.