6 Triple Quadrupole and Quadrupole Ion Trap Mass Spectrometers

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6.1 SUMMARY

Mass spectrometers based on ion separation using electrodynamic quadrupolar fields have been dominant in the evolution of the field of mass spectrometry. This chapter discusses triple quadrupole mass spectrometers and quadrupole ion trap mass spectrometers, two of the most important and commercially successful instruments in the history of mass spectrometry. An overview of the principles of operation is discussed in terms of the Mathieu stability diagram, a well-known graphical representation to determine whether ions will have stable or unstable trajectories in a radio frequency (RF) electric field. A brief introduction of tandem mass spectrometry (MS/MS) is also presented.

After the general introduction, each type of instrument is discussed individually. It should be noted that while both types of instruments are based on the same theory of ion motion in electrodynamic fields, there are very important differences in the way experiments are implemented. This is in large part because the triple quadrupole mass spectrometer is the so-called beam instrument where the different stages of an experiment are performed "in space" as the ions travel from the ion source to the detector. Conversely, quadrupole ion traps are "trapping" instruments and the stages of the experiment are performed sequentially "in time," with the ions being manipulated in the electric field.

A brief historical background is provided for each type of instrument, and then the method of operation is covered with an emphasis on the MS/MS capabilities. For the triple quadrupole mass spectrometer, its key MS/MS feature is the variety of MS/MS

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modes in which it can be operated: product ion scan, parent ion scan, neutral loss scan, and single and multiple reaction monitoring.

The discussion of quadrupole ion traps first covers how ions are manipulated in the device. The ability to manipulate ions ("ion taming") is one factor that makes the quadrupole ion trap a very useful instrument. The ion manipulation is critical in MS/MS operation. Differences between the operation of 2D and 3D instruments are also covered. Finally, there is an overview of the wide array of methods to perform MS/MS experiments: collisional activation using resonance or nonresonance techniques, infrared multiphoton dissociation (IRMPD), and gas-phase chemical reactions, namely, ion–molecule and ion–ion reactions.

6.2 INTRODUCTION

There are two types of quadrupole mass analyzers that use rf electric fields to confine ions in two or three dimensions: the beam-type quadrupole mass filter and the trapping quadrupole ion trap mass spectrometer. The quadrupole mass filter and two-dimensional (2D) quadrupole ion trap are physically composed of four parallel rods, which are typically hyperbolic (ideal) or round [1]. While the quadrupole name is widely applied to rods themselves, it is the electric fields produced between the electrodes (shown in Fig. 6.1) that are of analytical value, hence the three-dimensional (3D) ion traps consisting of three electrodes are referred to as *quadrupole ion traps*, although sometime also referred to as *Paul traps*, after the inventor Wolfgang Paul [1]. Because

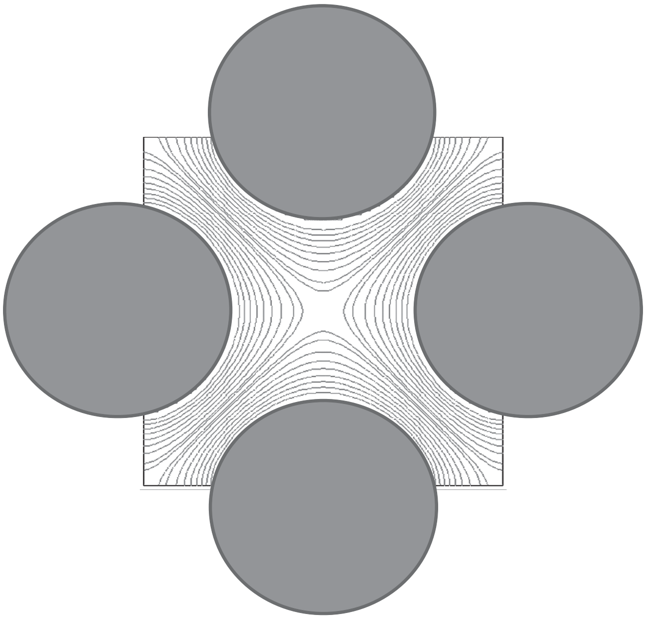

Figure 6.1 Axial view of a quadrupole mass filter rod assembled with an equipotential electric field plot. In an ideal system, the electric field is quadratic. The plot for a three-dimensional quadrupole ion trap would look very similar, with differences mainly in electrode spacing.

the electric field is created with an rf potential, the quadrupole field is dynamic. At any given point in time, the ions experience a field that forces them toward the center of the device in one dimension (trapping) and a corresponding field that forces them away from the center in the other dimension (repelling). As the phase of the rf voltage changes, the dimension in which the ions are trapped and the dimension in which they are repelled alternate back and forth at the frequency of the applied rf voltage. If this frequency is in the appropriate range, typically in the hundreds of kilohertz to a couple of megahertz, the ions have a stable trajectory that periodically goes through the center of the device, or as is commonly stated, the ions are trapped.

Of the mass spectrometers most commonly used today, those containing quadrupole mass filters are the oldest and most mature. The most common configurations for mass spectrometers using the quadrupole mass filter are the single quadrupole instrument (typically coupled to some form of chromatography), the triple quadrupole instrument (which for current instruments may instead consists of two quadrupoles and a higher order multipole), or a quadrupole used in conjunction with another type of mass analyzer to create a hybrid instrument. By grouping quadrupoles in tandem or coupling with another mass analyzer MS/MS experiments can be performed. Most of the modern day MS/MS instruments using quadrupole fields found their origin in work 25–30 years ago.

6.2.1 General Theory of Operation

The physics of ion motion in a quadrupolar field is the same whether the ions are analyzed by a single quadrupole mass filter or multiple quadrupole mass filters in series. In addition, the principles of a quadrupole mass filter can be modified slightly to produce 2D and 3D ion traps. All quadrupoles require the use of rf waveforms to confine ions to stable trajectories allowing them to be either transmitted from entrance to exit or trapped within the volume of the device. At a given value of rf voltage, a dc voltage can be added to define the range of ion mass-to-charge ratios that will have stable trajectories. In the case of beam-type quadrupole mass filters, rf and dc voltages of the same phase or polarity are applied to pairs of rods directly opposite each other, with adjacent rods being 180° out of phase for rf voltages and of opposite polarity for dc potentials. The stability of ions in a linear quadrupole is achieved when the ion motion in the radial dimensions of the quadrupole never exceeds the inscribed radius, r_0, of the rods. The motion of the ions can be described by a second-order differential equation. The details of the equation of motion are not critical to the general understanding of how a quadrupole works; it is sufficient to know that a general solution to the problem can be derived from the Mathieu equations developed in the late 1800s [2]. Ion trajectories fall into one of the two following classes: stable, where the motion periodically passes back through the origin, and unstable, where the motion increases to infinity. Determination of whether an ion has a stable trajectory or unstable trajectory depends on what is known as the Mathieu parameters q_u and a_u (Eqs. 6.1 and 6.2).

$$q_u = \frac{4eV}{m\Omega^2 r_0^2} \tag{6.1}$$

$$a_u = \frac{8_e U}{m\Omega^2 r_0^2} \tag{6.2}$$

In these equations, m is the mass of the ion, Ω is the frequency of the rf voltage applied to the rods (which is called hereafter as the *drive voltage* to differentiate it from other rf voltages that can be applied to manipulate ions), r_0 is the radius of the circle inscribed in the rod assembly, V is the amplitude (0-to-peak) of the rf drive voltage, U is the amplitude of the dc voltage, e is the charge, and u is the dimension to which the equation applies (x or y—the z-dimension is along the length of the rods). The set of solutions calculated by the Mathieu equation for stable trajectories of ions is often plotted in what is referred to as the *Mathieu stability diagram*. In Fig. 6.2, the stability diagram for a quadrupole mass filter is plotted. Ions having a and q values within the dashed lines have stable trajectories and those outside the dashed lines are unstable. It should be emphasized that this treatment does not provide information about the actual physical x- and y-positions of a given ion, only that the values of x and y are always less than the distance from the origin to the rods (stable) or whether the ion trajectory will at some point in time exceed the radius of the device in either the x- or y-dimension.

When a quadrupole mass filter operates with just rf drive voltages applied to the rods ($U = 0$), it behaves as a high pass filter, allowing the transmission of all ions with mass-to-charge ratios greater than the low mass cutoff i.e. those mass-to-charge ratios

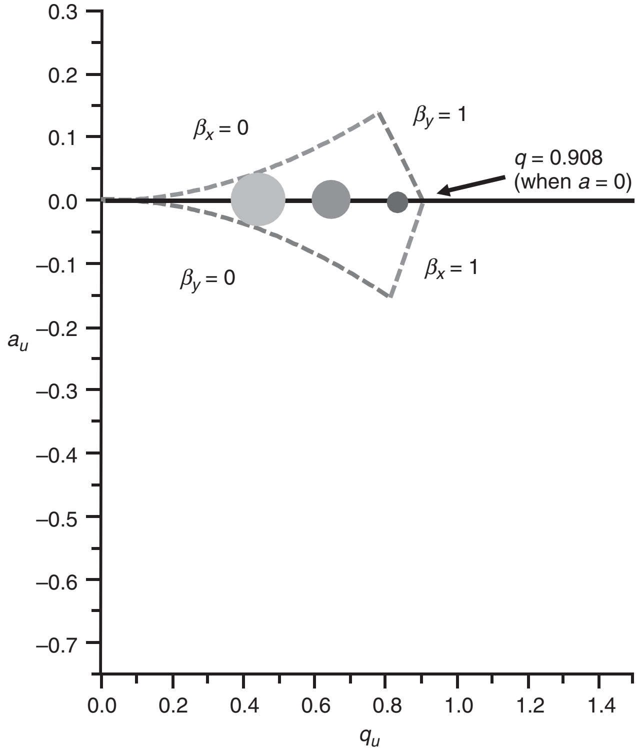

Figure 6.2 The Mathieu stability diagram for a quadrupole mass filter and a 2D quadrupole ion trap. The stability region is symmetrical about the q-axis. In this plot, the a and q values are shown for ions of different mass-to-charge ratios when there is no mass resolving dc voltage applied (rf-only mode). Mass-to-charge ratios are represented by the diameter of the dots.

that have a q_u value less than 0.908 (Fig. 6.2). When operated in such a manner, the quadrupole mass filter functions as an ion guide. This mode of operation has played a major role in the development of quadrupole systems for MS/MS.

Mass analysis in quadrupole mass filters is accomplished by the application of both rf and dc voltages to the rod sets. For a given value of V and U, each mass-to-charge value will lie along a "scan line." The ratio of the rf and dc voltages is selected such that the line passes through a small area at the apex of the stability region (Fig. 6.3). In this case, ideally ions of only a single mass-to-charge value will have stable trajectories in the device. As the rf and dc amplitudes are increased, keeping the ratio constant, successively larger mass-to-charge ratios are stable and will pass through the device to the detector.

For the 3D quadrupole ion trap, the same Mathieu equation applies, but in three dimensions. The x- and y-dimensions, which are the plane of the ring electrode, are combined in polar coordinates to give a radial dimension (r), and now, there is also an rf field in the z-dimension. To meet the conditions of the Laplace equation, there is an asymmetry between the r- and z-dimensions ($r^2 = 2z^2$), so the Mathieu

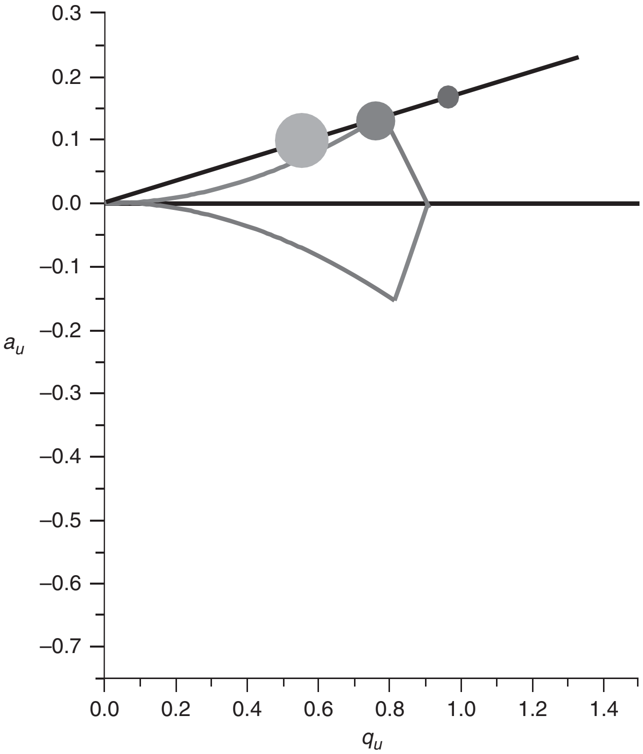

Figure 6.3 The Mathieu stability diagram for the operation of a quadrupole mass filter. The ratio of the rf to dc voltage determines the slope of the line on which lie the ions' a and q values. The values are selected such that only one mass-to-charge ratio lies within the stability region. That mass-to-charge ratio will be transmitted through the quadrupole to the detector. All other ions have a and q values that lie outside the stability diagram resulting in unstable trajectories though the mass filter.

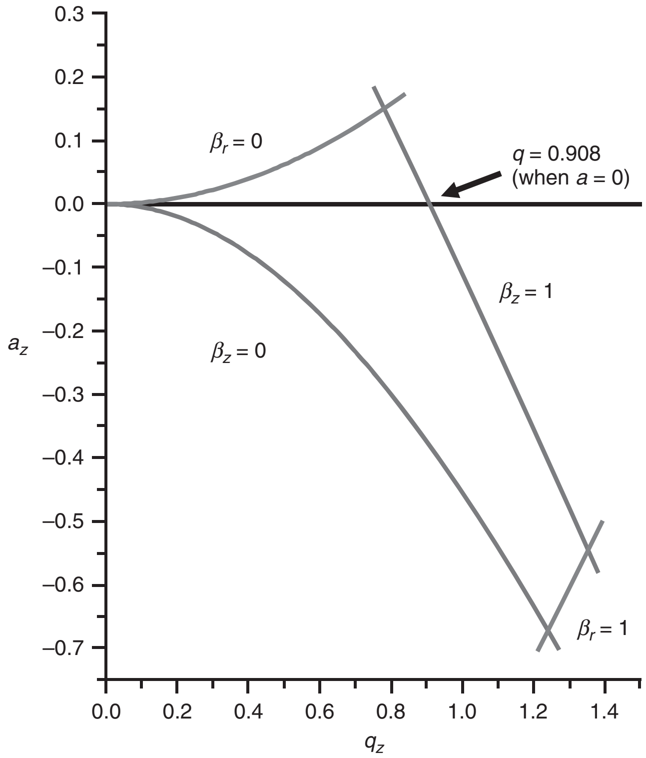

Figure 6.4 The Mathieu stability diagram for a three-dimensional quadrupole ion trap. The stability region is not symmetrical, but the intersection with the q-axis for $a = 0$ (rf-only mode) is still $q = 0.908$.

stability diagram for the three-dimensional ion trap (Fig. 6.4) is not symmetric like the quadrupole mass filter. The a_u and q_u equations are shown below.

$$a_z = -2a_r = \frac{16_e U}{m\Omega^2 r_0^2} \tag{6.3}$$

$$q_z = -2q_r = \frac{8_e V}{m\Omega^2 r_0^2} \tag{6.4}$$

It is important to note that while stable ions pass through the quadrupole mass filter and hit a detector, stable ions in a 3D quadrupole ion trap remain in the device and thus they need to be further manipulated to be detected (see later discussion). This added operational complexity hindered the development into a mass spectrometer of the 3D quadrupole ion trap for several decades after the quadrupole mass filter was commercialized.

A key feature of quadrupole-based instruments is the ease with which they can be interfaced to chromatography methods. Gas chromatography/MS and liquid chromatography/MS using single quadrupole devices are routine methods. However, the feature that is probably most important in the success of quadrupole-based devices is the ability to perform MS/MS [3]. In fact, the triple quadrupole was developed expressly for MS/MS. The MS/MS experiment can be divided into three distinct steps.

In the first step, an ion formed in the ion source is isolated in the first stage of MS. This ion, called the *parent ion* or *precursor ion*, is then reacted in some manner (step two). The ions thus produced from the reaction of the parent ions, called *product ions*, are analyzed in the second stage of MS (step three). This is often referred to as taking the mass spectrum of an ion in a mass spectrum. It also has analogies to chromatography/mass spectrometry experiments where the chromatography separation is replaced by the first stage of MS.

The reaction of the parent ions to generate product ions is a key component to the MS/MS experiment. Before quadrupole-based instruments, collision-induced dissociation [CID, also called *collision-activated dissociation* (*CAD*)] was the only reaction used. The development of quadrupole-based instruments expanded the types of reactions that could be used for MS/MS. Current reactions used include ion–molecule reactions, ion–ion reactions, ion–electron reactions, and photodissociation. However, CID is still by far the most widely used reaction.

The CID reaction involves colliding the parent ion with a target (collision) gas. To avoid chemically reactive collisions, a nonreactive gas is typically used, such as He, Ar, or N_2. The collision between the parent ion and the target can convert kinetic energy of the parent ion into internal energy of the parent ion, internal energy of the target gas, or kinetic energy of the target gas. Only the first process is useful in the MS/MS experiment. If the parent ion acquires sufficient internal energy, it will dissociate to give product ions that can be used to identify the parent ion structure. Conversion of the parent ion's kinetic energy to target gas internal energy can be minimized using a monoatomic target gas to eliminate the possibility of vibrational excitation of the target gas. N_2 is often used as a compromise target gas because of its low cost, and while not monoatomic, it has a limited number of degrees of freedom.

While the dynamics and kinematics of the ion-neutral collisions are quite complex, at a basic level, fragmentation of the parent ion is dependent on the amount of ion kinetic energy that can be converted into ion internal energy. The maximum amount of internal energy that can be obtained in a collision with a target gas is the center-of-mass collision energy (Eq. 6.5)

$$E_{\mathrm{com}} = E_{\mathrm{lab}}\left(\frac{m_{\mathrm{t}}}{m_{\mathrm{t}} + m_{\mathrm{i}}}\right) \tag{6.5}$$

where E_{com} is the center-of-mass collision energy, E_{lab} is the parent ion laboratory kinetic energy, m_{t} is the mass of the target gas, and m_{i} is the mass of the parent ion. Given that the most commonly used collision gases have masses from 4 to 40 Da and parent ions have masses in the hundreds of daltons, only a small fraction of the ion kinetic energy can be converted to internal energy, even in the best case when all the center-of-mass collision energy is converted to internal energy. For quadrupole-based MS/MS instruments, the ion kinetic energy usually is in the range of 5–20 eV. Thus, multiple collisions are necessary to produce dissociation.

Both triple quadrupole mass spectrometers and ion trap mass spectrometers are powerful MS/MS instruments, but there are very substantial differences in their capabilities and methods of operation. The rest of this chapter is divided into two major sections, one dealing with the quadrupole mass filters in MS/MS and the other, quadrupole ion traps, both 3D (the original quadrupole ion trap) and 2D (a common component of mass spectrometers at present).

6.3 TRIPLE QUADRUPOLE MASS SPECTROMETERS

6.3.1 Origin

The linear quadrupole mass analyzer was first described in the scientific literature in 1958 [4] and has been used since as a mass filter, ion guide, and ion trap. The first commercial single quadrupole mass analyzer instrument, used as a residual gas analyzer, became available in 1961 [5]. In 1974, the first arrangement to be used for MS/MS, three quadrupoles in series, was published [6]. This instrument used a mass-analyzing quadrupole (Q1) to isolate parent ions for transmission into the second quadrupole operated as an rf-only ion guide. As these ions passed through the second quadrupole (q2), they were photodissociated. The products of the photodissociation and any remaining parent ions were then mass analyzed with the third mass quadrupole (Q3). It was the work of Yost and Enke in 1977 that produced the basic design for all the so-called triple quadrupole instruments to come. The key aspect of the QqQ geometry is the middle quadrupole (q) being operated in the rf-only mode and with a collision gas introduced to effect CID [7]. From an instrumental perspective, CID is much simpler to implement than photodissociation and is also much more efficient in a beam-type experiment, so the development of CID in a quadrupole ion guide made possible a commercially viable instrument.

A generic schematic of a triple quadrupole is shown in Fig. 6.5. One of the attractive features of triple quadrupoles is the simplicity of operation. The mass-analyzing quadrupoles (Q1 and Q3) are operated as shown in Fig. 6.3; that is, a scan line determined by the ratio of the rf and dc voltages intersects the apex of the stability diagram and ions of only a single mass-to-charge ratio are transmitted though the quadrupole. To obtain a mass spectrum, Q1 can be mass-selectively scanned while q2 and Q3 are operated in rf-only mode, producing a mass spectrum identical to the operation of a single quadruple analyzer. Conversely, the same can be achieved by operating Q1 and q2 in the rf-only mode while mass scanning Q3 to produce a single stage of mass analysis.

6.3.2 Tandem Mass Spectrometry

Each stage of the MS/MS experiment in a triple quadrupole is performed in a different quadrupole, and thus, the experiment is sometimes referred to as *tandem-in-space*. The quadrupole in which the dissociation is effected (q2) functions as an ion guide

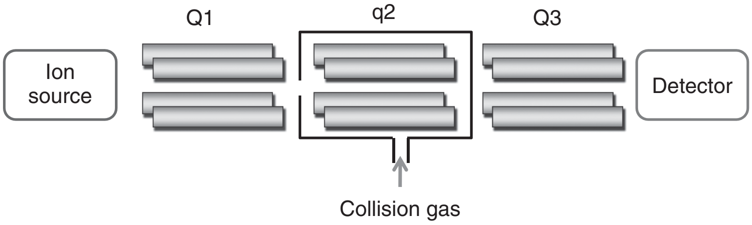

Figure 6.5 A schematic of a triple quadrupole mass spectrometer. Q1 and Q3 can be operated in either a mass filter mode (Fig. 6.3) or the rf-only mode (Fig. 6.2). q2 is operated in the rf-only mode at an elevated pressure to effect collision-induced dissociation for MS/MS experiments.

and operates with just rf drive voltages applied (Fig. 6.2), and thus, ions of all mass-to-charge ratios above a certain value are transmitted. There have been few changes to the triple quadrupole since they were first described in the 1970s and essentially all the changes have involved modifications of q2. The present triple quadrupole mass spectrometers often use a hexapole or octapole as the ion guide. Although these higher order fields are not suitable for mass analysis because of the coupling in the ion motion in the x- and y-dimensions, while each dimension is independent in a quadrupole field, they have a wider range of transmission in the rf-only mode [8,9]. The other change in q2 that has become common is a mechanically nonlinear rod set, so that there is not a line of sight from the ion source to the detector. Because the ions follow the centerline of the field, it is possible to do anything from having a small bend [10] in the ion guide to making a 180° curve.

6.3.2.1 MS/MS Efficiency. A key performance feature that made the triple quadrupole a success compared to the sector instruments used at that time was the increase in MS/MS efficiency. MS/MS efficiency (Eq. 6.6) is defined as the ratio of summed product ion abundance ($\Sigma \mathrm{D}$) to the initial parent ion abundance before activation (P_0).

$$E_{\mathrm{MS/MS}} = \frac{\Sigma \mathrm{D}}{P_0} \tag{6.6}$$

The triple quadrupole has MS/MS efficiencies of 1–10%, which are typically 10–100 times greater than those of the sector instruments. There are several reasons for the increased MS/MS efficiency in triple quadrupoles compared to the sector instruments, which were the principal MS/MS instruments at the time of the development of the triple quadrupole. The major factor leading to improved MS/MS efficiency is the rf-only ion guide (q2) in which the CID reaction takes place providing strong focusing for ions after collision with a neutral gas. Thus, even if the ions are scattered substantially in the collision with the target gas (typically N_2 or Ar), product ions or undissociated parent ions are refocused back to the center axis and efficiently transmitted to Q3 for mass analysis. Because of the focusing characteristics of the rf-only ion guide, higher pressures of collision gas can be used than in older non-rf-confined collision cells, allowing a higher number of collisions between the parent ion and target gas, which further improves the MS/MS efficiency. Finally, an often overlooked effect is the time frame of the experiment. The transit time through q2 is on the order of hundreds of microseconds. This is in contrast to sector instruments where the CID process occurs in a time frame closer to 10 μs. Thus, kinetically slower reactions occur in the triple quadrupole that are not observed in sector instruments, which can further increase the MS/MS efficiency.

6.3.2.2 Modes of Operation. One of the key advantages of triple quadrupole mass spectrometers is the multiple types of MS/MS experiments that can be readily implemented. By far, the most common MS/MS experiment is the product ion scan. This scan is used to obtain structural information on ions in the mass spectrum with the goal of identifying the structure of the neutral compound from which the ion was formed. In a product ion scan, an ion formed in the ion source (parent ion) is selected in Q1 by fixing the values of the rf and dc voltages applied to the Q1 rods; the parent ion is reacted in q2, typically using CID; and then the product ions from the reaction are detected by scanning Q3 in the normal mode.

While any tandem mass spectrometer is capable of readily performing such product ion scans, triple quadrupole instruments are unique in their capability to perform other types of MS/MS experiments. In addition to product ion scans, triple quadrupoles can perform parent ion scans and neutral loss scans. The parent ion scan and neutral loss scans are used to screen for targeted compound types in a mass spectrum. For example, m/z 149 is a characteristic product ion of phthalates. By just reversing the modes of operation of Q1 and Q3, a parent ion scan is performed that will detect all the parent ions that dissociate to produce a product ion at m/z 149. This means that Q3 has fixed values of rf and dc voltages and Q1 is scanned in the normal manner.

The neutral loss scan, is useful when a compound type loses a common neutral fragment, such as phosphorylated peptides losing 98 Da (H_2PO_4). In a neutral loss scan, both Q1 and Q3 are scanned in the normal manner, but the scans are offset by the neutral mass of interest (e.g., 98 for singly charged and 49 for doubly charged phosphorylated peptides). This offset means that if the rf and dc voltages are at values to transmit m/z 656 through Q1, the Q3 rf and dc voltages would be set to transmit m/z 558 (singly charged ions) or m/z 607 (doubly charged ions). Thus, if m/z 656 dissociates by the loss of 98 Da, the product ion will be transmitted through Q3 and detected. If it loses any other mass (e.g., 120 to give an ion at m/z 536), that ion will not be transmitted and no there will be no output signal from the detector. Because these scans are performed using identical hardware and need only specific software written to control the electronics via computer, all these scans can be implemented on any commercial triple quadrupole mass spectrometer.

In addition to the above scanning modes of operation, another very useful mode of operation is reaction monitoring. In reaction monitoring experiments, Q1 and Q3 are operated in a static mode to filter all ions but one parent ion and one product ion. This mode of operation is termed as *selected reaction monitoring* (*SRM*). SRM scans maximize the sensitivity for the detection of known or targeted compounds in complex mixtures. Multiple SRM experiments can be performed where Q1 and/or Q3 vary, but do not scan. One form of MRM provides increased specificity. Q1 is fixed to transmit a give parent ion, while Q3 "hops" between selected mass-to-charge ratios of known product ions. The specificity is increased not only due to the detection of multiple product ions but also by the ratio of the intensity of the product ions. Another form of MRM involves both Q1 and Q3 hopping between predetermined parent ion and product ion mass-to-charge ratios. This approach can be implemented to screen for multiple targeted compounds in a single sample. This is often used for quantification where one parent ion is the target analyte (e.g., drug metabolite) and the other compound is an internal standard. Commonly, the internal standard will be an isotopically labeled version of the target analyte. In this case, if the isotope label is lost as a neutral, only Q1 will need to be changed, by the mass of the isotopic label, while Q3 will be held constant because the product ion will have the same mass-to-charge ratio for both parent ions. If the isotopic label or part of the label is retained in the product ion, both Q1 and Q3 will be hopped.

An important but subtle consideration in performing these various MS/MS experiments is the transit time of the ions through each quadrupole. As an example in the case of an MRM where Q1 is rapidly switched between the parent ions m1 and m2, the rate at which Q1 can be switched is limited by the time it takes product ions to transit through q2 and Q3. In the event that Q1 is switched too rapidly from parent ion m1 to parent ion m2, product ions of m1 could be identified as having come from m2 if m1 and m2 share common product ions. In the case where m1 and m2 have different product ions, the scan speed will be limited by the time it takes for ions to be transmitted through to the detector, as any product ions present in Q3 when the mass is switched will be lost and sensitivity will be reduced.

6.4 QUADRUPOLE ION TRAP MASS SPECTROMETERS

6.4.1 Origin

Although the 3D quadrupole ion trap was first described at the same time as the quadrupole mass filter and used for trapping charged particles in physics experiments and to study ion–molecule reactions, it took several decades before it became a useful mass spectrometer. The transition from a quadrupole mass filter to a 3D quadrupole ion trap can be visualized as taking the quadrupole rod assembly and bending it into a vertical circle, joining the entrance and exit ends of the rods. The two rods in the vertical plane will form a donut-shaped ring, while the two rods in the horizontal plane will each form an "endcap" that is solid with a hyperbolic surface. Figure 6.6b shows the electrodes taken apart, and Fig. 6.6a is a cross-sectional drawing. It should be noted that to transfer ions into and out of the quadrupole ion trap, holes have to be drilled in the endcap electrodes. These holes, along with the fact that the electrodes do not extend to infinity, cause the electric field to deviate slightly from an ideal quadrupole field. These deviations can be expressed in terms of higher order fields, which can have positive and negative effects on the operation of the ion trap [11].

The key challenge to the development of the quadrupole ion trap as a mass spectrometer was an efficient way to detect mass-selected ions. Ions with stable trajectories in a quadrupole mass filter pass through the quadrupole to a typical mass spectrometry detector, an electron multiplier. Conversely, ions with stable trajectories in a quadrupole ion trap remain in the ion trap, and it is necessary to make them unstable to eject them to a detector. A simple but elegant solution to this problem was developed by Stafford and colleagues [12] in the early 1980s and led to the commercialization of the quadrupole ion trap by the Finnigan Corp. Operating the quadrupole ion trap with only an rf drive voltage means ions of increasing mass-to-charge ratio can be made sequentially unstable and ejected by increasing (ramping) the amplitude of the rf drive voltage. Because there is no dc voltage, the Mathieu a_u parameter is zero, and whether an ion has a stable trajectory or not is determined by the Mathieu q_z parameter. As noted in Fig. 6.4, ions with a $q_z < 0.908$ have stable trajectories. From Equation 6.3, q_z is proportional to the amplitude of the voltage (V) and inversely proportional to mass-to-charge ratio. For a given V, the mass-to-charge ratio that has a q_z of 0.908 is known as the *low mass cutoff*. As V is increased, the low mass cutoff increases and ions of increasing mass-to-charge value are ejected in the z-dimension. Placing an electron multiplier outside the electrode assembly on the z-axis allows the ions to be detected. This mode of operation is termed as *mass-selective instability*. While commercial quadrupole ion traps no longer use mass-selective instability, this invention catalyzed the development of the quadrupole ion trap mass spectrometer.

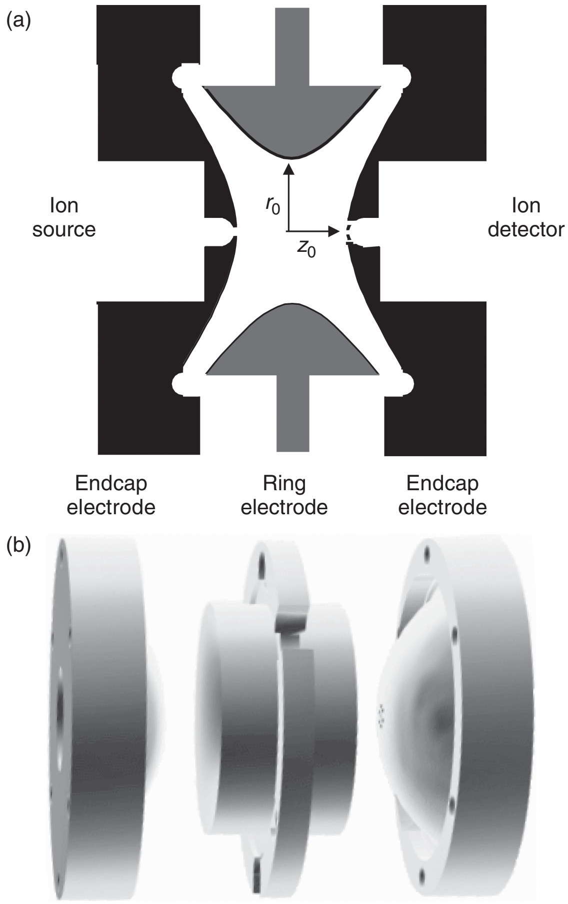

Figure 6.6 A cutaway schematic of a 3D quadrupole ion trap mass spectrometer. (a) Ions are injected into the ion trap through one of the endcaps and ejected through the other endcap to an electron multiplier for detection. (b) A three-dimensional model of the hyperbolic trapping electrodes commonly used in commercial 3D traps.

The original quadrupole ion traps were not even called mass spectrometers. They were marketed as Ion Trap Detectors™ and were used for gas chromatography/mass spectrometry. A rather unique operating feature of the quadrupole ion trap is the improvement of both resolution and sensitivity when operating with helium bath gas pressures in the millibar range [12]. The improvement in performance is a result of collisionally cooling the ions to the center of the electrodes that leads to more efficient ejection of the ions to the detector.

6.4.2 Ion Manipulation in Quadrupole Ion Traps

The power of the quadrupole ion trap lies in the ability to manipulate the trapped ions in time. The parameter that allows the ions to be controlled is what is termed the *secular frequency*. At a given set of operating parameters, V and U, ions of each mass-to-charge have a unique frequency at which they move in the z- and r-dimensions. For a given drive frequency, Ω, the secular frequency is determined by Equation 6.7

$$\omega_u = \frac{\beta_u \Omega}{2} \tag{6.7}$$

where β_u is given in Equation 6.8:

$$\begin{aligned}\beta_u^2 = a_u + &\cfrac{q_u^2}{(\beta_u + 2)^2 - a_u - \cfrac{q_u^2}{(\beta_u + 4)^2 - a_u - \cfrac{q_u^2}{(\beta_u + 6)^2 - a_u - \cdots}}} \\ + &\cfrac{q_u^2}{(\beta_u - 2)^2 - a_u - \cfrac{q_u^2}{(\beta_u - 4)^2 - a_u - \cfrac{q_u^2}{(\beta_u - 6)^2 - a_u - \cdots}}}\end{aligned} \tag{6.8}$$

which for $q_z < 0.4$ and $q_r < 0.2$ can be approximated by Equation 6.9:

$$\beta_u = \sqrt{a_u + \frac{q_u^z}{2}} \tag{6.9}$$

The plot in Fig. 6.7 shows the iso-β lines, that is, sets of values of a and q that give a constant β and thus a constant secular frequency of ions of a given mass-to-charge ratio. The limits of the stable trajectories are actually bounded by $\beta_u = 0$ and $\beta_u = 1$ (as noted in Figs. 6.2 and 6.4), so ions will have a secular frequency up to one half the drive frequency, Ω. From a practical perspective, only the secular frequency in the z-dimension is useful. Because in the typical operating mode of quadrupole ion traps $U = 0$, Equation 6.9 reduces to Equation 6.10.

$$\beta_z = \frac{q_z}{\sqrt{2}} \tag{6.10}$$

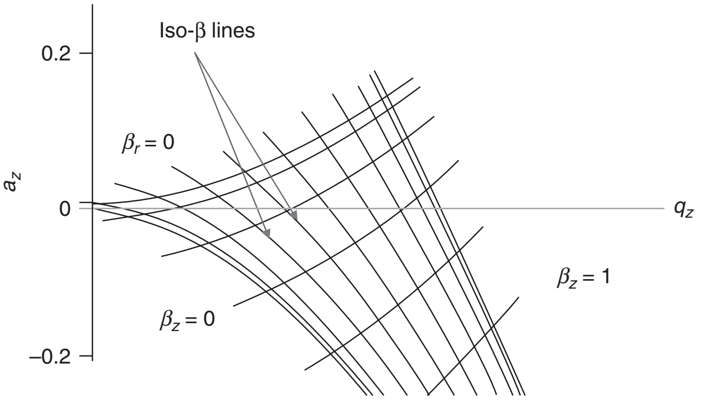

Figure 6.7 Upper part of the Mathieu stability diagram for a quadrupole ion trap showing the iso-β lines. These lines are plots of a and q values that correspond to a given secular frequency. The secular frequency is the main parameter used to manipulate ions in a quadrupole ion trap.

The ions in a quadruple ion trap are manipulated by applying a supplemental rf voltage at the secular frequency of the ions to the endcaps (z-dimension).

When the frequency of the supplemental rf voltage is the same as the secular frequency of a given mass-to-charge ratio, the ions that are in resonance will absorb power. This is termed as *resonant excitation* and increases the kinetic energy of the resonant ions. Resonance excitation is the key to almost every aspect of experiments performed in quadrupole ion traps. When resonance excitation is large enough, the ions will gain sufficient kinetic energy to be ejected from the trapping region; this process is often called *resonant ejection*.

Resonant ejection is how all commercial quadrupole ion trap systems obtain mass spectra, as it provides several advantages over mass-selective instability. First, it allows a larger range of mass analysis. With mass-selective instability, the ions have to reach a q_z value of 0.908 to be ejected and detected. With standard-sized electrodes (e.g., $r_0 = 1.0$ cm) this leads to a practical upper mass-to-charge ratio limit of around 1000. By performing resonant ejection at a lower q_z value, the mass range is extended proportionally. This allows the mass range to be extended to the 2000–4000 range, although much higher is possible [13]. The actual scan is analogous to the mass-selective instability scan, ramping up the rf drive amplitude so that the ions sequentially come into resonance and are mass-selectively ejected, from low to high mass-to-charge ratio, with the applied supplemental rf voltage. Another advantage of resonant ejection for mass analysis is increased resolution. A somewhat unique capability of the quadrupole ion trap is the ability to change resolution by changing the scan speed. By reducing the rate at which the rf drive voltage is ramped, the relative width of the peak is decreased, increasing the resolution [14]. For significant gains in resolution, the ramp rate must be drastically reduced, making impractical the simultaneous collection of a wide mass range and high resolution mass spectra. However, for narrow ranges, such as the isotopic distribution of a multiply charged ion, the slower scan is useful to obtain resolution allowing charge-state determination.

6.4.3 Linear Quadrupole Ion Traps

The linear ion trap, or 2D ion trap, is a quadrupole mass filter that traps the ions in two dimensions, x and y (or radial), using the rf drive voltage and traps ions in the axial dimension (z) with a dc voltage. The major advantage of the 2D ion trap versus the 3D ion trap (quadrupole ion trap) is the efficiency of ion injection. In the quadrupole ion trap, because there is an rf field in all three dimensions, the ions must be injected through an rf electric field. Only during a small phase angle of the rf cycle are the voltages compatible with efficient injection. Also, the injection is very sensitive to the amplitude of the rf potential. However, with the 2D ion trap, there is no rf electric field in the axial dimension, and as a result, ions can be injected over all 360° of the rf cycle. This leads to substantial reduction in the time required for ion injection.

Once in the trapping field, there is very little difference in the way ions are trapped in a 2D ion trap and a quadrupole ion trap. The Mathieu stability diagram also applies for 2D ion traps, and in the absence of dc potentials applied to the quadrupole rods, ions with a $q_z < 0.908$ are trapped radially. Ions will be prevented from exiting axially by applying to an electrode at the exit of the 2D trap a dc voltage slightly greater than the entering ions' kinetic energy. These blocked ions then turn around and travel back toward the lower dc potential applied at the entrance of the 2D trap. This dc potential is lower than the ions' kinetic energy when they are entering the 2D trap. However, collisions with the bath gas will have reduced the ions' kinetic energy, so by the time ions return to the entrance, too little kinetic energy remains to overcome the entrance barrier and they will be trapped axially.

There are two means of implementing dc axial trapping. The most common method involves using plates/lenses with apertures at the front and back of the quadrupole rod assembly. This truncates the rf electric field, introducing higher order fields near the entrance and exit of the rods. Alternatively, short sets of quadrupole rods can be used in front of the entrance and at the exit of the main trapping rod assembly. The rf voltage can be capacitively coupled to the end segments, yielding minimum distortion of the rf electric field in the center segment, which is where the ions typically reside. Simulations of the electric fields obtained when applying a supplementary resonance excitation voltage to a pair of rods resulting from these two designs are presented in Fig. 6.8 [15]. Although the resulting electric fields generated by these two approaches result in only minor differences for trapping ions, they are significant for mass analysis.

One approach to mass analysis with the 2D ion trap is very similar to resonant ejection used in the quadrupole ion trap [15]. In this case, a supplementary rf voltage is applied to a pair of rods opposite to each other to resonantly eject the ions radially. Slots must be machined into the rods to allow the ions to exit to external electron multiplier(s). Homogeneity of the field is essential in this design, and thus, the use of end segments and precise alignment of the rods are critical. This design is the basis for the Thermo linear ion trap systems [15]. Although ions are ejected through both opposing rods to which the resonant frequency is applied, this is a highly efficient form of mass-selective ion ejection. In some cases, two electron multipliers are used to increase detection efficiency.

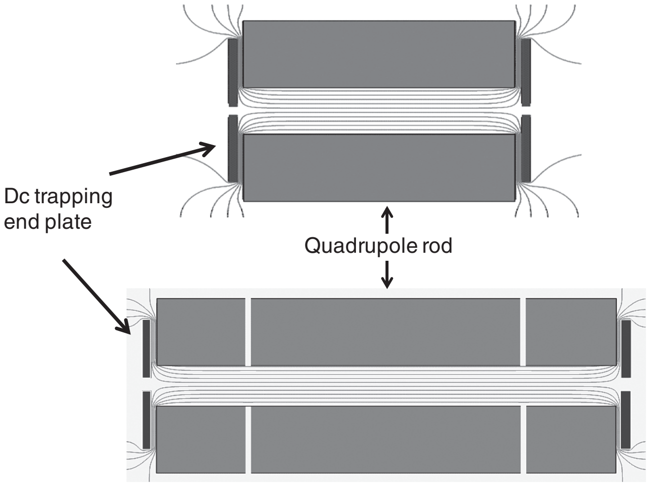

Figure 6.8 Simulation results showing the dipolar electric field in a single-segment 2D ion trap and in a three-segment 2D ion trap. The nonlinearity of the field where the end plate truncates the electric field can cause axial ejection. In a three-segment ion trap when the ions are trapped in the center segment, this is not a problem. In a single-segment ion trap, the axial ejection can be used to mass-selectively eject the ions axially.

An alternate mass analysis method takes advantage of the higher order fields that result from truncating the rf electric field with an aperture plate to perform axial ejection [16]. As noted earlier, ion motion in higher order fields is coupled; that is, when an ion is excited in one dimension, the initial kinetic energy gain is in that dimension, but it can then couple into other dimensions. This is in contrast to a pure quadrupole field where an ion increases its kinetic energy (and amplitude of motion) only in the dimension of the resonance excitation voltage. In the axial ejection 2D traps, ions are bunched toward the exit of the 2D ion trap and resonantly excited in the radial dimension. The increase in radial motion is coupled into axial motion, and the ions gain enough axial kinetic energy to overcome the dc potential at the trap end plate, resulting in ejection and detection by the electron multiplier. The coupling of radial excitation to axial ejection is not as efficient a process as radial excitation and ejection but more than 20% of the ions can be detected. This method of mass analysis is used in the AB Sciex Q-Trap® systems.

Another form of axial ejection has been introduced by researchers from Hitachi [17]. In this implementation, vanes are added between the quadrupole rods that produce a field that is approximately harmonic along the central axis. Ions can then be axially resonated and ejected. This is a more efficient ejection process than coupling between radial and axial motions but is still not as efficient as radial resonant ejection.

6.4.4 Tandem Mass Spectrometry

In the quadrupole ion trap, the different stages of the MS/MS experiment are performed in the same analyzer volume but at different points in time. Thus, MS/MS in trapping instruments is sometimes referred to as being *tandem-in-time*. A key feature of tandem-in-time experiments is that multiple stages of MS/MS (MS^n) can be performed. MS^n is not going to be specifically discussed here, but it can be a very powerful experiment to obtain ion structure/differentiation of isomers.

6.4.4.1 Ion Isolation. There are a number of approaches to isolating the parent ion in a quadrupole ion trap. To isolate parent ions of a given mass-to-charge ratio, ions of both lower and higher mass-to-charge ratios must be ejected. Lower mass-to-charge ions that have a $q_z < 0.908$ at the maximum amplitude of the rf drive voltage can be ejected by simply ramping the rf drive to a sufficient amplitude such that low mass-to-charge ions are ejected at $q_z = 0.908$. Higher mass ions can be simultaneously ejected during the ramp to ejection ions with mass-to-charge ratios less than that of the parent ion by applying a supplemental rf voltage at a frequency corresponding to a mass-to-charge ratio just higher than that of the parent ion at the start of the ramp. As the rf drive amplitude increases, sequentially higher mass-to-charge ions will be resonantly ejected, while those masses lower than the parent ion are ejected via mass-selective instability.

To this point, only single frequency resonance excitation waveforms have been considered. However, there is no reason that waveforms with multiple frequencies cannot be used. A standard means for manipulating ions with a waveform containing multiple frequencies is known as *SWIFT*, which is the acronym for Stored Waveform Inverse Fourier Transform [18]. In SWIFT, a frequency domain waveform is generated to achieve the desired purpose of the ion manipulation, for example, to eject all ions except parent ions for an MS/MS experiment. Such a waveform includes all secular frequencies except those of the parent ion. Then, an inverse Fourier transform of the frequency domain spectrum is performed to obtain a time domain supplemental rf voltage signal that can be applied to the quadrupole ion trap. This time domain signal will cause all the unwanted ions in the quadrupole ion trap to be ejected. Very complex ion manipulation can readily be performed by this method, but one has to be careful in how the waveform is calculated, that is, the phases of the time domain signal must be such that a physically unattainable amplitude is not produced mathematically.

A simple way to approximate a SWIFT waveform is to add a series of sine waves separated by 500 Hz over the range of the resonant frequencies of ions (0 to one half the rf drive frequency). Then the frequencies of the parent ion can be "notched" out. Because the parent ion q_z value is controlled by the rf drive amplitude, a constant value of q_z for parent ion isolation can be used by changing the rf drive amplitude to set the parent ion at the desired q_z for isolation, and thus, the same waveform is applicable to all parent ions.

6.4.4.2 Ion Activation.

6.4.4.2.1 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 *CID*. There are several methods to induce energetic collisions between ions and neutral gas molecules where kinetic energy can be converted into internal energy. As mentioned earlier, the deposition of internal energy into parent ions leads to dissociation and formation of product ions, from which structural information can be deduced.

The type of dissociation products observed in collisional activation methods used in ion traps can be different from those observed in beam-type mass analyzers. It should be noted that the collision energy in a quadrupole ion trap is ill-defined. The ions' kinetic energy is constantly changing due to the dynamic nature of the electric trapping field, the acceleration of ions due to supplemental voltages, and the deceleration of ions due to collisions with the He bath gas. While the collision energies in triple quadrupole instruments can be similar in magnitude to those in the quadrupole ion trap, two fundamental differences often lead to different MS/MS spectra being observed. First, helium is typically the collision gas in the quadrupole ion trap, whereas nitrogen or argon is more commonly used in triple quadrupoles. With heavier gases as the collision gas, much more kinetic energy can be converted to internal energy per collision (Eq. 6.5), leading to a different internal energy distribution. The second difference between the quadrupole ion trap and triple quadrupole is the time frame for the reaction. Ions must dissociate (react) in 100 μs or less in the triple quadrupole instrument to be observed in the next stage of analysis. However, in ion traps, the times are two or three orders of magnitude longer. This longer time frame allows low internal energy, but kinetically slow reactions to occur, and in fact can significantly favor such reactions.

6.4.4.2.2 Resonance Excitation. The first and by far the most common method for collisional activation is resonance excitation [19–22]. This method employs the same phenomenon of resonance as the resonance ejection method discussed previously. As noted above, when a supplemental rf voltage is applied to the endcap electrodes at an ion's secular frequency, that ion's kinetic energy increases. The difference between excitation and ejection is one of degree. By judiciously choosing the amplitude of the supplementary rf voltage and the time it is applied, the ion's kinetic energy can be increased without supplying sufficient energy for ejection because kinetic energy is also lost from collisions with the He bath gas. A typical excitation voltage would be a few hundred millivolts (peak-to-peak) and 10–40 ms long.

Unfortunately, determining the frequency needed for resonance excitation is not always straightforward. The equation for determining the secular frequency of an ion (Eq. 6.8) is not exact. The resonant frequency is affected by higher order fields and can change as the ions gain kinetic energy [23]. The number of trapped ions also affects the secular frequencies of those ions [24]. The sum of the charge within the trapping volume or the "space charge" of the ions acts as a dc potential. The effective a_z value no longer equals zero, and the secular frequency shifts (Eq. 6.9). To address this problem, most commercial quadrupole ion traps are designed to control the number of ions trapped. Alternatively, the rf drive amplitude can be scanned over a narrow range.

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 [22]. 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. 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 favor 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 *pseudopotential well*. The "depth" of this well is the maximum kinetic energy the ion can have and still remain trapped. The deeper the well, the more kinetic energy the ion can gain before ejection. Ions in deeper wells can undergo more energetic collisions as well as 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 pseudopotential well model for q_z values less than 0.4 (Eq. 6.11).

$$D = \frac{q_z^2 m r_0^2 \Omega^2}{32e} \tag{6.11}$$

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 drive amplitude, an ion's q_z value can be increased (Eq. 6.3). However, at higher q_z values, the $\mathrm{q_z}$ values of lower mass-to-charge ions may exceed 0.908 becoming unstable, so the smaller mass-to-charge product ions are not trapped if they are formed. To balance between the well depth and trapping of the product ions, a q_z of 0.2–0.4 for the parent ion is generally chosen. As a result, product ions less than about one-third the mass-to-charge of the parent ion are generally not observed in CID experiments performed on trapping mass analyzers.

A variant of resonance excitation that allows lower mass product ions to be trapped is called *HASTE* (*high amplitude short time excitation*) [25] or *PQD* (*pulsed q dissociation*) [26]. The implementation of these techniques involves using a much larger amplitude resonant supplementary rf voltage (>1 V), but for a very short time (1–2 ms). The parent ion q_z value during the resonance excitation is similar or greater than that used in conventional resonance excitation, but as soon as the excitation step is complete, the rf drive amplitude is decreased to put the parent ion q_z at around 0.1, thus allowing the slow-forming low mass product ions to be trapped.

Another variation of typical resonance 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. As discussed earlier, the maximum amount of laboratory frame kinetic energy (E_{lab}) that can be converted to internal energy through a collision, E_{com}, is given by Equation 6.5. For a parent ion with a given E_{lab}, a heavy gas provides a higher m_{t} and a greater percentage of energy deposition than helium. The addition of heavy gas molecules to the quadrupole ion trap 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 [27]. With heavy collision gases, q_z values as low as 0.05 can be used, allowing low mass-to-charge products to be detected [28]. However, the heavy gas also deteriorates the mass spectral resolution and sensitivity of the quadrupole ion trap because of the greater scattering on collision during ejection. Pulsing in a 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).

6.4.4.2.3 Nonresonance Excitation. Frequency shifts due to the number of trapped ions are part of the motivation for finding other means of dissociation, although resonance excitation CID is still by far the most common method for ion dissociation. Controlling the number of trapped ions may be problematic if the ion source output fluctuates significantly, as can be the case with matrix-assisted laser desorption ionization (MALDI). This maybe one reason why no commercial quadrupole ion traps currently offer MALDI as an ionization option. One nonresonance technique less affected by the number of ions is boundary-activated dissociation (BAD) [29–31]. In this technique, a dc voltage pulse is applied to the endcaps instead of an ac voltage, causing a change in the ions' a_z values. As the a_z value become large enough, the ion's a and q points approach the boundary of the stability diagram. The a_z value is chosen so that the ion is not ejected, but because it is near the boundary, its trajectory increases in amplitude. As the ions' motions become larger in amplitude, there is greater force acting on the ions, causing their kinetic energy to increase. Collisions with the bath gas molecules can then lead to 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 *mass-to-charge* 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.

Another activation technique using CID also involves a nonresonant approach [32]. Nonresonance excitation is capable of increasing an ion's kinetic energy to 40 eV and can do so in only a few microseconds. Like HASTE, the ions remain trapped with a higher kinetic energy than that that can be achieved via resonance excitation. Thus, the internal energy deposition can also be greater. This nonresonance excitation involves applying a low frequency square wave (50–500 Hz) to the endcaps. Because this frequency is so low in comparison to the secular frequencies of the ions (~100,000 Hz), this square wave can be more reasonably 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. Nonresonance 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 do. 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.

Nonresonance excitation differs from resonance 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 nonresonance techniques, higher energy dissociation channels can be accessed. Thus, nonresonance excitation can form a different group of product ions from those seen with resonance excitation.

6.4.4.2.4 Infrared Multiphoton Dissociation (IRMPD). Another method for inducing dissociation differs from all the previously discussed techniques, in that it does not make use of collisions. Instead of increasing the kinetic energy of ions and then using collisions to convert that to internal energy, IRMPD uses photons to deposit energy into the ions [33–38]. 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.

IRMPD offers several advantages over other dissociation techniques. First, it is not a resonance 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.

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 mass-to-charge product ions are easily trapped and observed. However, a complication with IRMPD in the quadrupole ion trap 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. All the early IRMPD experiments either reduced the bath gas pressure, which compromises sensitivity and resolution, or had a supplemental activation method concurrent with the IRMPD. However, recently, it has been demonstrated that appropriate focusing of the laser beam allows IRMPD to be performed at normal operating conditions without any supplemental ion activation [38].

6.4.4.3 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 quadrupole ion trap is particularly versatile for these types of analyses due to the combination of controlled timing of the reactions, mass-selecting capabilities for both reactants and products, and MS^n for both the formation of the reactants [39] and examination of the products [40]. Often, these reactions are between the trapped ion and a volatile neutral introduced into the vacuum chamber. However, reactions between two ions have also been reported [41–45]. 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.

6.4.4.3.1 Ion–Molecule Reactions. Ion–molecule reactions in which a chemical reaction occurs rather than just the transfer of energy (CID) can be useful as a means of interrogating ions in a quadrupole ion trap. Reactions that are specific for a certain functional group or structure can be used to provide information about an analyte [46]. While ion–molecule reactions can be performed in a triple quadrupole instrument [47,48], the ability to easily control and vary the reaction time in a quadrupole ion trap allows additional information, such as reaction rate constants, to be determined.

Two simple ion–molecule reactions are hydrogen/deuterium (H/D) exchange and proton transfer. The gas-phase H/D exchange can be used to probe structures of ions, in particular, larger ions that may have multiple conformations [49]. H/D exchange is also useful in distinguishing functional groups [50]. While H/D exchange just comprises a change in mass of the analyte ion, proton transfer reactions involve transferring charge from the analyte ion to the neutral molecule. For singly charged ions, this is not a useful reaction, as a loss of charge results in the formation of a neutral that cannot be detected by the mass analyzer, but for multiply charged ions, deprotonation can be a very useful reaction [39]. [51]’ There are thermodynamic limits to proton transfer ion–molecule reactions, which lead to the development of ion–ion reactions.

6.4.4.3.2 Ion–Ion Reactions. Reactions between two oppositely charged species can also be used. A quadrupole ion trap is particularly useful for these types of reactions, as both positively and negatively charged ions can be trapped simultaneously under normal operating conditions. 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 declutter charge-state convoluted spectra. By decreasing the charge on trapped ions, the mass-to-charge 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 [43].

The ion–ion proton transfer reactions lead to the development of ion–ion electron transfer reaction [41,52,53]. Electron transfer dissociation (ETD) is becoming an increasingly popular method for the analysis of peptides and proteins. ETD is closely related to electron capture dissociation (ECD) [54] where a multiply protonated ion captures an electron to reduce the charge state by one. This reaction is exothermic and thus, after capture (or transfer) of the electron to the ion, dissociation can occur. When an electron is added to a multiply protonated ion, the resulting ion is a radical. The dissociation chemistry of radical ions is significantly different than that of even electron ions and thus ETD is often complementary to CID. A particular advantage of ETD is that the dissociation chemistry has the ability to retain and identify sites of posttranslational modification [55–57]. In CID, functional groups added as posttranslational modifications to peptides and proteins are often facilely lost as neutral fragments and limited sequence information is obtained. With ETD, the radical dissociation chemistry is such that the posttranslational modification remains attached to the peptide/protein and typical sequence ions are produced. This allows identification of the site of posttranslational modification.

6.5 CONCLUDING REMARKS

The triple quadrupole mass spectrometer and the quadrupole ion trap mass spectrometer are probably the two most widely used instruments for MS/MS. Both are quite mature technologies, having been in existence for over 25 years. There are still improvements being made, but, in general, they are incremental and often more related to peripheral aspects of the mass spectrometry experiment rather than the actual mass analysis step.

The triple quadrupole instruments excel in the area of quantification and targeted compound analysis. The single reaction monitoring and MRM modes of operation of triple quadrupoles provide high duty cycles, maximizing sampling efficiency. Also, the quadrupole ion traps have some limitations in the area of quantification because of ion statistics. A limited number of ions can be trapped before space charge effects impact performance of ion traps. Development of 2D ion traps and newer 3D ion traps taking advantage of higher order fields have increased the ion capacity, but the triple quadrupole is still usually the instrument of choice for quantification.

The quadrupole ion traps excel in compound identification using MS/MS. The high sensitivity of ion traps combined with the fast scan rates and very high MS/MS efficiency make the ion trap uniquely compatible with chromatography/MS/MS experiments. quadrupole ion traps are also very powerful research instruments due to the variety and ease of implementation of ion activation methods.

Of course, triple quadrupoles and quadrupole ion traps do have limitations. In particular, neither the mass resolution nor mass accuracy is particularly high. While both these performance metrics have been improved over the years, in the near future, at least, quadrupole-based instruments will not compete in terms of resolution or mass accuracy with time-of-flight (TOF), ion cyclotron resonance (often referred to as *FTMS*), or orbitraps. However, one can get the best of all performance metrics by using hybrid instruments, that is, quadrupole-based analyzers combined with these other high performance analyzers. The hybrid QTOF instrument is similar in popularity to triple quadrupoles and quadrupole ion traps and the technology has been around about as long [58]. Quadrupoles interfaced with FTMS was also first reported over 25 years ago [59]. More recently, quadrupole ion traps combined with TOF [60,61], FTMS [62], and orbitraps [63] have become commercially available. Another hybrid instrument of importance is the hybrid triple quadrupole 2D ion trap. This is a multipurpose instrument that provides performance capabilities of a stand-alone triple quadrupole mass spectrometer along novel modes of operation that take advantage of a 2D ion trap [64]. As the instruments become more sophisticated and data systems more powerful, if cost is not a concern, only imagination will limit the possibilities. However, because of their MS/MS capabilities, it is likely that quadrupole-based analyzers will almost always have a role in mass spectrometry instrumentation.

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