CHAPTER 4

Tandem Mass Spectrometry

4.1 BASIC PRINCIPLES; PRECURSOR AND FRAGMENT IONS

With the exception of electron ionisation, all of the ionisation methods described in Section 3.2 lead to the formation of molecular ions (or pseudo-molecular ions) with little fragmentation. This is advantageous from the perspective of measuring the molecular weight of a compound, but a disadvantage if one wants to obtain structural information.

Tandem mass spectrometry was developed to derive structural detail originally through the use of two mass analysers between the ion source and the detector. A region between the two mass analysers is used to effect the dissociation of incoming ions. A schematic representation of a tandem mass spectrometry experiment is shown in Figure 4.1. The first mass analyser is used to transmit only ions of a particular *m/z* ratio into the dissociation region at any point in time. Within the dissociation region or chamber, ions are "excited" energetically through a number of different processes described within the next section. This leads to the production of fragments through bond cleavage of the precursor ions. The second mass analyser is scanned to pass, in turn, the products of the dissociation onto the detector. Since two mass analysis steps are involved, tandem mass spectrometry is often referred to as an MS/MS experiment and the resulting fragment or product ion spectra are known as MS/MS (or MS^2) spectra.

In practice the components of a sample are first surveyed in the MS-mode, usually by scanning the first mass analyser (MS-1) and passing all ions through MS-2 onto the detector. A series of tandem mass spectrometry experiments are then performed to detect the *fragment* (or *product*) *ions* (historically, *daughter ions*) for each of the *precursor* (or *parent*) *ions* formed in the ion source. The term *fragment ion* is preferred over *product ion* to differentiate them from the products of an ion-molecule reaction that have higher masses. The products of dissociation, in contrast, always have a lower mass since they represent a fragment or part of an intact molecular species.

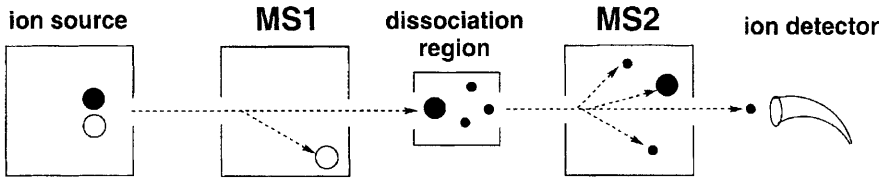

Figure 4.1 *Schematic representation of a tandem mass spectrometry experiment*

To derive structural information from MS/MS spectra one usually has to know some general information about the nature of the compound. Beyond this, the vast numbers of tandem mass spectra that have been obtained and interpreted for known compounds enable dissociation pathways and products to be assigned for an unknown compound with significant reliability. Studies over several decades have shown that certain bonds are more susceptible to dissociation than others, and particular structural features in a molecule can drive the fragmentation of ions in a predictable manner. Where bond cleavage takes place in the vicinity of a charged group, the fragmentation is termed *charge localised.* This is not to say that all molecular ions have charges fixed or localised at only one position; rather the molecular ions formed in the source may represent a mixture in which charge is positioned at different locations within the complete set of ions formed. In contrast, when bond dissociation takes place far removed in the molecule from a charge-bearing site the fragmentation is referred to as *charge remote*. The energy gained during the dissociation process may also lead to some migration of charge in an ion prior to bond cleavage, so some care has to be taken in defining a fragmentation process as charge remote.

These issues will be returned to later in Chapter 7 in tandem mass spectrometry studies of peptides, but first the ways in which an ion can be dissociated on its journey to the detector are considered.

4.2 DISSOCIATION PROCESSES AND THEORY

There are a number of dissociation methods utilised to add energy to ions and cause them to dissociate by bond cleavage. By far the most common of these is collisional activation.

4.2.1 Collisional Activation (CA)

If an ion collides with a neutral atom or molecule, some of the ion's kinetic energy is lost as its translational velocity decreases. The energy lost is converted into internal energy causing the ion to fragment. In the

collisional activation process, ions are collided with gas molecules (N) held at a moderately high pressure in a chamber or region between the mass analysers. Depending on the velocity of the ions and the density of the gas in the chamber, ions will undergo a few, or multiple, collisions with the gas. The overall dissociation process occurs in two steps, illustrated for positive-charged ions in equation 4.1.

$$m_p^+ + N \rightarrow m_p^{+*} \rightarrow m_f^+ + m_n \quad (4.1)$$

The first step involves energetic excitation of the precursor ion (m_p^+) through both electronic and vibrational processes, and the second involves the dissociation of the energetically-excited precursors (m_p^{+*}) to a fragment ion (m_f^+) and neutral portion (m_n). In the strictest sense, the first step is known as collisional activation (CA) and the second is referred to as *collisionally-activated dissociation* (CAD) or *collision-induced dissociation* (CID). It is necessary in a CAD experiment to maintain conditions such that the ions are fragmented into a few discernible species rather than obliterated into individual atoms. The latter would serve little use in deciphering the structure of the analyte.

The choice of gas is important in order to prevent the reaction of ions with gas molecules. The size of the gas molecule further impacts how the fragment ions are scattered and this needs to be minimised in order to assist in ion detection. Charge transfer to the gas should also usually be avoided. For these reasons, helium, argon and xenon are common collision target gases. They are all unreactive, monoatomic gases with high ionisation potentials.

4.2.2 Collisional Activation Theory

In the laboratory frame of reference, the description of a collision between an ion and a molecule involves the motion of each in three co-ordinates (x,y,z) and the internal energy of an ion can be defined as E_{lab}. To simplify the description of an ion-neutral collision, a centre of mass (CM) reference is adopted and the energy of an ion (E_{CM}) that is available for fragmentation depends on its mass (m_p) and that of the target gas (m_g) according to equation 4.2.

$$E_{CM} = E_{lab} \times m_g/(m_p + m_g) \quad (4.2)$$

If sufficient excess internal energy is deposited into an ion during collision to break chemical bonds, the ion will fragment. Increasing the ion's initial kinetic energy and/or the mass of the target gas can increase

the energy available for fragmentation. Multiple collisions with target gas molecules can also increase the internal energy of an ion to promote fragmentation, but at the same time also results in an increase in the probability of undesirable rearrangement reactions.

A principle shortcoming of the CAD process is a limitation in the amount of energy that can be deposited into a molecule. It has been calculated that as the mass of an ion increases, the internal energy it gains increases to a mass limit of approximately 1,500. Above this value the energy deposited during a collision begins to fall, leading to a practical limit for dissociation of a molecule of the order of 2,500 Da.

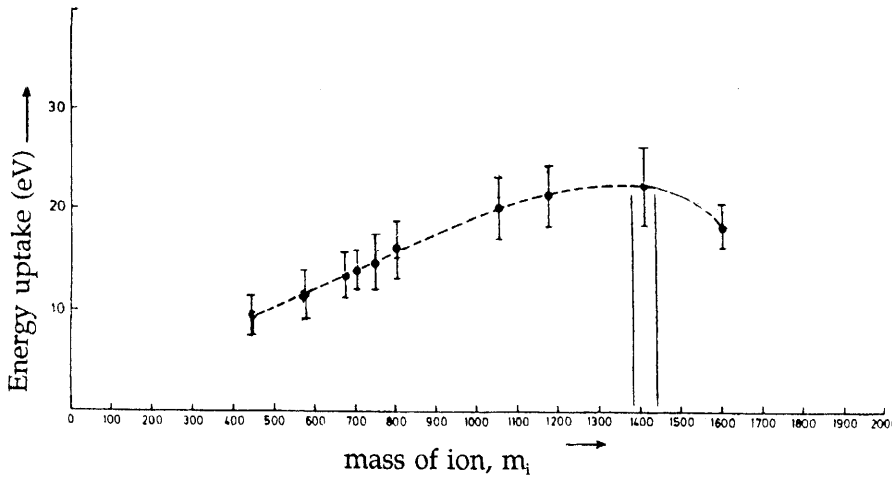

Figure 4.2 *Dependence of internal energy on the mass of the precursor ion* (Source: G.M. Neumann, M.M. Shiel and P.J. Derrick, Collision-induced decomposition of multiatomic ions, *Zeitschrift fuer Naturforschung, Teil A: Physik, Physikalische Chemie, Kosmophysik*, 1984, **39A(6)**, 584–92)

4.2.3 High (keV) and Low Energy (eV) Collisions

The term *high energy collisions* refer to those collisions between a neutral gas molecule and a precursor ion accelerated to kinetic energies of several kiloelectronvolts (keV). This is the case in magnetic sector and time-of-flight mass spectrometers where ions leave the source with energies of typically 3–30 keV. High-energy collisions lead to the excitation of electronic states in most molecules such that their ions have a broad range of internal energies. As a consequence, most structurally viable fragmentation processes are possible.

Because ions move rapidly through the collision chamber in high-energy experiments, ions are subjected to single or only a few collisions

with the target gas molecules. As a result, the mass of the target gas has a relatively small effect in high-energy CAD experiments because the centre of mass energy is a small fraction of the larger kinetic energy. This means that changes in the collision conditions (nature of the collision gas and its pressure in the collision chamber) do not result in significant changes in the MS/MS spectrum.

Low energy collisions, in contrast, occur when precursor ions have kinetic energies from a few eV up to a few hundred eV. Low-energy collisions are thought to excite vibrational states in a molecule and result in a much narrower range of internal energy distributions over high-energy collisions. A tandem mass spectrum resulting from 10 eV collisions can be dramatically different from one that results from 100 eV collisions and the nature of the target mass has a strong influence on the appearance of fragments in a low-energy CAD MS/MS spectrum. Heavier gases such as xenon and argon are often used in low-energy collision experiments to increase the probability of observing fragments. Ions undergo multiple collisions (tens to hundreds) as they pass through the chamber and internal energies are deposited in a stepwise manner and accumulated during each collision.

4.2.4 Charge Reversal and Stripping

Where an ion undergoes little to no dissociation during the collision process, it may lose electrons by *charge stripping* (equation 4.3) and undergo what is described as *charge inversion* or *charge reversal* (CR) (equation 4.4). The process of charge reversal can be useful for distinguishing the structures of ions where the CAD spectrum of the precursor ion exhibits few or no fragment ions, but its charge-reversed form readily dissociates.

$$M^{+\bullet} + N \rightarrow M^{2+\bullet} + N + e^- \tag{4.3}$$

$$M^{-\bullet} + N \rightarrow M^{+\bullet} + N + 2e^- \tag{4.4}$$

Collision energies of approximately 1 keV are required to effect the charge stripping of collisionally-activated ions.

Depending on the nature of the neutral gas, a charge exchange process (equation 4.5) can also occur. These reactions are exploited in *neutralisation/reionisation* (NR) experiments performed on tandem mass spectrometers. The neutralisation of positive ions is achieved using gaseous neutrals that have high ionisation efficiencies.

$$M^{+\bullet} + N \rightarrow M + N^{+\bullet} \tag{4.5}$$

4.2.5 Photon-Induced Dissociation (PID)

Ions that contain a chromophore, or light-absorbing unit, can absorb energy in the form of photons when irradiated. Single or multiple interactions with photons can lead to the uptake of energy greater than that required to break a chemical bond. Nearly all applications of PID make use of laser light delivered to the dissociation regions through ports in the instrument by means of conventional optics of the mass spectrometer or fibre optic cables.

One of the challenges of this method is to effect the interaction of significant numbers of ions with photons within the dissociation region. Quadrupole ion trap and ion cyclotron resonance mass spectrometers are useful in this regard, since ions are trapped or held for extended periods such that repeated interactions can take place. PID, however, has been applied on both magnetic sector and quadrupole instruments usually by slowing the ions as they pass through the dissociation chamber or by passing the light along the axis of the ion beam. Unlike molecules in solution, the ions cannot lose energy to the solvent and so may remain activated for sufficient time for dissociation to occur.

PID can also be selectively administered by irradiating ions at wavelengths at which only certain components absorb the light. It can also be used to direct the fragmentation of ions, in some cases simplifying the interpretation of MS/MS spectra through the generation of fragments of a common ion series.

4.2.6 Surface-Induced Dissociation (SID)

In the mid 1980s, Cooks and co-workers developed an alternate dissociation method known as *surface-induced dissociation* (SID). In SID, ions are collided or bounced off a solid or viscous liquid surface. Ions are projected perpendicular or at some angle off the normal axis (typically 25–30°) to a planar surface, or by passing ions through narrow channels.

Ions gain internal energy from the collision and subsequently fragment. The internal energy distributions of the fragments are usually quite narrow, and the collision energy can be controlled by the initial kinetic energy of the ions, the nature of the surface and the angle of deflection.

The approach offers the potential to dissociate much larger ions than has been possible by CAD. This, however, has largely been unrealised and a significant problem with the approach stems from the degree of scattering of the ions from the surface. This can lead to an appreciable loss in ion current at the detector. Nonetheless, the approach has an

advantage over CAD in that no gas is used for dissociation, thereby avoiding problems in maintaining low pressures within the mass analyser.

4.2.7 Electron Capture Dissociation (ECD)

An approach that has been successfully applied to the dissociation of large molecules, including intact proteins, is *electron capture dissociation* (ECD). Ions that pass through a high-current electron beam can be activated by ion-electron collisions.

The mechanism for this prompt fragmentation has been suggested to involve intramolecular proton transfer and ECD has been applied to sequence intact proteins by a so-called *top-down approach* in an impressive demonstration of the power of mass spectrometry. The observation that a significant proportion of backbone bonds are cleaved suggests the ECD process is *non-ergodic* (i.e. the energy is not randomised or distributed) since the localisation of a large proportion of the deposited energy is required to effect cleavage. Ions activated by collisional activation can be simultaneously subjected to ECD to enhance the level of fragmentation and thus the degree of structural detail. In the case of the protein cytochrome c (of ~12 kDa), all but 9 of the 103 amide bonds have been cleaved in activated-ion ECD experiments (Figure 4.3).

4.3 TANDEM MAGNETIC SECTOR MASS SPECTROMETERS

The first tandem mass spectrometers were those constructed of magnetic (B) and electric (E) sectors. Reverse-geometry BE mass spectrometers, in which a collision cell is located between the magnet and electric sector, were a common early form. The magnetic field strength is applied at a fixed value at any point in time leading to the transmission of ions of only one m/z ratio (equation 3.22). The products of CAD are then passed in turn onto the detector by scanning the electric field potential. The resulting MS/MS spectra are referred to as *mass-analysed ion kinetic energy spectra*, or MIKES.

4.3.1 Mass-Analysed Ion Kinetic Energy Spectra (MIKES)

Since an electric field separates ions according to their velocity only, the fragment ion peaks in MIKES are substantially broader than in other tandem mass spectra. This is because ions lose velocity in the direction of the precursor ion during collision. This leads to a broadening

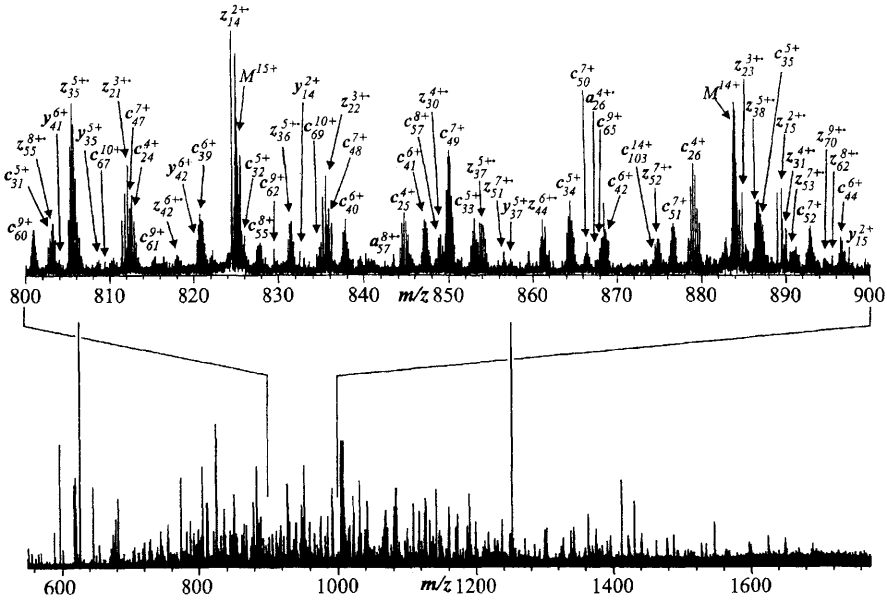

Figure 4.3 *Activated-ion electron capture dissociation MS/MS of the multiply-protonated ions of cytochrome c*
(Source: D.M. Horn, Y. Ge and F.W. McLafferty, Activated Ion Electron Capture Dissociation for Mass Spectral Sequencing of Larger (42 kDa) Proteins, *Anal. Chem.*, 2000, **72(20)**, 4778–4784)

of the ion signal over and above that associated with the distribution of ion energies from the source. A fragment ion m_f^+ formed from the unimolecular dissociation of the precursor ion m_p^+ will possess an energy of $z(m_f/m_p)eV$ where V is the accelerating potential used to extract precursor ions of charge z from the ion source. The fragment ions will be passed through the electric sector to the ion detector if the electric field strength is equal to $E_f = (m_f/m_p)E_p$. Thus MIKES are obtained by scanning the electric field from the value required to transmit the precursor ion (E_p) down to zero (or a value close to zero). The mean kinetic energy released during the formation of each of the fragment ions is measured based on the width of the peaks (at half maximum).

The peak shapes of the ion signals for the fragment ions are usually guassian in appearance. This is because each fragment ion is usually produced through a single dissociation pathway. However, where several pathways are involved and large kinetic energy releases occur, the peaks can have flat-top or dish-top shape (Figure 4.4).

To avoid broad ion signals in MS/MS spectra that lead to difficulties in assigning the correct m/z ratio to fragment ions, linked scans were

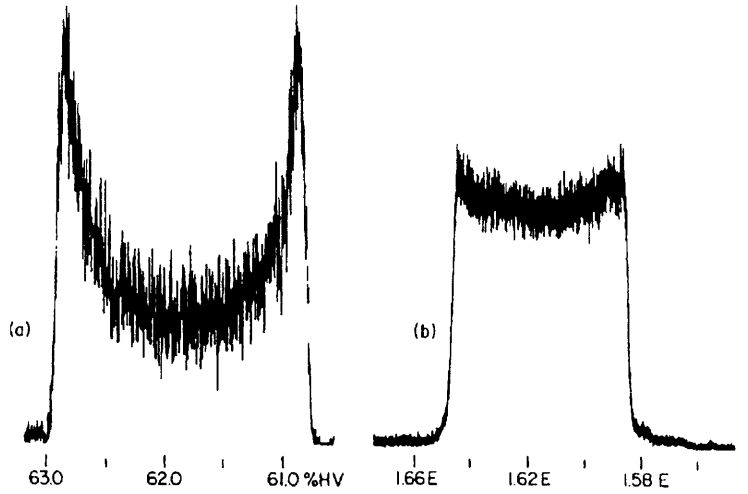

Figure 4.4 *Dishtop (a) and flattop (b) shaped ion signals evident in MIKES mass spectra* (Source: R.G. Cooks, J.H. Beynon, R.N. Caprioli and G.R. Lester, *Metastable Ions*, Elsevier, NY, 1973)

developed on two-sector mass spectrometers. These scan functions have subsequently been extended to a number of different instrument configurations.

4.3.2 Linked Scans

4.3.2.1 Fragment Ion Linked Scan. Linked scan experiments involve scanning the electric and/or magnetic fields of a mass spectrometer such that their values are always related or linked. One common linked scan is where the magnetic and electric field strengths are varied such that the ratio of their magnitudes is held at a constant value (B/E = constant). The dissociation of precursor ions is effected in a chamber immediately following the ion source on a two-sector BE or EB instrument. The initial values of B and E are those required to transmit a particular precursor ion to the detector. The B and E fields are then both successively reduced where the ratio of their magnitude (B/E) remains a constant value at all times. In this manner, all of the fragments for precursor ions of a single m/z ratio are brought into focus onto the detector. Since the fragment ions are both momentum and velocity focused the resulting MS/MS spectra achieve superior mass resolution over those obtained in MIKES. Yet since some translational energy is released during fragmentation, the mass resolution and accuracy is typically of the order of 1000. As the precursor ions are not mass-selected prior to collision,

they possess a range of velocities and energies and it is typical for all isotopes of all precursor ions and fragments to be detected in the MS/MS spectrum.

The mass-selection of precursor ions, however, can be achieved in multi-sector mass spectrometers. Instruments featuring a three-sector configuration (EBE or BEE) can record MIKES type spectra in which the precursor ions are both momentum and energy focused using the first two-sectors. A collision cell preceding the last electric sector provides a region to dissociate the precursor ions. Four-sector mass spectrometers have also been constructed for high resolution MS/MS experiments in both EBEB and BEBE configurations. In these mass spectrometers, momentum and energy focusing is achieved for both the precursor and fragment ions where a dissociation chamber is positioned in the centre of the instrument between the first and last two sectors. High-resolution four-sector tandem mass spectrometers were among the first to be applied to the sequencing of entire proteins by mass spectrometry.

4.3.2.2 Precursor Ion Linked Scan. A second common linked scan performed on magnetic-sector mass spectrometers is the so-called *precursor ion* (or B^2/E = constant) *scan*. In the case of two-sector BE or EB instruments, this scan enables the precursor ion from which a particular fragment is formed ahead of the mass analyser to be identified.

If the square of equation 3.18 is divided by equation 3.23 (see Chapter 3), one arrives at equation 4.6. Therefore, all ions that dissociate to a given fragment ion mass (m) are passed to the detector. This enables the precursor ion(s) from which a fragment originates to be determined.

$$B^2/E \propto m \tag{4.6}$$

In contrast to the B/E = constant scan, both B and E field strengths are scanned proportional to the square of the ions' velocities (v^2). Hence the magnetic field does not correct for the velocity spread of the precursor ions, and information on the kinetic energy released during an ion's fragmentation is retained. As a consequence, the mass resolution in the MS/MS spectrum is degraded, but in this case it is associated with the precursor ions (not the fragment ions as in MIKES).

4.3.2.3 Neutral Loss Linked Scan. A third linked scan useful in tandem mass spectrometry on magnetic-sector instruments is the *neutral loss linked scan*. Here, the identity of the precursor ion from which a neutral molecule was lost during dissociation is derived by using a scan in which the value for $B^2(1 - E)/E^2$ is held constant. In this experiment, a fragment ion formed between the ion source and the mass analyser of a two-sector

BE or EB instrument is passed to the detector only if it differs by a constant mass from the precursor ion from which it originates. This scan is useful for identifying the nature of the analyte, since certain neutral mass losses in MS/MS spectra can be characteristic of the fragmentation of particular classes of compounds (see Appendix 5).

The linked scans described in the above sections are just some of the linked scans that can be performed on magnetic-sector mass spectrometers. A further parameter that can be scanned alone, or in a simultaneous linked fashion, is the accelerating voltage. However, since this field has a dramatic effect on the successful extraction of ions from the source (low accelerating voltages remove fewer ions), its use is more limited.

4.4 TANDEM QUADRUPOLE MASS SPECTROMETERS

Early multi-quadrupole instruments were constructed to study photodissociation processes. A triple-quadrupole (QqQ) instrument in Melbourne, Australia was the first adapted for tandem mass spectrometry in the late 1970s. The first and third quadrupoles (Q) of such an instrument are operated using a combination of RF and DC voltages. The region about the second quadrupole serves as the dissocation chamber for CAD, surface and photodissociation, and is operated in the *RF-only mode*. For this reason, it is generally denoted by the lowercase letter, q. In the case of CAD experiments, either a closed cell is situated about the second quadrupole or a more open configuration is used in which the collision gas is passed into the region of the quadrupole and maintained at an appropriate pressure (Figure 4.5).

In a tandem experiment, the RF and DC voltages of the first quadrupole are set to values that allow only ions of particular mass-to-charge ratios to be transmitted. After dissociation of these ions within the second quadrupole, the fragment ions are passed in turn to the detector by scanning the RF and DC voltages applied to the third quadrupole (see Figure 4.1).

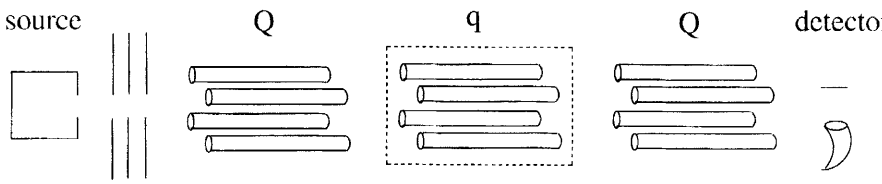

Figure 4.5 *Representation of a triple quadrupole mass spectrometer used for MS/MS experiments*

In the MS-mode, voltages applied to the first quadrupole are scanned to transmit all ions in turn through the third quadrupole to the detector.

In practice, mass discrimination effects impact the detection of some ions over others. Two modes of operation are usually employed to minimise these effects. One is a precursor ion transmission mode in which the amplitude of the RF voltage applied to the second quadrupole is set to a value that is a fraction of that required to transmit precursor ions. This results in the non-uniform transmission of fragment ions, which in the extreme may leave some products of dissociation undetected. The second fragment ion transmission mode involves scanning the RF amplitude of the second quadrupole to transmit each fragment ion with similar efficiency. It does, however, lead to a loss of precursor ions in the collision region that has a corresponding effect on fragment ion production.

Precursor ion scans can be performed on a triple quadrupole mass spectrometer by fixing the RF and DC voltages of the last quadrupole while scanning the voltages applied to the first quadrupole to transmit only fragment ions of a particular *m/z* ratio onto the detector. Neutral loss scans are performed by offsetting the voltages applied to the first and last quadrupole such that they transmit only fragment ions formed from precursors by a designated mass loss when scanned simultaneously.

In general, tandem mass spectrometry experiments are easier to perform on quadrupole based mass spectrometers over magnetic-sector instruments and operate at lower voltages. The former instruments, however, achieve lower mass resolution than sector instruments and are less able to exclude ions of similar *m/z* ratios to one another from the collision region. Triple quadrupole instruments also have found widespread use for studies of ion-molecule chemistries where a mass-selected precursor ion is studied in terms of its reactivity with a reactive gas added within the second quadrupole. Instruments featuring as many as five quadrupoles in tandem have been constructed to investigate successive reactions and also to enable MS^n (such as MS/MS/MS where $n = 3$) experiments to be performed.

4.5 TANDEM MASS SPECTROMETRY ON ION TRAPS

The MS/MS experiments described above are performed on instruments in which the mass-selection of precursor ions, their dissociation and fragment ion transmission and detection stages of tandem mass spectrometry are conducted in discrete sectors or regions of the mass spectrometer (Figure 4.1). In contrast, all stages of tandem mass spectrometry within ion traps (either quadrupole ion traps or ion cyclotron

resonance instruments) are conducted within the same physical space. In these experiments, precursor ion mass-selection, dissociation and fragment ion detection events are separated over time. Ion traps offer the advantage that, since ions are more confined in a common region of the instrument during tandem mass spectrometry, a greater number of ions can be fragmented and detected.

While there are practical differences in the operation of QIT and ICR mass spectrometers for this purpose, the concepts are the same. Ions are first formed and injected into the trap or cell after acceleration and the lowering of the voltage applied to the entrance to the trap. Ions are then excited to larger trajectories by applying an RF potential. In the case of QIT's, the resonant excitation of ions is based on increasing the amplitude of the RF potential to the ring electrode, while in an ICR it depends on the RF frequency applied to opposing plates perpendicular to the ion's initial motion. These extended paths cause the ions to collide with more gas molecules already present (in the case of QIT) or added as a pulse at high pressure (in the case of the ICR) to effect collisional-activation. The fragment ions from dissociation are then ejected sequentially from the trap onto the ion detector in the case of the QIT.

4.5.1 TANDEM MASS SPECTROMETRY ON QUADRUPOLE ION TRAPS

MS/MS spectra were first reported on a quadrupole ion trap in 1987. A typical tandem mass spectrometry experiment involves:

(i) the transmission of ions from the source into the trap where they are prevented from exiting by the voltages applied to the end caps
(ii) the selective isolation of the precursor ions by ramping the RF voltage to the ring electrode voltage above and below a particular value to store ions of a specific *m/z* ratio
(iii) the application of a resonance excitation RF voltage to the end-caps to induce faster and more extensive ion trajectories of the selected precursor ions
(iv) a lowering of the voltage applied to the end caps, with simultaneous ramping of the RF voltage applied to the ring electrode, to eject the remaining precursor ions and fragments.

The helium bath gas in the ion trap is used to both stabilise ion trajectories through collisional cooling and act as the collision gas to effect the dissociation of activated ions. Ion traps allow product ions

from the first stage of an MS/MS experiment to be trapped and reactivated thereby allowing multiple stages of mass analysis and dissociation to be carried out in so-called MS^n (*e.g.* MS/MS/MS *etc.*) experiments. As many as ten stages ($n = 10$) of tandem mass spectrometry have been performed on commercial instruments, though these experiments typically have little practical application.

4.5.2 TANDEM MASS SPECTROMETRY ON FT-ICRs

Tandem experiments are performed in a similar manner on FT-ICR mass spectrometers. However, since a FT-ICR cell operates at a significantly lower pressure to that of a quadrupole ion trap, the collision gas is delivered into the cell at high pressure only during the dissociation event. The pulsing of gas in and out of the cell during tandem mass spectrometry imparts a significant burden on the instrument's pumps. To overcome this, photon-induced and electron-capture induced dissociation approaches are frequently employed since they require no such variation in the pressure within the trap.

4.6 Tandem Mass Spectrometry on TOF/TOF Instruments

Mass spectrometers consisting of two time-of-flight mass analysers have recently been developed for tandem mass spectrometry. These instruments can consist of combinations of both linear and reflecting TOF mass analysers.

Particular advantages of this type of instrument for tandem mass spectrometry are that they are relatively inexpensive to construct and also achieve high transmission and detection sensitivities. They are more limited, however, in their ability to mass select precursor ions with unit mass resolution.

In a typical tandem experiment, precursor ions have flight times in the first flight tube given by equation 3.16 (Chapter 3) when accelerated from the source with a voltage, V. Mass-selection plates are positioned ahead of the collision cell within the flight tube. A high voltage is applied to these plates to deflect all ions from the flight path except at a time when ions of a particular m/z ratio (or range) reach them. At this time, the voltage applied to the plates is switched off allowing the mass-selected ions to enter the collision cell held at voltage, V_c. Intact precursor ions leaving the cell have kinetic energies given by equation 4.7. Their time-of-flight in the second TOF analyser can be expressed by equation 3.16 where l is the length of the second flight tube.

$$1/2m_p v^2 = ze(V - V_c) + zeV_c = zeV \tag{4.7}$$

In contrast, fragment ions of mass, m_f, leaving the cell have kinetic energies given by equation 4.8.

$$1/2m_f v^2 = zem_p/m_f(V - V_c) + zeV_c \tag{4.8}$$

Their time-of-flights will be given by equation 4.9, where l is the length of the second TOF mass analyser.

$$t^2 = m_p/z(l^2/2eV)\, l^2/[l + V_c/V(m_p/m_f - 1)] \tag{4.9}$$

These equations ignore contributions from the time ions spend in the deceleration and acceleration regions about the collision cell. It is usually desirable to slow ions as they pass into the cell to maximise the time available for dissociation.

A schematic representation of a tandem TOF instrument RTOF/RTOF featuring two ion reflectors is shown in Figure 4.6. These mass spectrometers are finding wider user for investigations of biological molecules, particularly in proteomics applications (see Chapter 7).

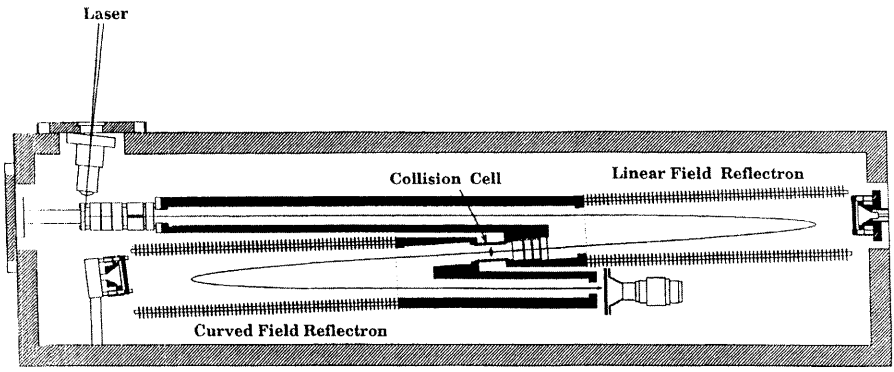

Figure 4.6 *Schematics representation of a tandem RTOF-RTOF mass spectrometer featuring both homogeneous field and "curved-field" reflectrons*
(Source: T.J. Cornish and R.J. Cotter, *Rapid Commun. Mass Spectrom.*, 1994, **8**, 781–785)

4.7 TANDEM MASS SPECTROMETRY ON HYBRID INSTRUMENTS

Replacing the first TOF analyser of a TOF/TOF instrument with a double-focusing sector mass spectrometer or a quadrupole mass filter enables mass-selection to be effected with unit mass resolution. A number

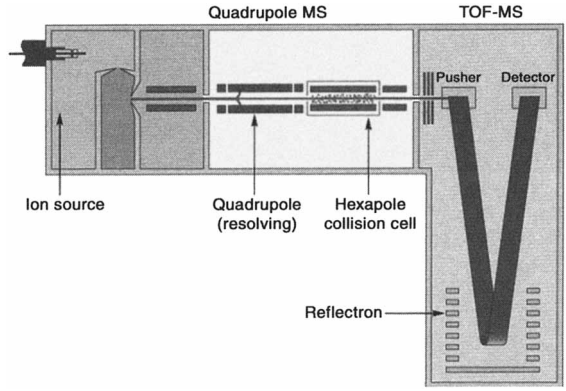

Figure 4.7 *Schematic representation of a tandem Q-TOF mass spectrometer featuring an orthogonal ESI ion source, hexapole collision cell and reflecting time-of-flight mass analyser*
(Source: Courtesy Micromass)

of hybrid instrument designs have been constructed including those of EB-TOF and Q-TOF (Figure 4.7) geometry.

Since ions are typically produced in a continuous manner from the source, it is necessary to extract packets of ions in order that time-of-flight measurements can be performed. This can result in a considerable loss in sensitivity if only small portions of the ion beam are subjected to dissociation and ultimately detected by TOF-MS. To minimise this, it is typical to project ions down the time-of-flight tube orthogonal to their initial trajectory. Since the TOF mass analyser in effect detects all fragment ions produced within a few hundred microseconds, the analysis is more sensitive than on scanning instruments that transmit only ions of a specific *m/z* value to the detector at any point in time.

In the tandem experiment, the mass-selected ions from the magnetic-sector or quadrupole analyser are decelerated prior to entering a dissociation chamber. The fragment ions are extracted using a pulsed accelerating voltage at right angles to their initial path. Since the distribution of velocities of the fragments is minimal in the direction of the TOF tube, reasonable focusing and mass resolution in the MS/MS spectrum is achieved.

Parent ion scans are performed on a Q-TOF instrument by fixing the time at which fragment ions of a particular *m/z* value reach the detector. The quadrupole is then scanned to determine values of *m/z* for the precursor ions during which fragment ions are detected.

FURTHER READING

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