Tandem Mass Spectrometry in Quadrupole Ion Trap and Ion Cyclotron Resonance Mass Spectrometers

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Abstract

Instruments that trap ions in a magnetic and/or electric field play a very important role in the analysis of biomolecules. The two predominant instruments in the category of trapping instrument are the quadrupole ion trap mass spectrometer (QIT-MS) and the ion cyclotron resonance (ICR) MS. The latter is also commonly called Fourier transform MS (FT-MS). The QIT is an inexpensive, simple, and rugged MS used for various routine applications. The ICR-MS is an expensive, high-performance instrument with figures of merit for resolution and mass accuracy surpassing all other mass spectrometers. This chapter covers the basic principles of operation of these instruments, including the trapping/manipulation/detection of ions and various approaches used to activate ions to perform tandem mass spectrometry experiments.

Introduction

The world of mass spectrometers can be divided into two general types of instruments: ion-trapping instruments and beam instruments. The major distinguishing characteristic of ion-trapping instruments versus other types of MSs is that tandem mass spectrometry (MS/MS) is performed via a tandem-in-time method, rather than tandem in space. This means that each stage of mass spectrometry is performed in the same analyzer, sequentially in time. In contrast, a beam instrument, such as a triple quadrupole, has each step of MS/MS performed in different analyzers that are sequentially separated in space, one analyzer for each stage of MS/MS. In beam instruments, each subsequent stage requires the addition of another reaction region and mass analyzer. An immediately obvious advantage of trapping instruments is that multiple stages of MS/MS (MSn) can be performed without instrumental modifications. The number of MS/MS stages possible in a trapping instrument is limited only by the ion intensity (Louris et al., 1990). An added benefit of trapping instruments is that 80–90% of MS/MS product ions can be trapped, whereas in linear quadrupole instruments, MS/MS efficiencies are typically an order of magnitude lower (Johnson
et al., 1990), and efficiencies in other beam instruments are even worse (Yost et al., 1979). The high MS/MS efficiency allows ion traps to perform even more stages of MS/MS. In our laboratory, for example, up to MS$^8$ has been performed to provide a great deal of information about the sequence of peptides (Lin and Glish, 1998).

There are two common types of ion-trapping mass analyzers: the QIT-MS and the ICR instrument. These two instruments operate using very different principles but have some similar characteristics. This chapter discusses the basic principles of operation of these two instruments and the variety of approaches to MS/MS that are available. It will start with the QIT, which is much less expensive and, thus, much more commonly found in bioanalytical laboratories. It is worth noting, however, that the ICR is a much more mature technology and that many of the methods used with the QIT have been adapted from ICR experiments.

Quadrupole Ion Trap Mass Spectrometer

Overview

Although the QIT was patented in 1960 (Paul and Steinwedel, 1960), it was not widely used until the 1980s. Today, interest in and applications for the QIT-MS have dramatically expanded. The versatility and availability of this instrument can make the QIT-MS the workhorse of a laboratory. Typical operation of commercial ion traps gives a resolution of about 2000 and a mass range of 4000–6000 Da/charge, although the QIT-MS is capable of resolutions of more than $10^6$ and a range of 70,000 Da/charge (Kaiser et al., 1991; Londry et al., 1993). Whereas the figures of merit of commercial QITs are significantly lower than those for an ICR, the QIT-MS is operationally and mechanically much simpler. In addition to the inherent advantages of a trapping instrument, the QIT-MS also boasts relatively simple vacuum requirements, a small footprint, fast analysis, and ruggedness, and it is relatively inexpensive. The QIT-MS can be interfaced with a wide variety of ionization methods, including continuous sources such as electrospray ionization (ESI) (VanBerkel et al., 1990) and secondary ion mass spectrometry (SIMS) (Kaiser et al., 1989), as well as pulsed methods like laser desorption (LD) (Glish et al., 1989) and matrix-assisted laser desorption ionization (MALDI) (Chambers et al., 1993; Jonscher et al., 1993). The QIT-MS also has the greatest sensitivity of any MS (Cooks et al., 1991). These features make the QIT-MS a valuable tool for analysis of biological molecules.
Trapping Ions in a QIT-MS

Apparatus. The QIT-MS operates analogously to a linear quadrupole, but in three dimensions rather than two. A QIT-MS is composed of three hyperbolic electrodes: two end-caps and one ring (Fig. 1). In a linear quadrupole mass filter, certain combinations of alternating current (AC) and direct current (DC) voltages allow an ion to have a stable trajectory, which means they pass through the filter and strike a detector. In the QIT-MS, those ions with stable trajectories are trapped in the volume encompassed by the electrodes. The donut-shaped ring electrode takes the place of one pair of poles. The end-caps replace the other pair of poles. The dimensions of the QIT-MS are described in terms of the distance from the center of the trap to the closest point on each of these electrodes. The ring electrode defines the radial direction, \( r_o \). The end-cap electrodes define the axial direction, \( z_o \). One end-cap electrode will usually have a hole in it to allow ions to enter the trapping volume. In rare instances, ions may also be injected through the ring, in which case the ring electrode would have an entrance hole. Ions are typically detected by ejecting them so they will strike a standard electron multiplier detector. The detector is located

![Diagram](image)

**Fig. 1.** Cross-sectional view of the quadrupole ion trap. The entrance end-cap has one larger hole for ion injection of focused ions while the exit end-cap has several smaller holes to allow ejection.
beyond the end-cap opposite of where the ions are injected, and thus, this end-cap also has holes, to allow the ions egress.

To generate the trapping field, an AC voltage is applied to the ring electrode, the end-cap electrodes or both the ring and the end-caps. In the latter arrangement, the voltage applied to the ring is 180 degrees phase shifted relative to the voltage applied to the end-cap. An optional DC voltage may be applied to either the ring or the end-caps. The movement of an ion in the trapping field is described by a second-order differential equation. A general solution to this type of differential equation was discovered by Mathieu more than a century ago. From this solution, the combinations of AC and DC voltages that result in a stable trajectory for an ion can be found (March and Londry, 1995). From Mathieu’s solution, the Mathieu parameters, \(a_u\) and \(q_u\), are used to describe the stability of an ion in both the axial \((z)\) and the radial \((r)\) direction, where

\[
a_z = -2a_r = \frac{-16eU}{m(r_0^2 + 2z_0^2)\Omega^2}
\]  

and

\[
q_z = -2q_r = \frac{8eU}{m(r_0^2 + 2z_0^2)\Omega^2}.
\]

These equations incorporate the ions’ characteristics of mass \((m)\) and charge \((e)\), the trap dimensions both radial \((r_0)\) and axial \((z_0)\), the AC frequency \((\Omega)\), and the AC \((V)\) and DC \((U)\) amplitudes. The AC voltage used is in the radiofrequency (RF) range and, thus, is commonly referred to as RF. The combinations of \(a_u\) and \(q_u\) values that result in a stable trajectory in both the axial and the radial direction are shown in the stability diagram in terms of the axial \((z)\) direction (Fig. 2). For practical reasons, \(r_0\), \(z_0\), and \(\Omega\) are fixed, and ion stability is controlled by changing the magnitudes of the RF and DC voltages.

**Higher Order Fields.** Electric trapping fields are generated by the applied RF voltage, which creates a three-dimensional (3D) trapping field. With prescribed dimensions of \(r_0\) and \(z_0\) \((r_0^2 = 2z_0^2)\), and a specific hyperbolic shape of the electrodes, the trapping field exerts a restoring force that increases linearly as ions move away from the center of the trap. This force pushes the ions back to the center of the ion trap when they move away and results in their trapping. However, because of field imperfections such as those caused by the entrance and exit holes and intentional distortions in \(z_0\), higher order fields are also present (Doroshenko and Cotter, 1997b; Wang et al., 1993).
The electric field is the first derivative of the electric potential. Thus, the quadrupole potential, which is quadratic, provides a linear field, whereas a hexapole potential, which is cubic, provides a quadratic field as shown in Fig. 3. These fields affect the motion and stability of the ions in the trap. Higher order fields are weakest at the center of the trap but become more significant toward the edges (greater slope). Therefore, ion motion can most easily be described when the ions are close to the center of the trap, where they experience mostly linear fields.

Resonance. An ion is trapped in the QIT-MS by maintaining stable periodic motion induced by the applied voltages. Each m/z ion has a unique periodicity of its motion, known as the secular frequency. The frequency in the axial direction ($\omega_z$) can be approximated ($q_z < 0.4$) in terms of the ion’s $a_z$ and $q_z$ parameters by

![Stability diagram](image)

**Fig. 2.** Stability diagram. Ions with $a_z$ and $q_z$ values that fall in the shaded region will have a stable trajectory and, thus, be trapped within the quadrupole ion trap mass spectrometer.
This characteristic frequency can be used to increase the kinetic energy of selected ions via resonance with a supplementary AC voltage applied across the end-cap electrodes. An ion moving at the frequency of the supplementary AC voltage will gain kinetic energy in the axial direction.

**Bath Gas.** In contrast to the ICR and most other mass analyzers, the QIT-MS benefits from a somewhat higher background gas pressure than is typically used for mass spectrometry (Stafford *et al.*, 1984). This is another advantage to the QIT-MS in that pumping requirements are not as stringent, and bath gas molecules are already present for use in CID. Bath gas molecules provide collisions with the trapped ions. These low-energy collisions damp the kinetic energy of the ions and restrict their movement to closer to the center of the trap. This is referred to as collisional cooling. At the center of the trap, the ions form a smaller cloud, and contributions of higher order fields are less. By condensing the ions to the center of the trapping volume, ion loss is reduced, and ions are ejected more coherently. Both sensitivity and resolution are improved by the presence of the bath

\[
\omega_z = \frac{(a_2^2 + \frac{a_0^2}{2})^{1/2} \Omega}{2}.
\]
gas (Louris et al., 1989). Generally, helium is the gas used, as collisional scattering is lower than with more massive gas molecules. The helium is typically added to a level of approximately 1 m Torr.

Ion-Trapping Capacity and Space Charge. The QIT-MS electrodes define a finite trapping volume. Because like charges repel each other, a finite number of ions can be trapped. It has been estimated that $10^5$ ions can be trapped at once (Cooks et al., 1991). Whereas this capacity is more than sufficient for detection of an analyte, problems can arise if the trap “fills up” with extraneous ions. If the trapping limit is reached by extraneous ions, the dynamic range for analyte detection will be reduced, and in the worst case, analyte ions may not be observed at all.

The presence of a large number of ions results in an effective DC charge, known as space charge. Space charging is the result of overfilling the ion trap and begins to have an effect on performance long before the ion-trapping capacity is reached. Beyond a certain point, not only will increasing the ion accumulation time not increase the signal, but an overfilled trap also detrimentally affects the resolution of the QIT-MS. Resolution in the QIT-MS depends on ejecting all ions of the same $m/z$ as a coherent packet to a detector. Ions in a space-charged trap will be forced by coulombic repulsions to spread out into a larger cloud. As the ion packet becomes more spread out, the resolution suffers. Because the $m/z$ is determined by the RF level at the time the ions strike the detector, if the ions are spread out over time, the signal intensity of a single $m/z$ is read over a longer time. This results not only in degradation in resolution but also as reduced signal current at the detector. The observation of broader than normal peaks is often indicative of a space-charged trap. Solutions include lowering ionization times or ejecting extraneous ions, either resonantly or by increasing the RF amplitude before mass analysis.

Ion Injection

All discussion up to this point has involved analysis and manipulation of ions. However, ions must first be formed, injected, and successfully trapped before they can be analyzed (Doroshenko and Cotter, 1997a; Louris et al., 1989). Previous chapters have discussed ionization techniques. Pairing these with the QIT-MS, however, is not entirely straightforward. The complication is that these ions must have sufficient kinetic energy to enter the trap, overcoming the fringing fields created by the RF voltage that obscure the entrance. However, when ions have sufficient kinetic energy to enter the trap, they have sufficient energy to also exit the trap.

Trapping Ions. There are two possible solutions to this problem: increasing the trapping field strength or decreasing the ions’ kinetic energies,
once the ions have entered the trapping volume. Ions can be injected through a hole in either an end-cap electrode (axially) or the ring electrode (radially). For methods in which the RF is increased, injection through the ring is superior \( (O \text{ and Schuessler, 1981c}) \). When the RF is constant, injection through an end-cap is preferred \( (O \text{ and Schuessler, 1981a}) \).

If the RF is changed as the ions are injected, it can either be pulsed on \( (Kishore \text{ and Ghosh, 1979}; O \text{ and Schuessler, 1981b,c}) \) or ramped to higher voltages once the ions are inside the trap \( (Doroshenko \text{ and Cotter, 1997a}) \). For practical reasons, ramping up the RF voltage is the easier technique to implement. However, only those ions in the trapping volume at the instant the RF is raised will be trapped. Therefore, these methods are ill-suited for pairing with continuous ion sources such as ESI because the duty cycle is very poor. However, these methods have seen use with MALDI, a pulsed ionization source. By timing the laser pulse with the RF, significant ion trapping can be achieved.

The more common method for trapping ions is to remove kinetic energy from ions in the trap so that the trapping fields are sufficient to hold them. Although the trapping process is not well understood, significant increases in trapping efficiencies are seen when a bath gas is present in the ion trap. In this case, ions are given sufficient kinetic energy to enter the trap. These ions are then thought to lose energy due to collisions with the gas molecules in the trapping volume \( (Quarmby \text{ and Yost, 1999}) \). Sufficient kinetic energy is removed from the ions so that they are effectively trapped. This method requires a balance so that the RF level is low enough to admit the ions but high enough that the field can trap the ions once they have lost some of their kinetic energy through collisions. With a helium pressure of 1 mTorr, an RF level corresponding to a lower trapping limit of 40–50 Da/e is appropriate.

A potential problem with ion injection is that the population of ions trapped can be biased \( (Louris \text{ et al., 1989}) \). There appears to be some correlation between the injected ions’ \( m/z \) and the RF level of the trap during injection. By using a higher RF level during ion injection, the lighter ions are either no longer stable in the trap or not able to pass the fields at the trap entrance while the heavier ions are more effectively trapped by the stronger field. The ion population can be shifted to favor heavier ions in this way. In general, this effect is small and a single RF level during injection is generally acceptable, but caution should be used when deducing the relative solution concentrations of species, keeping in mind this potential \( m/z \) bias.

Selective Accumulation. One strength of the QIT-MS is that ions can be accumulated over long periods of time to increase the number of ions present when they are ejected to the detector. However, once the trap is
full, extending accumulation times will not improve the signal and can even be detrimental (see the section “Ion-Trapping Capacity and Space Charge,” earlier in this chapter). If the analyte of interest is a small component of a mixture, this can be problematic, as the trap will fill up with the extraneous ions. However, if the extraneous ions could be ejected during ion accumulation, the trap would not fill up, and the analyte population could be increased. This can be done by resonantly ejecting the unwanted ions.

Selective accumulation can most easily be achieved using stored waveform inverse FT (SWIFT) (see the section “Ion Manipulation,” in the section “Ion Cyclotron Resonance” section) because a large band of frequencies can be used to eject the unwanted ions. The waveform does not include those frequencies corresponding to the analytes so that these ions are not ejected. This technique can be particularly useful with MALDI, which produces a great excess of matrix ions that can quickly fill up the QIT-MS. Selective accumulation works best when the analyte ions are significantly different in m/z than the unwanted ions because at the RF levels used for injection, the higher mass ions have closely spaced secular frequencies. When the secular frequencies are too closely spaced, it is difficult to eject ions of unwanted m/z that are near the analyte m/z without also ejecting analyte ions. In such cases, ions can be injected for a period of time, the RF level raised to increase the spacing of the secular frequencies, and then the unwanted ions ejected. After ejection of the unwanted ions, another accumulation period can be used and the cycle repeated.

Ion Detection

Methods of Detection. In the first couple of decades after the invention of the QIT, the main mode of operation was a method termed mass selective stability. In this mode, the QIT-MS is operated analogous to the linear quadrupole; the parameters are set so that one m/z at a time has a stable trajectory. The trapped m/z is then ejected to the external detector. The next m/z is then trapped and ejected, and the process continues until the mass spectrum is acquired. This mode of operation offers little or no advantage over a linear quadrupole. Alternative modes of operation offer advantages over linear quadrupoles. When these modes became routine, the use of the QIT-MS was greatly increased.

Mass Selective Instability. The first commercial QIT-MSs were operated in a mode known as mass selective instability. In this mode of operation, the DC = 0 V (a_z = 0) and an AC voltage is applied to the ring electrode while the end-caps are grounded, so all ions lie along the q_z axis (Kelley et al., 1985) (Fig. 4). All ions with q_z < 0.908 (the boundary of the stability diagram) are trapped. Because m/z \propto 1/q_z (Eq. [2]), smaller
$m/z$ ions have a larger $q_z$ and are closer to the boundary of the stability diagram. The $m/z$ which corresponds to $q_z = 0.908$ is the lowest mass trapped and is typically referred to as the cutoff mass. The amplitude of the RF determines this cutoff mass, so often the RF level is given in terms of the cutoff mass rather than by the voltage amplitude. Mass analysis can be achieved by increasing the amplitude of the RF. As the amplitude increases, each ion’s $q_z$ value increases. When an ion’s $q_z$ value becomes just greater than 0.908, that ion’s trajectory becomes unstable, and it is ejected from the trap. Low $m/z$ ions will be ejected first, followed by higher $m/z$ ions. An exit hole in one end-cap allows the ions to escape from the trap and strike the detector. By correlating the resulting signals at the detector to the RF amplitude, the $m/z$ of the ions can be determined. This is known as the mass selective instability mode of operation (Stafford et al., 1984).

RESONANCE EJECTION. The operation mode known as resonance ejection has made the QIT-MS a practical instrument for the analysis of biological molecules. In the mass selective instability mode of operation, the mass

![Diagram showing mass-selective instability mode](image-url)
range is limited to about 650 Da/e because of practical limitations in the amplitude of the RF achievable without electrical breakdown. However, the resonance ejection mode of detection can circumvent this problem (Kaiser et al., 1989). By applying a sufficiently large or long supplemental voltage at the resonant frequency, the ion gains enough kinetic energy to exit the trap. The first use of resonance ejection involved scanning the resonance frequency and holding the RF voltage constant (Ensberg and Jefferts, 1975). The most common implementation now involves applying a supplemental voltage at a fixed frequency and ramping the RF amplitude. Because secular frequencies are dependent upon the RF amplitude (Eqs. [2] and [3]), the secular frequencies of the trapped ions change as the RF amplitude changes. As the ions’ frequencies come into resonance with the supplemental voltage, from lower \( m/z \) to higher \( m/z \), they will be ejected. Resonance ejection can provide superior resolution and sensitivity compared to mass selective instability (as discussed in the section “Resolution”). More importantly, resonance ejection can be used to increase the mass range. Because of these features, resonance ejection is used on most current commercial QITs.

**Nonlinear Resonance Ejection.** As mentioned previously, higher order fields are naturally present in the QIT, and they are also intentionally induced by changes in the geometry from the mathematically ideal geometry. The higher order fields induce nonlinear resonances at specific locations in the stability diagram. One such location, due to hexapole resonance, is at a secular frequency, \( \omega_z \), equal to one-third the RF trapping frequency, \( \Omega \). The strength of this resonance can be increased by increasing the hexapolar contribution. This is done by distorting the geometry of the trapping electrodes in a nonsymmetrical manner.

The nonlinear resonances have little effect on the ions if they are at the center of the trapping volume; however, as the ion motion moves further from the center, the nonlinear resonances can cause rapid ejection of the ions from the trap (Franzen et al., 1995). Thus, by applying a supplementary voltage at a frequency of \( \omega_z = \{1/3\}\Omega \), very rapid ejection of the ions can be achieved. This nonlinear resonance ejection provides improved performance such as faster scan rates and better resolution. At least one commercially available QIT operates using nonlinear resonance ejection.

**Image Current Detection.** Another mode of detection is also possible in the QIT-MS. Image current detection is the type of detection used in ICRs (see the section “Ion Detection” in the “Ion Cyclotron Resonance” section for details). Because each \( m/z \) has a characteristic secular frequency, the image current is a function of the secular frequencies of the trapped ions. The secular frequencies (and thus the \( m/z \)) in the image current can be determined via FT. This type of detection has particular
advantages in that it is nondestructive, can detect very high \( m/z \)s, and ion populations can be remeasured to either detect a change in the ions or achieve better sensitivity.

Image current detection is not currently used in commercial QIT-MS instruments but has been demonstrated (Badman et al., 1999; Goeringer et al., 1995; Soni et al., 1996). A complication of this mode of detection is isolating the detector electrodes from the RF on the ring electrode. The trapping RF is large enough to obscure the ions’ image currents if not properly shielded. Ions can be excited either resonantly (Goeringer et al., 1995) or by a high-voltage DC pulse (100 V, 1 \( \mu s \)) applied to one end-cap (Badman et al., 1999; Soni et al., 1996) to cause them to move coherently. Both of these methods function analogously to the broadband excitation used before detection in an FT-ICR. FT detection enables ICRs to provide vastly improved resolution and sensitivity, and this detection method could potentially enhance these performance characteristics in the QIT-MS as well. However, the best resolutions achieved thus far have been approximately 1000 (Badman et al., 1999). Complications such as RF interference and space-charge–induced frequency shifts (covered in the section “Space Charge”) will have to be overcome before image current detection will see widespread use.

Resolution. Resolution in the QIT-MS in either mass selective instability or resonance ejection mode depends on how quickly and coherently a packet of ions can be ejected to the detector. In both of these modes, the RF amplitude is increased at a constant rate. As the ions are ejected and strike the detector, the resulting signals can be correlated to an RF level at which the ions were ejected and the mass can be determined. Resonance ejection not only increases the mass range of the QIT-MS but can also provide higher resolution spectra (Goeringer et al., 1992).

Scan rate is also related to resolution in resonance ejection. A faster scan rate results in lower resolution. A small amount of time is necessary for ions to become unstable and be ejected as they come into resonance with the ejection frequency. If the RF amplitude increases too quickly, all ions of a given \( m/z \) do not all have time to become destabilized equally and are not ejected together. Normal scan rates for unit mass resolution are 5000–13,000 Da/s. Slower scan rates are used to obtain higher resolution.

**Ion Manipulation**

Once ions have been trapped, analysis via MS/MS can follow. This is accomplished in three steps: isolation of the desired parent ion, activation of that ion, and detection of the product ions. Isolation of an ion is achieved using resonance ejection, which has already been discussed as a mode of
ejection and detection. To isolate a parent ion, ions of both lower and higher $m/z$ must be ejected. Lower $m/z$ ions can be ejected by simply ramping the RF to a sufficient voltage so low $m/z$ ions are ejected at $q_z = 0.908$. However, higher mass ions can be simultaneously ejected by applying a supplemental frequency corresponding to an $m/z$ just higher than the parent ion’s. As the RF amplitude increases, sequentially higher $m/z$ ions will be resonantly ejected while those masses lower than the parent ion are ejected via mass selective instability. Ions can also be isolated using SWIFT in the same manner described in the section “Selective Accumulation.”

**Ion Activation**

*Collisional Activation.* Several methods can be used for ion activation, each with different advantages and limitations. The most common form of activation is by collision with a neutral target gas as part of the overall process known as collision-induced dissociation (CID), which is discussed in more detail in Chapter 5. There are several methods to cause ions to undergo energetic collisions where kinetic energy is converted into internal energy. The excess internal energy then induces the parent ions to dissociate to product ions, from which structural information can be deduced.

The type of dissociation products seen with these collisional activation methods in ion traps can be different from those seen in other mass analyzers. There is a dramatic difference in the collision energies accessed in a sector instrument, typically in the $3–10$ keV range, versus the $10$s of electron volts in a QIT-MS. (It should be noted that the collision energy in a QIT-MS is ill-defined because the ions’ kinetic energy is constantly changing due to the dynamic nature of the electric trapping field.) This difference in magnitude of the collision energy in a sector versus a QIT-MS leads to substantial differences in the amount of internal energy deposited and the internal energy distribution, with the result being notable differences in MS/MS spectra.

Although the collision energies in triple quadrupole instruments can be similar in magnitude to those in the QIT-MS, two other differences often lead to different MS/MS spectra being observed. First, helium is typically the collision gas in the QIT-MS, whereas argon is more commonly used in triple quadrupoles. With argon as the collision gas, much more kinetic energy can be converted to internal energy per collision, again leading to a different internal energy distribution. The second difference between the QIT-MS and triple quadrupole (and sector instruments) is the time frame for the reaction. Ions must dissociate (react) in $100$ ms or less in the triple quadrupole instrument to be observed in the next stage of analysis.
However, in the ion trap, the times are two to three orders of magnitude longer. This longer time frame allows low energy, but kinetically slow reactions to occur, and can significantly favor such reactions.

**Resonance Excitation.** The first and most common method for collisional activation is resonance excitation (Charles et al., 1994; March et al., 1990; Splendore et al., 1996; Williams et al., 1993). This method employs the same phenomenon of resonance as the resonance ejection method discussed previously. When a supplemental AC voltage is applied across the end-cap electrodes at an ion’s secular frequency, that ion’s kinetic energy increases. The difference between excitation and ejection is one of degrees. By judiciously choosing the amplitude of the supplementary voltage and the time it is applied, the ion’s kinetic energy can be increased without supplying sufficient energy for ejection. A typical excitation voltage would be 300–500 mV_p-p and 10–40 ms long. Because the ions are manipulated by changing the amplitude of the RF, the sequence of events, or scan function, can be shown in terms of the RF amplitude. A scan function including isolation and resonance excitation is shown in Fig. 5.

Unfortunately, determining the frequency needed for resonance excitation is not always straightforward. The equation for determining the secular frequency of an ion (Eq. [3]) is not exact. The resonant frequency can be affected by higher order fields and can change as the ions gain kinetic

![Fig. 5. Scan function for MS/MS using resonance excitation collision-induced dissociation.](image)
energy (Franzen, 1991). The number of trapped ions also affects the secular frequencies of those ions (Vedel and André, 1984). The space charge of the ions acts as a DC potential. The effective $a_z$ value no longer equals zero, and the secular frequency changes (Eq. [3]). To address this problem, some commercial QITs try to carefully control the number of ions trapped. Another approach is to use a narrow range of frequencies rather than a single frequency for resonance excitation.

Controlling the number of trapped ions is especially problematic if the ion source output fluctuates significantly, as can be the case with MALDI. This may be one reason that no commercial QITs offer MALDI as an ionization option. Frequency shifts caused by the number of trapped ions are part of the motivation for finding other means of dissociation, although resonance excitation CID is still the most common method for ion dissociation.

Once the appropriate frequency is determined, the ion’s kinetic energy can be increased. However, one must be cautious of the competition between ejection and excitation (Charles et al., 1994). If too much energy is supplied, a significant fraction of the parent ion population can be lost through ejection. The MS/MS efficiency is then decreased.

$$\text{MS/MS Efficiency} = \frac{\sum \text{(production ion intensities)}}{\text{(initial parent ion intensity)}}.$$ (4)

This not only hampers the identification of the product ions but also limits subsequent stages of MS/MS due to an insufficient number of ions.

To weight the experiment in favor of excitation over ejection, the parent ion should be trapped as strongly as possible. The ions can be thought of as being held in an energy well, called the pseudo-potential well. The depth of this well is the maximum kinetic energy the ion can have and still remain trapped. The deeper this well, the more kinetic energy the ion can gain before it is ejected. Ions in deeper wells can, thus, undergo more energetic collisions and a greater number of collisions. This allows the ion to gain enough internal energy to dissociate before it is ejected. The depth of the well, $D$, is related to the $q_z$ parameter and can be approximated by the Dehmelt pseudo-potential well model for $q_z$ values less than 0.4:

$$D = \frac{(q_z^2 m r_0^2 \Omega)}{32e}.$$ (5)

This equation shows that ions trapped with higher $q_z$ values reside in a deeper well and can be excited to higher kinetic energies without being ejected. By increasing the RF amplitude, an ion’s $q_z$ value can be increased
However, at higher $q_z$ values, lower $m/z$ ions are not stable, so the smaller product ions are not trapped. To balance between the well depth and trapping the product ions, a $q_z$ of 0.2–0.4 is generally chosen. Thus, product ions less than about one-third the $m/z$ of the parent ion are generally not observed.

**Heavy Gas CID.** A variation on typical resonant excitation CID involves the use of heavy gases (Ar, Xe, Kr) as the collision gas. Heavy gases offer the benefit of higher energy deposition per collision. The maximum amount of kinetic energy ($E_k$) that can be converted to internal energy through a collision ($E_{\text{com}}$) is given by

$$E_{\text{com}} = E_k \left( \frac{M_n}{M_n + M_p} \right),$$

where $M_p$ is the mass of the parent ion and $M_n$ is the mass of the neutral molecule. For a parent ion with a given $E_k$, a heavy gas provides a higher $M_n$ and a greater percentage of energy deposition than helium. The addition of heavy gas molecules to the QIT-MS allows higher energy deposition and a greater degree of dissociation via CID. Also, the frequency range for efficient excitation is wider with heavy gases, reducing the required precision of the secular frequency (Vachet and Glish, 1996). In addition, $q_z$ values as low as 0.05 can be used, allowing low $m/z$ products to be detected (Doroshenko and Cotter, 1996). However, the heavy gas also deteriorates the resolution and sensitivity of the QIT-MS due to greater scattering upon collision. Pulsing in the heavy gas for CID and allowing it to pump away before detection can alleviate these problems at the cost of increased time per scan (reduced duty cycle).

**Boundary-Activated Dissociation.** Methods of activation other than resonance excitation have also been explored to circumvent some of the problems. One such technique is boundary-activated dissociation (BAD) (March et al., 1993; Paradisi et al., 1992; Vachet and Glish, 1998). In this technique, a DC pulse is applied to the end-caps instead of an AC voltage. This causes a change in ions’ $a_z$ values. As the $a_z$ value becomes large enough, the ion’s $a$ and $q$ values approach the boundary of the stability diagram (Fig. 6). The $a_z$ value is chosen so that the ion is not ejected, but its trajectory increases in amplitude. As the ions’ motions become larger in amplitude, there is greater force acting upon the ions, causing their kinetic energy to increase. Collisions with the bath gas molecules can then induce dissociation just as with resonance excitation. Only the magnitude of the DC must be adjusted to optimize the dissociation. As in resonance excitation, the ion must reside in a sufficiently deep well that the large-amplitude oscillations do not result in ejection. So, again, the $q_z$ values used are
typically between 0.2 and 0.4, and low m/z product ions are not observed. However, the secular frequency is irrelevant in BAD, so no tuning is required, and any fluctuations in frequencies are inconsequential. Therefore, BAD is particularly useful for MS/MS when the ion intensity fluctuates significantly from scan to scan, as is common with MALDI.

**RED-SHIFTED OFF-RESONANCE LARGE-AMPLITUDE EXCITATION.** Another activation technique that has been shown to be particularly useful for larger ions (m/z > 1000) is called red-shifted off-resonance large-amplitude excitation (RSORLAE) (Qin and Chait, 1996a,b). Larger ions often require a large amount of energy before dissociation is induced due to the number of degrees of freedom. Because of the competition between ejection and excitation, it can be difficult to increase the ions' kinetic energy to allow more energetic collisions without sacrificing MS/MS efficiency due to ion ejection. RSORLAE involves first increasing the RF

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**FIG. 6.** Boundary-activated dissociation (BAD). The application of a direct current (DC) pulse changes the ion’s $a_z$ value. As the $a_z$ value approaches the boundary, the ion gains kinetic energy.
amplitude over a period of about 10 ms, immediately dropping the RF level, and applying an excitation voltage. This voltage has a very large amplitude ($21 \text{ V}_p\text{p}$ compared to $\sim 400 \text{ mV}$ in resonance excitation). However, the ions are not ejected because the frequency is shifted from the secular frequency by approximately 5% to lower frequencies (red shifted). The excitation times are variable from 30 ms to as long as 1 s.

The reason this technique can deposit larger amounts of energy in an ion without ejection lies in the higher order fields in the trapping field. These higher order fields get stronger closer to the edges of the trapping volume. The secular frequencies are affected by these higher order fields by being shifted to higher frequencies (blue shifted) as the ion moves out from the center of the trap. The initial ramping and drop in the RF amplitude serves to first compress the ions to the center of the trap under the stronger trapping field. Then, when the amplitude is dropped, the cloud expands, and the higher order fields have more effect on the ions. The excitation frequency is red shifted, so the ions are further excited to the edges of the trap, the blue-shifting secular frequencies move further off resonance. This allows the ions to experience a more sustained excitation period, building up internal energy, without gaining enough kinetic energy to be ejected.

RSORLAE results not only in a greater degree of dissociation than resonance excitation, but also a slightly lower $q_z$ value can be used ($q_z \approx 0.15$). Thus, somewhat smaller product ions can be trapped than those seen with resonance excitation or BAD. RSORLAE is of particular value when paired with MALDI. Because MALDI produces predominantly singly charged ions, ions of $m/z$ values more than 1000 are common. Also, because the excitation frequency does not have to exactly match the secular frequency, fluctuations in the secular frequency are not as problematic. RSORLAE has been used to dissociate MALDI-produced ions up to $m/z$ values 3500 with significant improvements over resonance excitation.

**Nonresonance Excitation.** One other activation technique using CID also involves a non-resonant approach (Wang *et al.*, 1996). Non-resonance excitation is capable of increasing an ion’s kinetic energy to 40 eV and can do so in only a few microseconds. Like RSORLAE, the ion remains trapped even with a higher kinetic energy than can be achieved via resonance excitation. Thus, the internal energy deposition can also be greater. This non-resonance excitation involves applying a low-frequency square wave (50–500 Hz) to the end-caps. Because this frequency is so low in comparison to the secular frequencies of the ions ($\sim 100,000$ Hz), this square wave can more reasonably be thought of as a series of DC pulses. The square wave causes the trapping field to change instantaneously. The ions will accelerate quickly to compensate for this change. While doing so,
collisions with the bath gas convert the kinetic energy to internal energy. This process is repeated as the square wave cycles. Non-resonance excitation does not result in ejection because the kinetic energy added to the ions does not continuously increase the ions’ periodic stable motions as each of the previously discussed methods does. Instead, the ions change from one periodic motion to another very quickly. The kinetic energy is gained as the ions adjust from one motion to the other, not as a periodic motion is increased in amplitude.

Non-resonance excitation differs from resonant excitation in that it is not selective for any one species of ions. All trapped ions will be excited simultaneously. This can be desirable or not, depending on the application. Also, because more energy can be deposited quickly by non-resonance techniques, higher energy dissociation channels can be accessed. Thus, non-resonance excitation can form a different group of product ions from those seen with resonance excitation.

SURFACE-INDUCED DISSOCIATION. A final type of ion activation that uses collisions to increase the internal energy of ions is surface-induced dissociation (SID) (Lammert and Cooks, 1991). However, this method differs from CID in that the collisions do not involve gas molecules. Rather, the collision is between the ion and a surface such as the ring electrode. SID has several advantages including larger energy deposition and the non-resonant nature of the excitation. Resonance excitation has been estimated to increase the average kinetic energy to around 9 eV (Williams et al., 1993), although depending on the experimental conditions, this can vary somewhat. RSORLAE and non-resonance excitation techniques can increase the kinetic energy somewhat more, to tens of electron volts. In contrast, SID kinetic energies are estimated to be in the hundreds of electron volts. The combination of such large kinetic energies and collisions with a massive surface, as opposed to light He atoms, allows SID to form products with very high threshold energy levels. The excitation is achieved via a very large DC pulse, typically 300–400 V, lasting 1–4 \( \mu \)s, applied to the end-caps. This pulse causes the ions to strike the ring electrode. The DC pulse must be carefully timed with the phase of the trapping RF frequency. The position of the ions upon acceleration must be such that the product ions are reflected into the trap and can subsequently be detected. Although this technique does show the aforementioned advantages, it also has drawbacks. Besides the experimental difficulty of implementing the fast, large, carefully timed DC pulse, SID has shown very poor efficiencies. The most abundant product ions observed are less than 5% that of the parent ion. And, as in most of the previously mentioned techniques, \( q_z \) values must be raised somewhat, to 0.2–0.4 in this case, which prevents the observation of low \( m/z \) product ions.
Infrared Multiphoton Dissociation. Another method for inducing dissociation differs from all the previously discussed techniques in that it does not use collisions. Instead of increasing the kinetic energy of ions and then using collisions to convert that to internal energy, infrared multiphoton dissociation (IRMPD) uses photons to deposit energy into the ions (Boué et al., 2000; Colorado et al., 1996; Stephenson et al., 1994). IR wavelength photons are sent into the trapping volume where they can be absorbed by the trapped ions. An IR-transparent window in the vacuum housing allows access to the trap. A laser beam can then be directed into a hole drilled through the ring electrode. An intense laser beam is used because multiple photons must be absorbed before an ion gains enough energy to dissociate. Either a 25- or a 50-watt CO$_2$ laser can be used, and irradiation times of approximately 100 ms are usually sufficient. IRMPD offers several advantages over other dissociation techniques. First, it is not a resonant technique, and any ion in the path of the laser will be excited. This can be used, for example, to dissociate multiple analytes simultaneously. The laser beam will also excite product ions. These ions will subsequently dissociate and can provide a richer product ion spectrum. However, the spectrum can also be more complicated and hide the genealogy of the ions. The order in which product ions are formed in this type of MS/MS is not as clear as in stepwise MS$^n$. These relationships can be revealed but require additional steps to the experiment (Colorado et al., 1996).

One very important advantage of IRMPD is that low q$_z$ values can be used. All other excitation methods described rely on increasing the kinetic energy to increase the internal energy of ions. Therefore, the ions must be able to absorb that kinetic energy without being ejected, which requires higher q$_z$ values. IRMPD has no such restrictions, and low m/z product ions are easily trapped and observed. However, a complication with IRMPD in the QIT-MS is that collisions with the bath gas molecules between absorption events can remove energy from the ions. If collisions occur quickly enough compared to the rate of photon absorption, the ions will not reach the critical energy for dissociation. As the bath gas pressure is increased from 1 to 4 \times 10^{-5} Torr, dissociation rates decrease. Almost no dissociation is seen at bath gas pressures above 8 \times 10^{-5} Torr, even with a 50-Watt laser pulse lasting hundreds of milliseconds. Heating the ion trap/bath gas to 160°C allows typical bath gas pressures to be used (Payne and Glish, 2001). This technique, termed thermally assisted IRMPD, can improve sensitivity by more than an order of magnitude versus IRMPD at ambient temperature and reduced bath gas pressure.

Gas-Phase Reactions. Another type of MS/MS reaction for which trapping instruments are particularly useful involves gas-phase ion–molecule and ion–ion reactions. The QIT-MS is particularly versatile for
these types of analyses because of the combination of controlled timing of the reactions, mass-selecting capabilities for both reactants and products, and MS\textsuperscript{n} for both the formation of the reactants (McLuckey et al., 1991) and the examination of the products (Donovan and Brodbelt, 1992). Often, these reactions are between the trapped ion and a volatile neutral introduced to the vacuum chamber. However, reactions between two ions have also been reported (Herron et al., 1996a,b; Stephenson and McLuckey, 1996a,b, 1997a). Ion traps allow the reaction to be controlled by holding the reactants together for a variable time before ejection and detection. Therefore, studying the kinetics is straightforward, and the extent of the reaction can be well controlled.

**ION–MOLECULE REACTIONS.** One very useful type of ion–molecule reaction for biological molecules involves hydrogen-deuterium exchange. A protonated analyte reacts with a deuterated species such as CH\textsubscript{3}OD, D\textsubscript{2}O, or ND\textsubscript{3}. As active hydrogens are exchanged for deuteriums, the m/z of the analyte is seen to increase because deuteriums are more massive than hydrogens. The number of exchangeable hydrogens can then be determined. This is of particular interest in evaluating the conformation of the analyte. For example, if a protein or peptide is folded in the gas phase, some hydrogens will be protected from exchange within the interior of the protein. However, if the same species is unfolded, more hydrogens are exposed, and the degree of exchange will be greater. H/D exchange can also be used to determine when exchangeable hydrogens are obscured by an adduct. This structural information is then used to deduce the analyte’s nature in solution and how the structure is affected upon transfer to the gas phase.

Ion–molecule reactions that are specific for a certain functional group or structure can also be used to provide information about an analyte. For example, multiply charged peptides can be reacted with HI (Stephenson and McLuckey, 1997b). The basic groups of the N-terminus, Arg, His, and Lys, will each gain an HI adduct. By monitoring the change in m/z, the number of HI adducts can be determined and the number of basic sites deduced.

Deprotonation reactions have been used with multiply protonated protein ions. These ions can be held in an ion trap in the presence of a basic reagent such as dimethylamine. The dimethylamine molecules will extract protons from the protein, reducing its charge. The rate of deprotonation changes as the charge state changes. The change in rate reflects the different structures of the multiply charged proteins (McLuckey et al., 1990). Further, deprotonation reactions between 1,6-diaminohexane and CID product ions have been used to determine the charge states of these ions by observing the m/z shift as z is changed (McLuckey et al., 1991).
ION–ION REACTIONS. Reactions between two oppositely charged species can also be used. A QIT-MS is particularly useful for these types of reactions, as both positively and negatively charged ions can be trapped simultaneously under normal operating conditions (this is not true for ICR instruments). Ion–ion reactions provide greater control in that ions are injected as needed, while neutral reactant molecules are usually present at a constant pressure. As with the ion–molecule reactions, deprotonation can also be achieved via ion–ion reactions. Ion–ion reactions have an advantage over deprotonating ion–molecule reactions in that anions are much stronger bases than neutral compounds. Anions can completely deprotonate multiply charged proteins and peptides in ion–ion reactions, whereas neutral compounds used in ion–molecule reactions typically cannot. Ion–ion deprotonation reactions have been useful to determine charge states and to de-clutter charge-state convoluted spectra. By decreasing the charge on trapped ions, the $m/z$ values shift. Where multiple charge states of proteins overlap, shifting to a lower charge state for each protein can separate the overlapping signals. Ion–ion deprotonation of multiply charged proteins can be done using fluorocarbon anions (Stephenson and McLuckey, 1996b). A similar reaction involving protonated pyridine ions allowed determination of the charge states of negative CID product ions (Herron et al., 1996b). Electron-transfer reactions have also been used to these ends (Stephenson and McLuckey, 1997a).

Ion Cyclotron Resonance

Overview

The modern era of ICR has its beginning in the work of Marshall and Comisarow (1974). By applying FT techniques, improved performance in numerous aspects of the experiment became possible. Today the ICR may be the most powerful mass spectrometer available. It is renowned for its unparalleled mass resolving power and provides accurate mass measurements as good as or better than any other type of mass spectrometer. The performance capabilities of the ICR have been steadily improving over the years as computers have become more powerful and as stronger magnetic fields have been obtained. Many of the performance characteristics, such as mass range and mass resolving power, are related to the strength of the magnetic field. As magnet technology has evolved, ICR instruments have gone from magnetic field strengths on the order of 1 Tesla, generated by electromagnets, to 3.0, 4.7, 7.0, and now 9.4 Tesla fields available with superconducting magnets. The desire for high magnetic fields provides two of the major contrasts between ICR and QIT-MS instruments—size
and cost. Whereas the QIT-MS is a benchtop instrument, an ICR requires significant laboratory floor space.

As mentioned, the performance characteristics are a function of the magnetic field strength. Current state-of-the-art instruments with 9.4-Tesla magnets can reach resolving powers in excess of $10^6$ for ions of $m/z$ of 1000. The theoretical mass range exceeds $10^5$ Da/e and mass accuracies can be in the sub–part-per-million range. Like the QIT, most any type of ionization technique can be coupled with an ICR. In many ways, the QIT-MS and ICR complement each other, and although QIT-MS instruments are found in a wide variety of laboratories, ICR is becoming an indispensable tool in state-of-the-art MS facilities involved in biotechnology-related areas.

**Trapping Ions in Ion Cyclotron Resonance**

**Principles.** The main trapping force in ICR is the magnetic field. However, unlike the QIT in which the RF electric field traps ions in all three dimensions, the magnetic field traps the ions only in two dimensions, the plane perpendicular to the magnetic field. The ion motion in this plane is the cyclotron motion and is simply described by the basic equation of motion of a charged particle in a magnetic field:

\[
\frac{mv^2}{r} = Bev. \tag{7}
\]

in which $m$ is the mass of the ion (kilograms), $r$ is the radius of the ion motion (meters), $v$ is the ion velocity (meters/s), $e$ is the charge on the ion (coulombs), and $B$ is the magnetic field strength (Tesla). Equation (7) can be rearranged to give:

\[
\frac{m}{e} = \frac{Br}{v} = B\omega_c, \tag{8}
\]

in which $\omega_c$ is the angular velocity (radians/s) at which the ion is orbiting in the magnetic field. The angular velocity is related to cyclotron frequency by

\[
f_c = 2\pi\omega_c. \tag{9}
\]

In most ICR instruments, the magnetic field is fixed, so each mass-to-charge ratio has a unique cyclotron frequency ($f_c$). It is worth noting that the cyclotron frequency of a given mass-to-charge ratio is independent of the velocity (kinetic energy) of the ions.

Because the magnetic field traps ions only in two dimensions, an electric field is used to trap the ions in the third dimension, the axis of the magnetic field. A potential of the same polarity as the charge of the ions
being trapped is applied to trapping plates, which are mounted perpendicular to the magnetic field. Ions with axial kinetic energies less than the potential applied to the trapping plates move in a potential as well as simple harmonic oscillators. This trapping motion is independent of the cyclotron motion.

In addition to cyclotron and trapping motion of the ions in an ICR instrument, there is also a motion termed the magnetron motion. The magnetron motion results from the combined effect of the magnetic and electric fields. Like the cyclotron motion, the magnetron motion has a frequency associated with it. However, the magnetron frequency is independent of the mass-to-charge ratio of the ions. Also, whereas the cyclotron frequency is typically in the range of a few kilohertz to a few megahertz, the magnetron motion frequency is typically less than 100 Hz and is given by

\[ f_m = \frac{\alpha V}{\pi a^2 B} \]  

where \( V \) is the magnitude of the trapping potential, \( B \) is the magnetic field strength, \( a \) is the distance between the trapping plates, and \( \alpha \) is a constant related to the analyzer cell geometry. Thus, the magnetron motion depends not only on the magnetic and electric fields, but also on the analyzer cell geometry. The magnetron motion can be thought of as coupling with the cyclotron motion and displacing the center of the cyclotron motion.

**Apparatus.** Although many analyzer cell geometries have been used in ICR, the most common is the cubic cell (McIver, 1970). This cell, as the name implies, is a six-sided cube. The two sides that are orthogonal to the magnetic field are the trapping plates discussed previously. The plates on the other four sides of the cube are not important in the trapping of the ions but are used for the ion manipulation and detection processes, which are discussed below. While the cube is the simplest arrangement, a cylinder cut in quarters along the magnetic field axis is also a common geometry. Again, the main purpose of the individual sections of the cylinder is to provide the means to manipulate and detect the ions that are trapped in the cell. There have been many variations of these two basic designs, typically implemented to shim the electric field to improve various aspects of performance. Schematics of these two basic cells are shown in Fig. 7.

**Ion-Trapping Capacity and Space Charge.** As with QIT-MS, there is a limit to the number of charges that can be trapped in an ICR analyzer cell due to the coulombic repulsion between the ions. The QIT-MS can hold slightly more ions than an ICR, but the difference is not great. And just like the QIT-MS, performance is degraded in the ICR before the space charge limit is reached. Shifts in the cyclotron frequency along with peak
broadening can occur. If ions of similar mass are present, the two peaks can coalesce into a single peak.

**Ion Injection**

*Ion Sources.* Since the early days of ICR, a common means to get ions into the trapping region is to form them by electron ionization within the trapping cell. A filament to generate the ionizing electrons is located just
outside the cell, within the magnetic field. This arrangement allows efficient ionization and trapping of volatile compounds. Most biological compounds, however, are involatile and require a desorption ionization technique. Desorption ionization techniques require that the sample be external to the cell. Thus, the ions formed must be injected into the cell.

For MALDI, it is possible to locate a sample probe just outside the analyzer cell and let the ions diffuse into the cell after being formed by the laser pulse (Hettich and Buchanan, 1991; Koster et al., 1992). However, the kinetic energy of the MALDI-desorbed ions, which increases with mass, limits the trapping efficiency of this approach and can cause discrimination based on the ion mass.

Coupling ESI with ICR has increased challenges due to the vast pressure differences between the source and the analyzer. ESI is an atmospheric pressure technique and optimum ICR performance is at very low pressures (e.g., $10^{-9}$ Torr or less), a pressure differential of 11–12 orders of magnitude. This can only be achieved through a number of stages of differential pumping. Although there has been one example of an ESI probe with multiple stages of differential pumping that can be inserted into the magnetic field near the cell (Hofstadler and Laude, 1992), all commercial instruments have the source located outside of the magnetic field, typically at least a meter away from the cell (Henry et al., 1989).

*Trapping Ions.* Injecting ions into the analyzer cell is not a trivial task. Not only do ions have to penetrate a large magnetic field, but they also must be slowed down enough to be trapped in the axial dimension. Several approaches have been used to help the ions traverse the magnetic field gradient. The earliest and probably most common approach is use of an RF-only ion guide (McIver et al., 1985). In addition to RF-only ion guides, DC wire ion guides have also been used (Limbach et al., 1993).

After traversing the magnetic field, the next issue is trapping the ions in the cell. To get the ions into the cell, the potential on the front trapping plate (closest to the ion source) must be lower than the kinetic energy of the ions. To keep the ions from going straight through the cell, there must be either a higher voltage on the rear trapping plate (i.e., the one farthest from the ion source), or the axial kinetic energy must be decreased after the ions enter the cell. By having the axial trapping plates at different voltages, ions that have kinetic energies lower than the potential on the rear trapping plate will be turned around and head back in the direction of the source. If there is no change in the ion kinetic energy, the front trapping plate potential must be raised to a level greater than the kinetic energy of the ions. When this is done, no more ions can enter the cell. The time it takes a given ion to travel from the front plate to the maximum distance toward the rear plate (depending on the potential applied to the rear plate)
and back to the front plate will be a function of the mass of the ion (smaller ions will have larger velocities). For a continuous beam of ions, this limits the number of ions that can be trapped but will not discriminate based on mass. However, for pulsed beams of ions, mass discrimination may occur. The gating period (i.e., the length of time that the trapping potential is lowered on the front plate to allow ions in) can be varied to optimize the trapping of a particular mass-to-charge ratio relative to the rest of the species present (Dey et al., 1995).

Another method for trapping ions is to decrease the kinetic energy of the ions through collisions with a background gas. This can allow longer ion accumulation times. However, the analyzer cell pressure is increased so that there is loss of resolution (Beu and Laude, 1991). A gas can be pulsed into the analyzer cell, but this substantially increases the duty cycle because of long pump-out times (20–120 s) to return to the low pressure needed for optimal resolution (Beu et al., 1993). As an alternative to the gated trapping or the use of collisions to trap ions, ions can be deflected off-axis in a technique known as “sidekick” (Caravatti and Allemann, 1991). The sidekick method provides the ability to accumulate ions in the analyzer cell for longer periods with reduced mass discrimination, increasing the number of ions that are trapped.

Whereas MALDI naturally forms a pulse of ions, ESI generates a continuous beam of ions. However, a common mode of operation of ESI is to accumulate the ions in an RF-only multipole external to the magnetic field and then inject the ions as a pulse (Senko et al., 1997). Because the ion injection portion of the ICR experiment is typically only a small fraction of the overall cycle time, accumulating the ions externally and injecting them in a pulse can greatly increase the effective duty cycle. One point of caution concerning external ion accumulation in an RF-only multipole is that it has been shown that above a certain space-charge value, dissociation of the ions can occur. This leads to a mass spectrum that is not representative of the ion species being formed in the ion source (Sannes-Lowery and Hofstadler, 2000).

The ability to inject ions from external ionization (EI) sources makes the combination of almost any ionization technique with ICR feasible. Although MALDI and EI can readily be performed in the magnetic field near the analyzer cell, it is now common to have these ionization sources external to the field. This allows ready access to many ionization techniques on a given instrument. In some designs, it is possible to rather quickly switch from one ionization method to another. This ability is facilitated by the fact that mass calibration is dependent only on the magnetic field, and thus, the same calibration applies regardless of the ionization technique, in contrast to many types of mass spectrometers. It
is also possible to form ions via EI with a filament within the magnetic field and, at the same time, inject ions from an external source. This allows even more accurate mass measurements to be obtained.

*Ion Detection*

*Image Current.* ICR is unique among MS techniques in that the ions can be readily detected without impinging them on an electron multiplier detector or any other type of detector external to the mass analysis region. They are detected inside the cell without destroying them, and therefore, after the ions have been detected, they can be manipulated further to perform more sophisticated mass spectrometric experiments. Two steps are involved in the ion-detection process: ion excitation and subsequent detection of the current induced on the analyzer cell plates by the ions (image current).

In the basic scheme, two of the four plates parallel with the magnetic field are used to excite the ions (excitation plates), and the other two are used to detect the ions (detection plates). The excitation involves applying a broadband signal to the excitation plates that spans the cyclotron frequency range of ions that are trapped. This excitation signal will cause the ions to gain kinetic energy. Because the cyclotron frequency remains constant, the ions will increase the radius of their orbit. By judicious choice of the excitation signal parameters, the orbit radius can be increased to just slightly less than the dimension of the cell. The excited ions are coherent in their motion (i.e., ions of each individual mass-to-charge move together in a packet).

If the ions are positively charged, as they approach a detection plate, electrons are attracted to that plate. As the packet of ions continues its orbit and approaches the opposite detection plate, electrons are attracted to that plate. A current that oscillates at the same frequency of the cyclotron motion of the ions can be detected in an external circuit between the two detection plates. This current is called the *image current* and is detected as a function of time. The current can then be mathematically processed using the Fourier equation to change from a time-domain signal to a frequency-domain signal. This process gives the technique its commonly used name, *FT-ICR* or *FT-MS,* although it should be noted that Fourier processes have been used with other types of mass spectrometers and are not unique to ICR. After the cyclotron frequency of the trapped ions has been determined, the mass-to-charge ratio can be obtained from Eqs. (8) and (9). Figure 8 shows theoretical time-domain signals and the corresponding frequency-domain spectra.
Resolution. The high-resolution capabilities of the ICR are based on the capability to measure the image current for a relatively long period (seconds). The longer the time-domain signal can be measured, the more precisely the cyclotron frequency can be determined. The higher the precision is, the better the resolution (i.e., the separation between two peaks) unless peak coalescence occurs due to space charge. In addition to scaling with the acquisition time, the resolution increases linearly with increasing magnetic field and decreases linearly as a function of m/z.

Fig. 8. Examples of Fourier Transform. (A) Long time domain signal gives high resolution in frequency domain. (B) Time domain is exponentially damped (as by collisions) to give a lower frequency resolution. (C) Several frequencies convoluted in time domain are separated into frequencies by Fourier transform.
The main limit on the resolution is the pressure in the system. The image current is damped (Fig. 8B) as ions undergo collisions with neutral molecules in the vacuum system. Several effects are responsible for the decreased signal due to ion–molecule collisions. First, the ions’ velocity is decreased, reducing their cyclotron radius, moving them farther from the detection plate. Thus, there is less charge induced on the detection plate. Also, as the cyclotron radius decreases, the magnetron radius increases, which can cause the ions to be lost from the cell. Finally, scattering resulting from the collisions dephases the coherent ion motion, again reducing the charge induced on the detection plates.

The effect of pressure on resolution (higher pressure, poorer resolution) leads to one of the biggest contrasts between the operation of an ICR and a QIT-MS. In an ICR experiment, pressures in the analyzer cell are typically in the $10^{-9}$ Torr pressure range (or lower), whereas the QIT-MS is typically operated in the range of $10^{-3}$ Torr. Thus, as noted earlier, the ICR requires significantly more vacuum pumping, especially when ionization techniques at atmospheric pressure are used.

**Ion Manipulation**

*Stored Waveform Inverse Fourier Transform.* A unique characteristic of the ion-trapping instruments is the ability to selectively manipulate the ions that are trapped. This is a result of the fact that each different mass-to-charge ratio has a unique frequency of motion in the trapping field. In the ICR, this motion is the cyclotron motion, discussed earlier. By appropriate application of an RF potential to the analyzer cell, the cyclotron motion can be resonantly excited, analogous to resonant excitation in a QIT-MS. The cyclotron motion is excited for ion detection but can also be excited for other purposes. A standard means for manipulating ions is known as SWIFT (Marshall et al., 1985). In SWIFT, a frequency-domain waveform is generated to achieve the desired purpose of the ion manipulation (e.g., to eject all ions except those of a selected $m/z$ that will be the parent ions for an MS/MS experiment) (Fig. 9). Then, an inverse FT of the frequency domain spectrum is performed to obtain a time-domain signal that can be applied to the appropriate (excitation) cell plates. This time-domain signal will then cause all the ions in the analyzer cell to increase in radius to the point at which they strike a plate and are lost. Very complex ion manipulation can readily be performed by this method.

*Quadrupolar Axialization.* Another technique used for ion manipulation is quadrupolar axialization (Bollen et al., 1990). This technique involves converting magnetron motion to cyclotron motion by electronic manipulation of the ions. The conversion of magnetron to cyclotron motion
decreases the magnetron radius while increasing the cyclotron radius. The cyclotron radius can simultaneously be decreased by collisions, as described previously. The net effect is to focus the ions to the center of the analyzer cell. This improves the performance of an ICR instrument for a number of different parameters and experiments, such as mass resolution and accuracy, MS/MS, and ion remeasurement (Speir et al., 1993).

Ion remeasurement is another unique feature of ion-trapping instruments. Because the ions are not destroyed when they are detected via image current measurement, they can be refocused to the center of the analyzer cell using quadrupolar axialization. Once in the center of the cell, the ions can be efficiently excited and detected again. This cycle, detection followed by axialization, can be done many times. This process can substantially improve the signal-to-noise ratio of a spectrum and the MS^n efficiency.

**Ion Activation**

Like the QIT-MS, there are numerous methods to increase the internal energy of ions to cause them to dissociate. Some of the techniques are identical, and others are similar in principle. In addition, just like the QIT, multiple stages of MS/MS can readily be performed.

*Collisional Activation.* As with all other instruments capable of performing MS/MS experiments, collisions of the selected parent ions with neutral gas molecules is by far the most common means of ion activation in ICR. However, a major drawback to this approach is the conflict between the need for a collision gas to be present to effect collisional activation and the need to have ultralow pressures to obtain the high mass resolution
characteristic of ICR analysis. Two approaches have been taken to address this conflict. The simplest approach involves pulsing the collision gas into the analyzer cell during the time period of the collisional activation and then delaying the detection sequence several seconds to allow the collision gas to pump out and the pressure to return to a level allowing the desired resolution to be obtained (Carlin and Freiser, 1983).

As an alternative to pulsing a collision gas into the vacuum system, a differentially pumped dual cell geometry can be used (Littlejohn and Ghaderi, 1986). In this arrangement, two cubic-trapping cells are used with a common trapping plate between them. This common plate separates regions of the vacuum system with a conductance-limiting aperture in the center of the plate. This aperture allows ions to be transferred back and forth between the two cells while keeping a pressure differential of over two orders of magnitude between the two cells. Thus, collisional activation can be performed at a higher pressure and then the product ions transferred to the low-pressure cell for high-resolution mass analysis.

The physical aspects of collisional activation in an ICR are the same as in the QIT-MS (see the section “Surface-Induced Dissociation”). One difference in the experiment implementation is the nature of the collision gas. Typically, helium is used in the QIT-MS, mostly because it is already present; for other purposes, argon is more commonly used in the ICR experiment, and thus, a greater fraction of kinetic energy can be converted to internal energy in the ICR. On some occasions, this may lead to different MS/MS spectra being obtained from the two instruments.

Gas-Phase Collisional Activation. As in the QIT-MS, the ions trapped in the ICR analyzer cell have low kinetic energies. Thus, some means to increase their kinetic energy to effect collisional activation must be used. There are a number of ways in which this can be done. The original collisional activation involved dipolar resonant excitation of the cyclotron motion (Cody et al., 1982), analogous to the resonant excitation that is the most common method used with the QIT-MS. The maximum kinetic energy that the ions can obtain during excitation is a function of the magnetic field strength and can be hundreds of electron volts. The actual kinetic energy can be calculated for this and other forms of resonant excitation (Schweikhard and Marshall, 1993).

A problem with resonant excitation in ICR is that product ions are formed with significant cyclotron radii, which complicates detection (and transfer between cells in a dual-cell system). There are alternative methods to standard resonant excitation that are currently used: sustained off-resonance irradiation (SORI) (Gauthier et al., 1991), very low energy (VLE) excitation (Boering et al., 1992), and multiple excitation collisional
activation (MECA) (Lee et al., 1993). The most common of these methods is SORI. This is a non-resonant technique in which a frequency slightly different than the ion cyclotron frequency is used to excite the ions. This difference in frequency causes the ion motion to be alternatively accelerated and then decelerated. This allows long excitation periods while keeping the ions near the center of the cell.

VLE also provides alternating periods of acceleration and deceleration. Resonant excitation is used to accelerate the ions for a period of time, after which the resonant frequency is phase shifted 180°, causing the ions to be decelerated. This sequence can be repeated many times, providing a very similar result as SORI. MECA is similar to VLE except collisional cooling is used to decelerate the ions rather than doing it electronically.

Surface-Induced Dissociation. Another activation method that avoids the use of a collision gas is SID. In this technique, the ions are forced to collide with a surface in the mass spectrometer. In the ICR, that surface is typically one of the trapping plates (Ijames and Wilkins, 1990; Williams et al., 1990). A voltage pulse is applied to one trapping plate, accelerating the ions so that they strike the opposite plate. The amplitude and duration of this voltage pulse are the important parameters. Although in theory this technique is probably the easiest of all the techniques to implement, requiring the least amount of modification or extra equipment, it is not commonly used. This is probably because of the relatively low efficiency with which product ions are collected and because the modified surfaces, which can enhance SID, are difficult to use. However, work has been focused toward these issues (Danell and Glish, 2000; Zhong et al., 1997), so SID may become a more widely used technique.

Photodissociation. Photodissociation is a logical combination with ICR. It obviates the need to add a collision gas to the instrument, and because the ions are trapped in a well-defined region and at low energy, relatively good overlap between the laser beam and ions can be achieved. Both UV and IR photodissociation have been used to dissociate biological ions in ICR experiments. UV photodissociation was the first method tried for biomolecules (Bowers et al., 1984; Hunt et al., 1987). Extensive dissociation is observed, in fact so much that it is difficult to interpret the resulting MS/MS spectra. As an alternative, infrared multiphoton photodissociation (IRMPD) has been explored (Little et al., 1994). This activation method is somewhat similar to the SORI method in that the ions gain internal energy relatively slowly, so the lower energy dissociation pathways predominate.

Blackbody Infrared Radiative Dissociation. A relatively new method to dissociate ions that also relies on photons to energize the ions is termed
blackbody infrared radiative dissociation (BIRD) (Price et al., 1996). This method basically involves heating the trapped ions by absorption of IR photons emitted (blackbody radiation) from the analyzer cell plates/vacuum chamber. By heating the vacuum system up significantly above room temperature (typically 150–200°C), trapped ions can be energized to levels sufficient for dissociation to occur at observable rates. The IR photon absorption (heating) process of the ion is in competition with IR emission, which cools the ions. It often takes tens to hundreds of seconds to effect dissociation by the BIRD technique. Obviously, BIRD is not a high-throughput method. However, fundamental properties of biomolecules, such as activation energies, can be determined under appropriate experimental conditions.

Ion–Molecule Reactions. Though sometimes not thought of as such, isolation of ions of a specific m/z and reaction of those ions with a neutral gas to form products is a classic MS/MS experiment. One of the main strengths of ICR from the outset has been the ability to use ion–molecule reactions. For many years, the main application of ICR was the study of gas phase bimolecular chemistry. As the instrumentation has improved and matured, ion–molecule chemistry has played a reduced role behind the high-resolution and accurate mass measurement capabilities. However, it is becoming apparent that using ion–molecule reactions for fundamental studies of biomolecules will be a great strength of ICR because of its resolution capabilities. In particular, H/D exchange of peptides and proteins to probe gas-phase conformations of these species is emerging as a major tool (McLafferty et al., 1998). Insight into the effect of parameters such as charge state can be obtained from comparing the number of exchangeable protons and the rate of exchange. A related experiment involves the stripping of protons from multiply charged peptides and proteins (Gross and Williams, 1995). Being able to control the reaction time allows kinetic measurements to be made, which provide yet another piece of data in the analysis of biomolecules.

Electron-Capture Dissociation. The newest activation technique that is unique to the ICR is electron-capture dissociation (Zubarev et al., 1998). In this method, low-energy electrons (<0.2 eV) are captured by multiply charged ions (typically proteins) in the analyzer cell. An intriguing feature of the electron-capture–induced dissociation is that the product ions formed are c and z ions that results from cleavage of the backbone amine bond, not b and y ions typically observed with other methods as a result of the peptide bond cleavage. In addition, dissociation is observed at more sites along the protein backbone relative to other activation methods.
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References


Collision-Induced Dissociation (CID) of Peptides and Proteins

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Abstract

The most commonly used activation method in the tandem mass spectrometry (MS) of peptides and proteins is energetic collisions with a neutral target gas. The overall process of collisional activation followed by fragmentation of the ion is commonly referred to as collision-induced dissociation (CID). The structural information that results from CID of a peptide or protein ion is highly dependent on the conditions used to effect CID. These include, for example, the relative translational energy of the ion and target, the nature of the target, the number of collisions that is likely to take place, and the observation window of the apparatus. This chapter summarizes the key experimental parameters in the CID of peptide and protein ions, as well as the conditions that tend to prevail in the most commonly employed tandem mass spectrometers.

Introduction

Evidence for the gas-phase CID of molecular ions is apparent in the first mass spectra recorded by Sir J. J. Thomson with his parabola mass spectrograph, and the phenomenon was a subject of study throughout the development of MS during the first half of the twentieth century. A summary of the early work on CID has been published (Cooks, 1995). The modern application of CID to the detection, identification, and structural