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SPECIAL FEATURE: TUTORIAL

Tandem Mass Spectrometry: a Primer

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Tandem mass spectrometry (MS/MS) employs two stages of mass analysis in order to examine selectively the fragmentation of particular ions in a mixture of ions. The various types of instruments which can be used to perform this experiment are described, including those based on separation of the mass-analysis events in time and those based on measurements in physically separate analysers. The several MS/MS scan types—product scans, precursor scans and neutral loss scans—are presented and examples of their applications are provided. These include the characterization of individual compounds and the recognition of groups of compounds with specified functional groups. Applications are shown to the determination of compounds present in complex mixtures. The improvement in signal-to-noise ratio achieved by MS/MS when compounds are ionized by methods which produce high chemical noise is illustrated for choline derivatives. Fundamental aspects of collisional activation in the low and high collision energy range are reviewed.

INTRODUCTION

A mass spectrum is a display of the relative abundances of ions produced in an ion source as a function of their mass-to-charge ratios. If a pure compound is analysed using a clean electron impact source, the spectrum will be devoid of peaks that do not belong to the analysed compound, and many fragments useful for its structural identification will be observed. However, electron impact ionization often does not produce a molecular ion, is not applicable to non-volatile compounds and is difficult to apply directly to mixtures. Chemical ionization is also applicable only to volatile compounds, in most cases produces abundant molecular ions, typically gives fewer and less informative fragment ions than electron impact and produces peaks originating from the reagent gas. Desorption and nebulization methods such as fast atom bombardment (FAB), atmospheric pressure chemical ionization (APCI), and electrospray ionization (ES) can be applied to non-volatile compounds. These ionization techniques give abundant ions of the molecular species by yield relatively few fragments which provide relatively little structural information. Most significantly, for the present discussion, these spectra are also complicated by the presence of ions derived from the sample matrix.

There is a need to be able to select ions of a given mass produced in the source and observed in a mass spectrum, to fragment these ions and to analyse the frament produced. The goals in such an experiment are to obtain more structural information on a particular ionic species, either (i) because its fragmentation is obscured by the presence of other compounds in the mixture introduced into the ion source, or (ii) because it is obscured by other ions generated from the matrix in the course of ionization or (iii) because the ionization method chosen yields relatively few structurally diagnostic fragments. Many different instruments and techniques are now in use to achieve these objectives. Furthermore, the selective detection of ions that yield a given fragment or lose a given neutral is also possible with appropriate tandem mass spectrometric (MS/MS) techniques. This capability has allowed the development of selective methods to detect targeted compounds or classes of compounds. The aim of this tutorial is to review the simple principles which underlie what is a diversity of instruments, activation conditions and scan modes. The most widely used techniques and instruments are described together with an introduction to the main scan modes, comments on the collision-induced dissociation process and examples of some applications.

Pioneering work in the area of MS/MS is due to the work of Beynon, Cooks and co-workers[1] on reverse-geometry magnet instruments, to the development of collision-activated dissociation by McLafferty *et al.*[2] and Jennings[3] and to the introduction of the triple quadrupole mass spectrometer by Yost and Enke.[4]

MS/MS INSTRUMENTATION

There are two main categories of instruments that allow MS/MS experiments.[5] The first category is made up of instruments in which two mass spectrometers are assembled in tandem. Two mass-analysing quadrupoles, or two magnetic analyser instruments or hybrids containing one magnetic and one quadrupole spectrometer are representative cases. The arrangement of sectors used in high-resolution instruments, consisting of a magnetic and an electric sector, can also be used as a tandem mass spectrometer, but with limited capabilities as discussed below. Coupling of other analysers has also been described.

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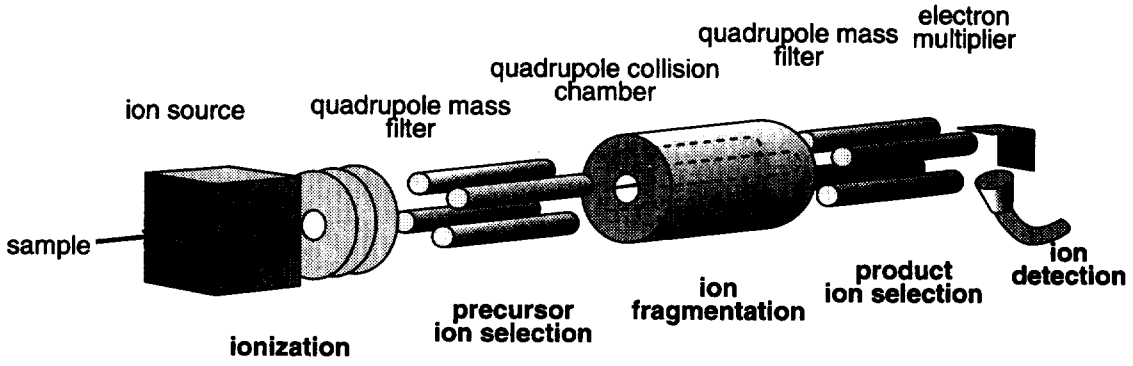


Figure 1. Schematic diagram of a triple quadrupole mass spectrometer.

The second category of MS/MS instruments comprises analysers capable of storing ions: the ion cyclotron resonance (ICR)[6] and the quadrupole ion trap[7] mass spectrometer. These devices allow the selection of particular ions by ejection of all others. The selected ion can be excited and caused to fragment during a selected time period, and the fragment ions can be observed in a mass spectrum. This process may be repeated to observe fragments of fragments, over several generations. The first category of instruments uses a sequence of mass spectrometers in space, while the second category uses one spectrometer with ion storage capability to exploit a sequence of events in time.

At present, the triple quadrupole[4] is the most widely used tandem mass spectrometer. It is a linear assembly of three quadrupoles as shown in Fig. 1. Only the first and the third quadrupoles are mass analysers, being operated with the combination of both r.f. and d.c. potentials necessary for mass selection. The second quadrupole, the central one, has a fixed r.f. voltage only. Thus, ions of every mass can pass this quadrupole, which is used as a collision cell with ion focusing properties. The focusing property results from the effect of the 'saddle field,' illustrated in Fig. 2. A positive ion will travel towards the negative rod but, owing to the frequency of the applied signal, the polarity of the positive rods quickly changes to positive and *vice versa*. This change in polarity can be compared to a saddle on which a ball has been placed. The ball will roll down the slope, but if the saddle is quickly rotated by 90°, the rolling ball will face a hill, and will roll back to the center of the saddle. If there are several balls and the trajectories change owing to collisions between them, they will be driven back to the centre by the effect of the rapidly rotating saddle. This is analogous to what happens to the ions in the central quadrupole. Even if they undergo collisions with a neutral gas in the cell, the effect of the r.f. potential will bring them back to the centre of the device. This means that loss of ions by scattering after collision is avoided.

An offset voltage between the source and this quadrupole collision cell can be adjusted, to allow the collision energy to be varied between zero and several hundred volts. This is low compared with magnetic instruments, where the usual values are fixed somewhere in the range 2–10 keV. However, a relatively large number of collisions are usually allowed to occur in a triple quadrupole collision cell, so that the conversion of the main beam of parent ions into product ions is normally much greater than the corresponding value for a sector tandem mass spectrometer. Important advantages of triple quadrupole instruments are relatively lower cost and ease of use. Once both quadrupole mass analysers have been calibrated switching between different scan modes and mass ranges can be done instantaneously, and with unit mass resolution in both analysers for all types of MS/MS experiments.

Instead of quadrupoles, two double-focusing magnetic instruments may be combined into a four-sector type[8] as shown in Fig. 3. These instruments allow one to achieve high resolution on the precursor ions with unit resolution on the fragment ions. Collisions occur in a collision cell, generally at high energy, but sometimes at lower energy to improve product ion resolution. Low-energy collisions require that the ion beam be decelerated and then re-accelerated as the ions need to

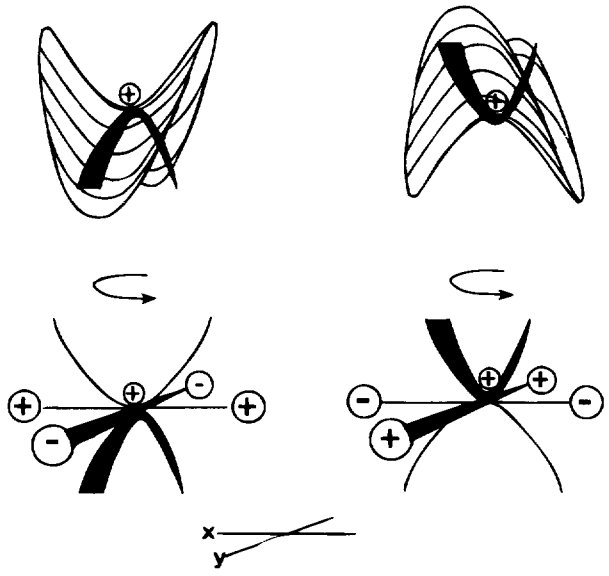


Figure 2. Ball-on-a-saddle-point model of ion trapping in a quadrupole electric field. (Top) the ball will tend to roll down, but if the saddle is rotated the ball will be constrained to roll back to the middle of the saddle. Similarly (bottom), a positive ion submitted to a quadrupolar field will move towards a negative electrode, and *vice versa* for a negative ion. However, if the field polarity changes sufficiently quickly, the ion will be brought back to the centre of the structure. This is the principle of the focusing effect of r.f.-only quadrupole collision cells and of ion trapping in the quadrupole ion trap. (Reproduced from Ref. 32, Fig. 2.8.)

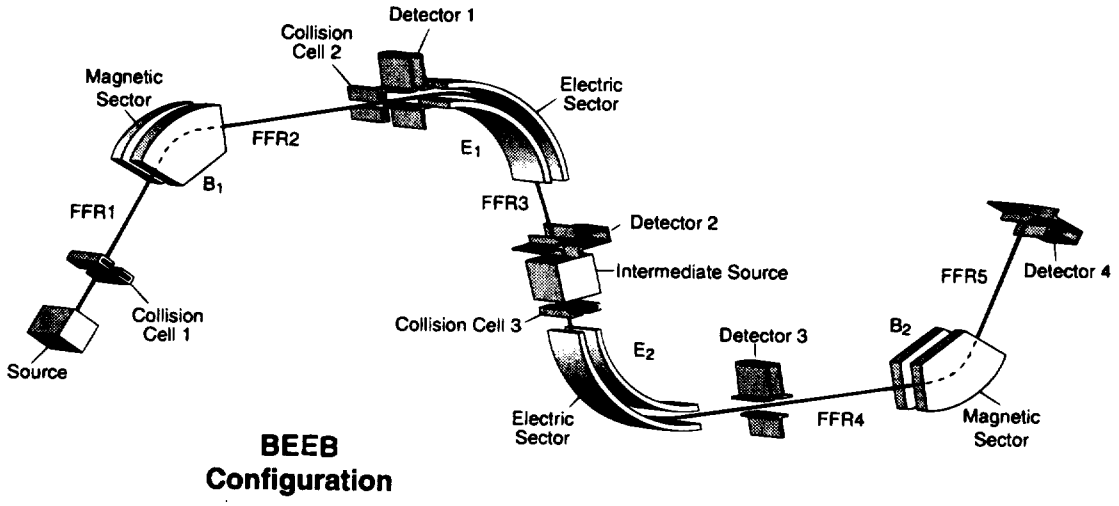


Figure 3. Schematic diagram of one design of a four-sector mass spectrometer of geometry BEEB, showing various field free-regions in which collisions can occur.

have high kinetic energy as they traverse the analysers. In a product ion scan, a precursor is selected by the first set of analysers and the fragment analysed by the second set. These sectors must be scanned in the *B/E* linked scan mode, as the fragments do not have the same kinetic energy. However, this method of scanning is not necessary if the fragment ions have been generated as a result of low-energy collisions and then reaccelerated.

The combination of a magnetic sector mass spectrometer with a quadrupole mass filter, to form a hybrid instrument[9] as shown in Fig. 4, has also seen considerable use. This instrument is much simpler than the four-sector type, allows high-energy collisions in addition to low-energy collisions and provides high-resolution parent ion selection and unit-resolution product ion detection. It has the advantages of high efficiency fragmentation associated with the quadrupole collision chamber and mass analyser. Both BEQ and EBQ configurations have been used.

Another type of instrument combines a magnetic mass spectrometer with an orthogonal time-of-flight analyser.[10] As already mentioned, when ions fragment during their flight, unimolecularly or upon collision-induced dissociation (CID), precursor and fragments have the same nominal velocity and will arrive together at the detector. If, however, they are accelerated perpendicularly to their flight into time-of-flight mass spectrometer, they will arrive at different times on an array detector, and can thus be detected according to their mass. This arrangement also has the advantage of minimizing the effects of kinetic energy release on resolution as explained in a recent Feature Article on time-of-flight spectrometry.[11] Figure 5, illustrates the principle of such an instrument.

SCAN MODES IN SEQUENTIAL ANALYSER-BASED MS*

The three main scan modes available using tandem mass spectrometers, illustrated in Fig. 6, are product ion, precursor ion and neutral loss scans. A convenient

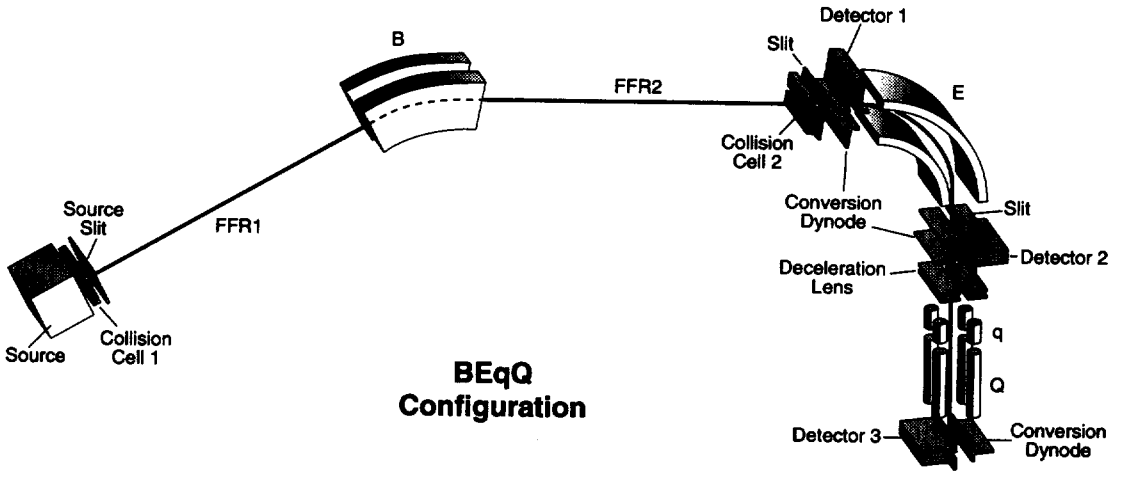


Figure 4. Schematic diagram of one design of a hybrid tandem mass spectrometer of BE(q)Q geometry, where B indicates a magnetic sector analyser, E and electric sector analyser, Q a quadrupole mass filter and q a collision (r.f.-only) quadrupole.

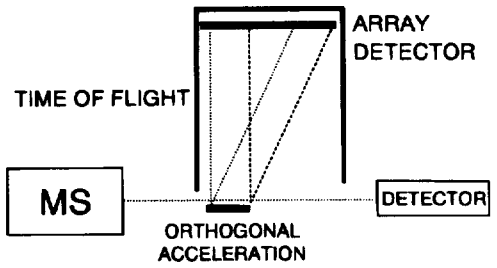


Figure 5. Combination of a sector mass spectrometer with an orthogonal time-of-flight mass spectrometer. Ions coming towards the detector from the sector mass spectrometer (MS) are accelerated orthogonally by a voltage pulse applied to the orthogonal acceleration repeller, and analysed by the time-of-flight instrument. Faster ions will impinge further down the array detector.

symbolism[12,13] for describing the various MS/MS experiments is presented in Fig. 7. The two mass spectrometers may, *a priori*, be of any kind. The most common type is that in which the analysers are quadrupole mass spectrometers and the collision cell includes a focusing quadrupole, hence the name triple quadrupole[4] (see above for further discussion of this instrument). Other frequently used types consist of either a magnetic and a quadrupole mass spectrometer or two magnetic mass spectrometers. Magnetic instruments consisting of one magnetic and one electric sector can also be used to perform MS/MS experiments, but they have limited capabilities. Discussion of these instruments is given below in the section on mass-analysed ion kinetic energy (MIKE) spectrometry and linked scans.

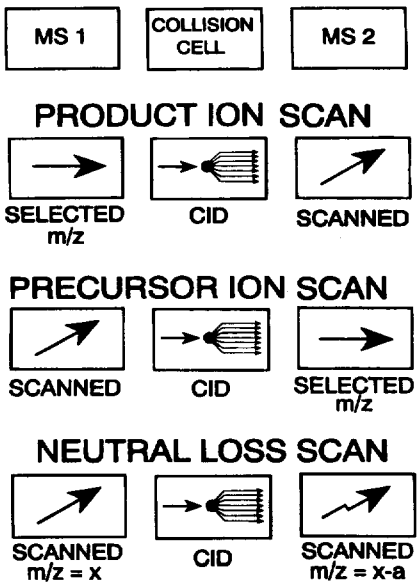


Figure 6. MS/MS experiments based on analysis using two spatially separate mass analysers, MS 1 and MS 2. Between the analysers a collision cell (CID) containing an inert gas is used to induce fragmentation.

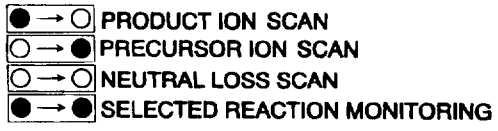


Figure 7. Symbolism proposed by Kondrat *et al.*[13] for the representation of various scan modes, where ● refers to a fixed mass analyser and ○ refers to a scanning mass spectrometer. Note that neither analyser is scanned in the reaction monitoring experiment.

Product ion scan

The most common tandem mass spectrometric experiment is the product ion scan. In this experiment, ions of a given m/z value are selected with the first mass spectrometer. The selected ions are passed into the collision cell, typically filled with helium, argon or xenon. The ions are activated by collision, and therefore are induced to fragment. The product ions are then analysed with the second mass spectrometer, which is set to scan over an appropriate mass range. A product ion spectrum, formerly known as a daughter ion spectrum, is obtained. Such a spectrum allows one to record fragments arising from the molecular ion of a specific compound present as a component in a mixture and generate fragmentation data that can be used to provide information on the structure of the selected ion when necessary.

If a reactive gas is introduced into the collision cell of a tandem-in-space mass spectrometer (or into an ion trapping instrument), ion–molecule reactions can be observed.[14] In multiple analyser mass spectrometers, the time allowed for reaction will be short and can be varied over only a limited range. Moreover, it is difficult to achieve the very low collision energies which promote exothermic ion–molecule reactions. Nor will equilibrium be achieved, except with very special dedicated instruments. Nevertheless, product ion spectra arising from ion–molecule reactions can be recorded, and increasing use is being made of these spectra as an alternative to CID in characterizing ions.

Precursor ion scan

Instruments based on the coupling of two mass spectrometers allow so-called precursor ion scans, also known as parent scans, to be recorded. In this scan mode, the second mass spectrometer is set to pass only ions with a particular, selected m/z value. The first mass spectrometer is scanned over a chosen mass range, with a collision gas present in the instrument. Ions which pass through the first mass spectrometer will be detected if, and only if, after fragmentation (or more generally, reaction) in the collision cell it produces the pre-selected product ion. This product ion is the only ion that the second mass analyser can transmit to the detector. For example, if the second analyser is set on m/z 77 ($[C_6H_5]^+$), the precursor ion scan will provide a record of compounds containing the phenyl group. Adventitious formation of m/z 77 by other ions or non-routine fragmentation by phenyl-containing ions which do not yield m/z 77 as a product, can of course interfere with this determination. Note that the experiment is selective for ions containing particular functional groups, and that it yields the masses of all the ions which satisfy this criterion. In many experiments, this information corresponds to the molecular masses of compounds containing the functional group in question.

Neutral loss scan

As in the case of the precursor ion scan, this scan mode cannot be performed with time-based tandem mass spectrometers. It is a form of functional group-selective scan but is more complex in practice than the precursor ion scan, since it requires that both analysers are now scanned together, but with a constant m/z difference between the two spectrometers. This scan allows the selective recognition of all ions which, by fragmentation, lead to the loss of a given neutral fragment.

SCAN MODES IN TIME SEQUENCE-BASED MS"

Spectrometers having ion storage capabilities, viz. ion traps or ICR spectrometers, allow the sequence of ion manipulation events displayed in Fig. 8.[15,16] CID occurs in the spectrometer cell itself, by collision with a gas which is continuously present or introduced for a short time for the purpose. The two first time sequences may be repeated before the mass scan, allowing one to select ions with a given m/z value, fragment it, then select a product ion and also allow it to fragment, and so on. Finally, the ions in the cell are analysed. In the case of ion storage spectrometers, the product ion scan is the only one available directly. However, with these instruments the process can be repeated: an initial precursor is selected and fragmented, then one of the fragments is selected as the new precursor and allowed to fragment in turn. This process may be repeated again and again. Finally, the product ions in the storage cell are analysed, yielding an MS" product ion spectrum. With hardware sequence experiments, this would necessitate the coupling of more than two mass spectrometers, and although this has been done, in the form of the pentaquadrupole spectrometer, it is not common. Time-based instruments greatly facilitate multi-stage product ion experiments, but on the other hand do not allow precursor or neutral loss scans to be performed.

Ion storage-based instruments facilitate the observation of ion–molecule reactions. Reaction times can be extended over appropriate time periods, typically as long as several tens of seconds. It is also possible to vary the reactant ion energy. This allows one to observe equilibrium processes in the gas phase. This method with ICR instruments has allowed the establishment of scales of basicities and acidities in the gas phase.[17,18] For example, the position of the proton transfer equilibrium between aniline and ammonia establishes that in the gas phase aniline is a stronger Brønsted base than ammonia.

A significant difference between the two types of trapping instruments is that in the ion trap mass spectrometer, ions are expelled from the trap to be mass analysed. Hence they can be observed only once, at the end of the process. In Fourier transform MS instruments, they can be observed non-destructively, and hence measured at each step in the sequential fragmentation process.

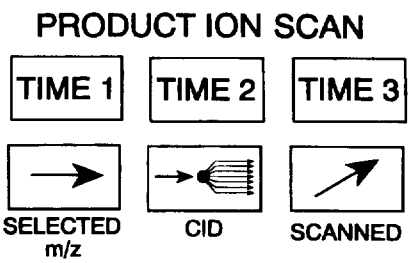


Figure 8. MS/MS based on temporally separate analyses in a single mass spectrometer.

LINKED SCAN AND MIKE SPECTROMETRY

Tandem mass spectrometry can be performed with magnetic instruments consisting of an electric (E) and a magnetic (B) sector, arranged in either BE or EB geometry. The former, the MIKES or reversed geometry instrument (so-named because its geometry is the reverse of the geometry normally used in high-resolution mass spectrometers up to that time) was the first type of instrument used for MS/MS experiments.[1] When an ion of high kinetic energy fragments in a field-free region in such an instrument, the product ions will have almost the same velocity as the precursor. A small amount of energy might be lost in the course of activation by collision and individual ions receive a small velocity component due to kinetic energy released during the fragmentation. However, to a reasonable approximation, the average velocity will be unchanged so that an electric sector which measures kinetic energy functions as a mass analyser since all the product ions are of fixed velocity. The situation is depicted in Scheme 1. There is a great deal of information on the fragmentation process embedded in the details of the shapes of the kinetic energy peaks, a subject exploited in MIKE spectrometry. However, this instrument also provides ready access to simple MS/MS data in the form of product and precursor scans.

In fact, it is possible to perform any of the three principal types of MS/MS experiment with either the BE or the EB geometry instruments by scanning the two analysers in the appropriate relationship to each other.[19] It is not necessary for the ion beam to be mass selected prior to passing through the second sector; the fragmentation may occur in the field-free region between the source and the first sector. In this experiment, the precursor ion will be focused by the magnetic sector operating at a magnetic field B, if $qr_B B_p = m_p v$, where q is the number of charges on the ion, r is the radius of curvature of the ion path in the magnetic field or electric sector, the subscripts B and (later) E refer to a given parameter in a magnetic field and electric sector, respectively, and the subscript p is used to denote precursor and (later) f to denote fragment. The fragment ion will have a mass m_f but the same velocity v, and the

	PRECURSOR ⟶	FRAGMENT +	NEUTRAL
Mass:	m_p	m_f	m_n
Velocity:	v	v	v
Momentum:	$m_p v$	$m_f v$	$m_n v$
Kinetic energy:	$m_p v^2$	$m_f v^2$	$m_n v^2$

Scheme 1. Properties of ions in high-energy collisions.

value B_p required to focus the fragment ion will be $qr_B B_f = m_f v$. It is obvious that the ratio of these two equations will give $B_p B_f = m_p/m_f$. For the electric sector, the corresponding equations are $qr_E E_p = m_p v^2$ and $qr_E E_f = m_f v^2$, leading to the ratio $E_p/E_f = m_p/m_f$.

Hence the condition under which a fragment ion, generated in the region before both sectors, can be focused through both is $B_p/B_f = E_p/E_f$ or $B_p/E_f = m_p/m_f$. Thus, scanning the two sectors simultaneously, while holding the ratio B/E constant, will allow the detection of all the product ions from a given parent. This is the principle of the B/E linked scan, a method of generating a product ion spectrum, and can be performed with instruments of either the BE or EB configuration. Analogous linked scan experiments can be used to perform precursor and neutral loss scans.

COLLISIONAL ACTIVATION

Ions fragmenting spontaneously during their lifetime are termed metastable ions.[1] As they generally yield few fragment ions, often in relatively low abundance, collision with an inert gas is often used to promote fragmentation and to increase the variety of product ions. The acronyms CAD, for collision-activated dissociation, and CID, for collision-induced dissociation, are both in use to describe this process. A recent Feature Article described the history of this technique,[20] and fundamental aspects are detailed in the recent literature.[21,22] To achieve collisional activation in MS/MS instruments with spatially separate analysers, a collision cell is placed between the two mass analysers to promote fragmentation. This cell, often simply a small chamber with entrance and egress apertures, contains an inert target gas at a pressure sufficient for collisions with ions to occur. Alternatively, the cell may contain an r.f.-only quadrupole, used as a focusing device to force scattered ions back to the centre of the analyser (the ion optical axis). In MS/MS instruments based on time-separated mass analysis steps, an inert gas is simply introduced into the ICR or ion trap instrument. In some elaborations of these experiments, it is introduced only into a certain region (e.g. in the dual-cell ICR) or at a particular time in the operating sequence using a pulsed valve. As a result of collisions, the internal energy of the ion may be increased by conversion of kinetic energy into internal energy. The choice of collision gas, its pressure, the collision energy and the angle over which scattered products are collected all affect the internal energy deposited in the projectile ion.

Two collision regimes have been explored thoroughly, those in which the ions have less than 100 eV kinetic energy, and those in which they have several keV. The maximum collision energy (E_{kin}) that can be converted into internal energy (E_{int}) in a single collision is given by the equation

$$E_{int} = E_{kin}\, m_{target}/(m_{ion} + m_{target})$$

where m_{ion} is the mass of the incoming ion and m_{target} the mass of the neutral gas atoms or molecules used as target. It must be emphasized that ions will be scattered after the collision. At low energy, one can make use of an r.f.-only quadrupole as a collision cell in order to bring scattered ions back to the ion optical axis, thus reducing ion loss by scattering. At low energies the interaction time between ion and target in the collision is long enough to allow direct vibrational excitation. This does not apply at high energies. At several keV collision energy, the interaction time for a small ion, of mass up to some hundreds of daltons, is of the order of 10^{-16} to 10^{-15} s. This is too short to be accompanied by a vibration (typically 10^{-14} s) but is about the time of an electronic excitation. Furthermore, at high energy, ions scattered by more than about 1° will not be observed. R.f.-only collision cells cannot be used in this energy range. As a result, products from grazing collisions, thus having small scattering angles, will be predominantly observed. Deliberate selection of ions scattered through larger angles results in the observation of the products of harder collisions, in which more internal energy is deposited in the projectile ion, a technique known as angle-resolved mass spectrometry.[23]

The pattern of the fragments observed in a mass spectrum will depend on the internal energy of the precursor ion. In MS/MS, this is chiefly controlled by the collision energy and the number of collisions. A first and obvious result is that at lower collision energies, fragmentation occurring in one step will dominate, while at higher energy fragments resulting from consecutive fragmentations will be more important. Another, less obvious, difference is that fragmentations that involve a rearrangement will be more important in spectra recorded at low energy. This is illustrated by the internal energy dependence of the rate constants for the fragmentation reactions of the tetramethylammonium cation as shown in Fig. 9. This ion undergoes successive loss of a methyl and a hydrogen radical, in addition to the loss of a methane molecule through a rearrangement process.[24]

Some ions, whose thermochemistry is well known, may be used to probe the actual internal energy of the excited precursors ions after collision.[25] One reaction

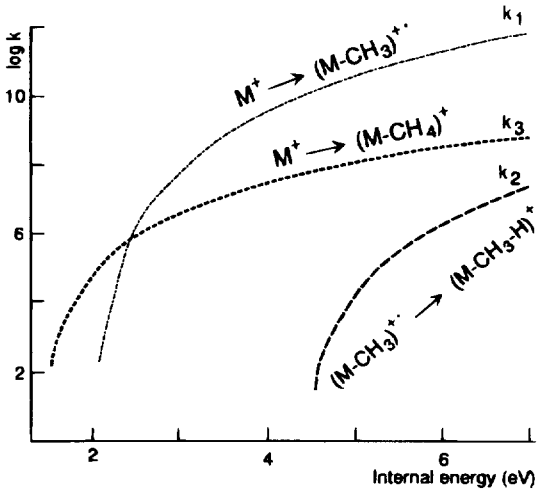


Figure 9. Fragmentation rate constant vs internal energy for three reactions of the tetramethylammonium cation. At very low energy, only the fragmentation accompanied by rearrangement (loss of CH_4) is fast enough to be observed. From about 2 eV energy, the loss of a methyl radical is observed, and from about 5 eV, the consecutive loss of a hydrogen atom occurs. (Adapted from Ref. 5, Fig. 3.2.)

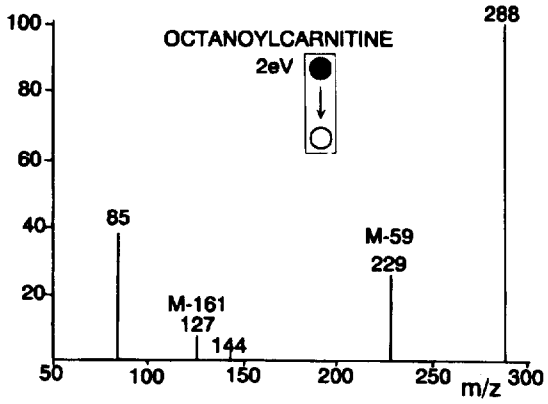


Figure 10. Product ion mass spectrum of the octanoylcarnitine cation. For the nature of the fragments, see Scheme 2. (Adapted from Ref. 32, Fig. 4.5.)

used is the fragmentation of triethyl phosphate, which undergoes consecutive fragmentations with well known energy requirements. A measure of the relative abundances of the fragments allows the determination of the precursor ion internal energy. Another often used 'thermometer' ion[26] is *n*-butylbenzene, the fragmentation of which produces ions of *m/z* 92 ($[C_7H_8]^{+\cdot}$) and 91 ($[C_7H_7]^+$) in relative abundances which are a measure of the internal energy distribution of the precursor ion.

Collisions at high energy not only increase the internal energy of an ion but also lead to changes in the charge state of the ion. Charge-inversion reactions (negative precursor to positively charged product ions) are readily observed in high-energy collisions:

$$M^- \rightarrow M^{+\cdot} + 2e^-$$

Charge stripping reactions also can occur:

$$M^{+\cdot} \rightarrow M^{2+} + e^-$$

As both of these reactions, by necessity, can only result from electronic excitation, they suggest that collision activation under similar conditions also results, at least in part, from electronic excitation. Note, however, that especially for larger molecules, the mechanisms of collisional activation is more likely to involve vibrational excitation. This may occur through impulsive collision of the target atom or molecule with a selected atom or group of atoms in the region of the collision site.[27] Alternatives do exist to the use of collisional activation to perform MS/MS experiments, particularly the use of photons for photodissociation, which has been performed particularly in ICR and in tandem time-of-flight instruments.[28–30] In addition, collisions with surfaces, a process known as surface-induced dissociation, have begun to be used more frequently.[31]

SOME APPLICATIONS OF MS/MS

Figure 10 shows the CID product ion spectrum of the quaternary cation octanoylcarnitine. The corresponding fragmentation scheme is given as Scheme 2. A typical feature of this product ion spectrum is the absence of isotopic masses. This is because the selected precursor ion (*m/z* 288) contains only the main isotopes ^{12}C, ^{1}H, ^{14}N and ^{16}O. At the low precursor ion kinetic energy (2 eV) at which this triple quadrupole spectrum is recorded, few fragments are observed, yet they are structurally informative. Moreover, the interpretation of this spectrum suggests that alternative scan modes might be useful to detect selectively carnitine conjugates with different fatty acids. This is important because the presence of carnitine conjugates in body fluids is diagnostic of several metabolic diseases and carnitine

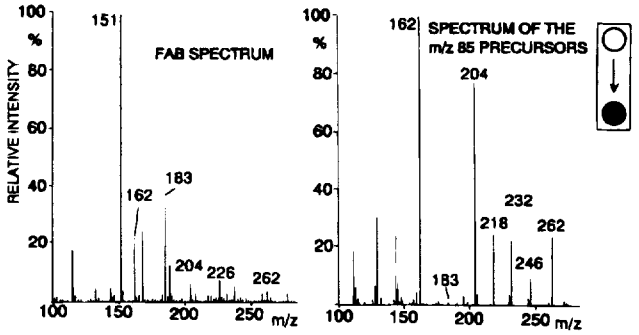
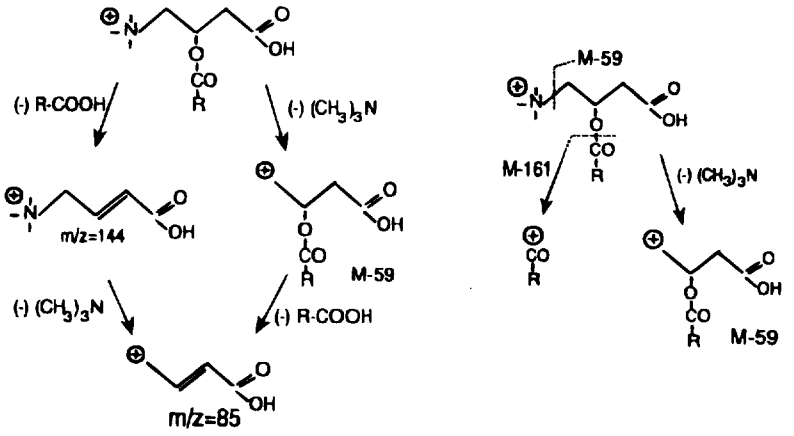


Figure 11. Left: FAB mass spectrum of a biological sample containing acylcarnitines. Right: precursor scan of *m/z* 85 for the same sample, showing *m/z* 162, free carnitine: *m/z* 204, acylcarnitine; *m/z* 218, propionylcarnitine, etc. A comparison of these two spectra shows that the selectivity of the MS/MS scan is accompanied by an increase in signal-to-noise ratio. (Adapted from Ref. 32, Fig. 4.7.)



Scheme 2. Fragmentation scheme of acylcarnitines, as deduced from the product ion spectra of octanoylcarnitine and other carnitine conjugates.

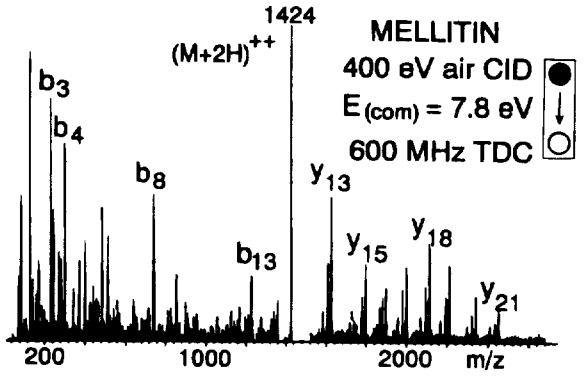


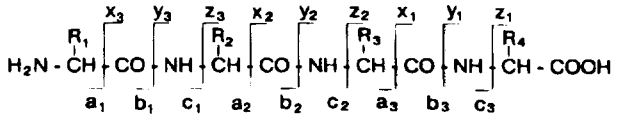
Figure 12. ESI tandem mass spectrum obtained using an orthogonal time-of-flight mass spectrometer. The doubly charged molecular species yields singly charged immonium (y-) fragments. (Courtesy Fisons Instruments.)

itself is used as a drug in treating some of these diseases.[32,33]

Referring to Scheme 2, it can be seen that a precursor scan of either *m/z* 144 or 85 is a good candidate to detect the entire set of functionally related compounds because they do not contain the fatty acids. The fragment ions at *m/z* 229 (loss of 59) and 127 (loss of 161) do contain the fatty acid chain, and therefore are not suitable choices for precursor ion scans to detect all carnitine conjugates. However, the neutral species lost in these fragmentations contain a portion of (59 Da) or the complete (161 Da) carnitine moiety. Neutral loss scans of either 59 or 161 Da should thus also allow the selective detection of all the carnitine conjugates.

Figure 11, displays both the FAB mass spectrum of a biological sample containing carnitines and the precursor scan of *m/z* 85. If it is followed by a product ion scan of each detected conjugate, individual spectra for each compound are recorded. This example illustrates how the precursor ion and neutral loss scans can be used in a methodology for the selective detection of compounds belonging to a given class. These procedures can be combined with chromatographic separation, allowing the selective determination of particular compounds in complex mixtures, although the chromatographic step can be avoided if amounts of material or time constraints require this. Tandem mass spectrometric experiments of the types described here are widely used in the pharmaceutical industry to monitor and determine selected compounds in pharmacokinetic studies.

Other aspects of MS/MS, especially its application to the elucidation of chemical structures, are illustrated in the ESI spectrum of mellitin recorded using a hybrid magnetic/orthogonal time-of-flight instrument and displayed in Fig. 12. The notation of the fragments is as given in Scheme 3. This example also illustrates the abundant fragments obtained from a doubly charged ion produced by electrospray ionization: doubly charged



Scheme 3. Nomenclature for fragmentation of peptides (from Ref. 36).

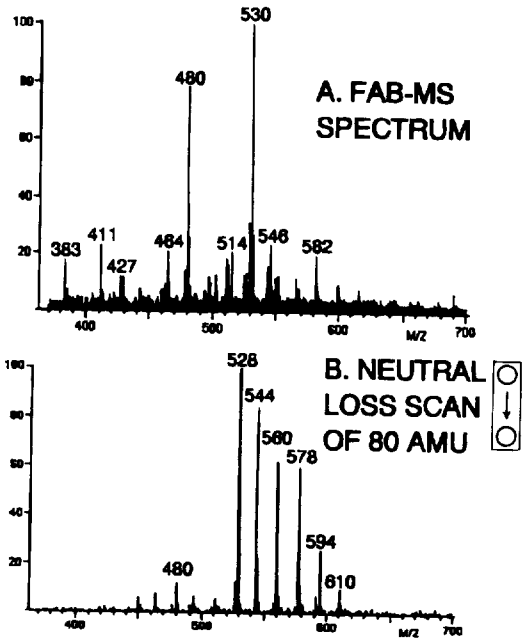


Figure 13. Top: FAB mass spectrum of a mixture of bile acids extracted from a physiological sample. Bottom: selective detection of the sulphated bile acids by the neutral loss scan of 80 Da (SO_3). This spectrum displays compounds completely buried in the chemical noise of the FAB spectrum. (Reproduced from Ref. 34, with permission.)

fragments, appearing at lower *m/z* values, belong to the b-series, while singly charged fragments, some of them appearing at *m/z* higher than the precursor ion, belong to the y-series.

For the detection of compounds present in mixtures, MS/MS often allows a remarkable improvement in the signal-to-noise ratio. This is illustrated by the spectra shown in Fig. 13. The first spectrum is a FAB mass spectrum (conventional mass spectrum) of a physiological sample of bile acids.[34] Some of these bile acids are sulphated, and for the diagnosis of biliary atresia it is important to detect them selectively. In the negative-ion mode, sulphated bile acids lose SO_3 (80 Da). A corresponding neutral loss scan is also displayed in Fig. 13. It shows that sulphated bile acids, buried in the chemical noise of the FAB mass spectrum, are now clearly detected, including those appearing at *m/z* 560, 578, 594 and 610. This is due to the fact that the selective detection does not apply to most of the components in the background, thus strongly reducing the background. The example shows that MS/MS can greatly improve the detection limits for selected compounds, especially when analysing complex mixtures, even though the total ion current associated with the MS/MS experiment is decreased relative to the normal mass spectrum. This characteristic of MS/MS, one of the first to be noted,[13,35] remains a major advantage of the technique.

CONCLUSION

The numerous tandem mass spectrometric techniques available have dramatically increased the power of mass spectrometry for structure elucidation and for selective

compound or compound-class analysis, and also for the study and the analytical use of ion–molecule reactions. These contributions are founded upon an increase in sensitivity which complements the high selectivity of the method. MS/MS is becoming a common technique with the appearance of relatively inexpensive instruments. However, even with the maturity of some MS/MS methods and instruments, the development of new instrumentation and new analytical methodologies continues.

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