REVIEW

The Quadrupole Ion Trap Mass Spectrometer — A Small Solution to a Big Challenge

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Innovations in ion trap mass spectrometry have extended the applicability of this technique to the analysis of biological molecules. This tutorial review discusses basic ion trap theory and provides practical examples of how the theory is used to perform different types of experiments such as molecular weight measurements, high resolution, and multiple stages of mass spectrometry (MS^n). Peptides generated from enzymatic digestion of α-casein, recombinant tissue plasminogen activator, and a cellular extract of proteins from Haemophilus influenzae illustrate the utility of ion trap mass spectrometers for the analysis of biochemical problems.

Analysis of biochemical systems comprised of interacting proteins and peptides typically involves elucidating the molecular weights of the biological molecules and obtaining their amino acid sequences. Covalent modifications to the primary sequence may affect protein function and must also be determined by some means. Several complementary techniques are typically used to obtain this sort of information. Gel electrophoresis provides an excellent visualization of the complexity of the biological process under study; however, anomalies in migration afford poor mass accuracy and the presence of posttranslational modifications may be difficult to ascertain. Amino acid sequence information has historically been obtained using automated Edman degradation. Chemical reagents are employed to remove one amino acid at a time from the amino terminus of an intact protein or peptide. The resulting amino acid derivative is purified and identification is obtained in a straightforward manner by comparing the HPLC retention time of the sample to those of standard amino acid derivatives. The technique requires a free amino terminus and a homogeneous sample. Typical sample quantities are at the 1- to 10-pmol level (1). Posttranslational modifications to amino acid residues cause anomalous retention times (2) and may be difficult to identify. Cycle times are ~30 min/amino acid (1); thus; a peptide containing 25 amino acids would take 12.5 h to sequence.

By contrast, mass spectrometry is a high-sensitivity, high-throughput technique used to acquire both molecular weight and sequence information for proteins and peptides. Typical sample quantities are at the low- to mid-femtomole level (3). Time-of-flight instruments can be employed to obtain protein molecular weights with two orders of magnitude improvement in mass accuracy over gel electrophoresis. Triple quadrupole mass spectrometers are utilized to analyze enzymatic digests of proteins and tandem mass spectrometry can be performed to elucidate amino acid sequence information for peptides through the use of collision-aided dissociation. The presence of posttranslational modifications is directly determined. Data may be generated in less than 1 min. Data interpretation has historically limited the throughput of the mass spectrometry approach; however, a number of algorithms have been developed to automatically interpret tandem mass spectra (4).

Because of these advantages, mass spectrometry is developing into an essential technique for biochemical and biological research. The range of problems that mass spectrometry is currently being applied to includes the analysis of posttranslational modifications of proteins (5); noncovalent protein–protein, protein–DNA, and protein–RNA interactions (6–9); study of peptides implicated in the functioning of the immune system (10–13); and the study of proteins involved in

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signal transduction pathways (14–16). A sensitive and versatile analytical system, capable of detecting both large and small molecules and determining aspects of molecular structure, is required to address the complex mixtures of molecules found in these types of biological problems. Of fundamental importance to the biochemist and biologist is the existence of robust, easy-to-use, and inexpensive instrumentation for application to their studies.

Developments over the past 10 years have made the quadrupole ion trap mass spectrometer an excellent tool for biomolecular analysis. A quadrupole ion trap is a mass analyzer roughly the size of a tennis ball whose size is inversely proportional to its versatility. Three hyperbolic electrodes, consisting of a ring and two endcaps, form the core of this instrument (Fig. 1). Using theory to drive instrument development, the nominal mass range of the instrument has been extended from m/z 650 to m/z 70,000 (17); up to 12 stages of tandem mass spectrometry (MS/MS) have been performed (18); and mass resolution that can allow the separation of ions of m/z 10^6 and m/z 10^5 + 1 has been implemented (19). Quadrupole ion trap mass spectrometers are also exquisitely sensitive. Molecular weight information has been recorded with as few as 1.5 million peptide molecules (20). Although not all of these features can be applied simultaneously, a judicious choice of parameters can afford sensitive molecular weight measurements and structural analyses of biopolymers. The goal of this tutorial is to review the theory of ion trap operation and give examples of the practical application of ion trap mass spectrometry to biomolecular analysis.

HISTORY OF THE DEVELOPMENT OF ION TRAPS

In the early 1950s, Wolfgang Paul and co-workers invented two instruments that could be used to deter-
TABLE 1
Time Line of Ion Trap Technology Development

<table>
<thead>
<tr>
<th>Year</th>
<th>Event Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1953</td>
<td>Invention of quadrupole mass filter and quadrupole ion trap by Paul.</td>
</tr>
<tr>
<td>1959</td>
<td>Storage of single microparticles.</td>
</tr>
<tr>
<td>1959</td>
<td>Used as a mass spectrometer. Detection by power absorbance.</td>
</tr>
<tr>
<td>1962</td>
<td>Single ions stored at low temperatures to set frequency standards.</td>
</tr>
<tr>
<td>1968</td>
<td>Used as a mass spectrometer with external detection.</td>
</tr>
<tr>
<td>1972</td>
<td>Characterization of the ion trap: Chemical ionization, study of ion/molecule kinetics. Used as a storage device with a quadrupole mass filter employed for mass analysis.</td>
</tr>
<tr>
<td>1976</td>
<td>Ions collisionally focused.</td>
</tr>
<tr>
<td>1978</td>
<td>Used as a selective ion reactor.</td>
</tr>
<tr>
<td>1979</td>
<td>Ions resonantly ejected.</td>
</tr>
<tr>
<td>1980</td>
<td>Used as a GC detector.</td>
</tr>
<tr>
<td>1982</td>
<td>Multiphoton dissociation of ions.</td>
</tr>
<tr>
<td>1983</td>
<td>Development of mass-selective instability mode of operation.</td>
</tr>
<tr>
<td>1984</td>
<td>Commercialization of ion trap detector (ITD).</td>
</tr>
<tr>
<td>1985</td>
<td>Commercialization of ion trap mass spectrometer (ITMS).</td>
</tr>
<tr>
<td>1987</td>
<td>High-performance mass spectrometry: Multiple stages of mass spectrometry, chemical ionization, photodissociation, external ion injection, mass range extension.</td>
</tr>
<tr>
<td>1990</td>
<td>Electrospray ionization of biopolymers.</td>
</tr>
<tr>
<td>1991</td>
<td>High resolution.</td>
</tr>
<tr>
<td>1992</td>
<td>Discovery of nonlinear effects.</td>
</tr>
<tr>
<td>1993</td>
<td>Matrix-assisted laser desorption ionization of biopolymers.</td>
</tr>
<tr>
<td>1993</td>
<td>Biological problem-solving using ion trap mass spectrometry.</td>
</tr>
</tbody>
</table>

mine mass-to-charge \( (m/z) \) ratios of ions (21, 22). The first was the quadrupole mass filter that rapidly was applied to a wide range of analytical problems (23). The second was the quadrupole ion trap, consisting of a ring electrode and two endcap electrodes with hyperbolic surfaces. As is shown in Table 1 (24), the quadrupole ion trap was primarily used by the physics community, notably Hans Dehmelt at the University of Washington, to investigate the properties of isolated ions (25–28). The ion trap was operated at that time in a “mass-selective stability” mode of operation. In this mode, analogous to the operation of a quadrupole mass filter, rf and dc voltages applied to the ring electrode were ramped to allow stability, hence storage, of a single (increasing) value of \( m/z \) in the ion trap (24). Ions were detected by resonance absorption from an external power source (29) or were ejected using a dc pulse applied to an endcap and detected using an electron multiplier (30). Due to limited mass range and resolution, these methods of mass measurement were not practical for many analytical purposes.

The chemistry community’s interest in the trap was confined to several research groups until 1983 when George Stafford and co-workers at Finnigan MAT made two major advances. First, they developed the mass-selective instability mode of operation (31). The fundamental difference between this mode of operation and previous methods is that all ions created over a given time period were trapped and then sequentially ejected from the ion trap into a conventional electron multiplier detector. Thus, all ions were stored while mass analysis was performed, unlike the mass-selective stability mode of operation that had been previously employed. This new method for operating the ion trap simplified the use of the instrument. Stafford’s group next discovered that a helium damping gas of \( \sim 1 \) mtorr within the trapping volume greatly improved the mass resolution of the instrument (32). Both of these discoveries led to the successful development of a commercial ion trap mass spectrometer. In later work, the addition of helium was observed to significantly improve trapping efficiencies, especially for externally injected ions (33). Subsequent innovations have been rapid. Cooks and co-workers at Purdue University have pioneered high-performance techniques such as external injection of ions (33), mass range extension (17, 34), MS\(^n\) (18), and high resolution (19) that improved the performance of the ion trap and created interest in its application to biological molecules.

**HOW THEORY IS PUT INTO PRACTICE**

**The Theory**

Quadrupole ion traps are dynamic mass analyzers that use an oscillating electric potential applied to the ring electrode, called the “fundamental rf,” to focus ions toward the center of the trap. This is accomplished by creating a parabolic potential, shaped like a saddle (35), inside the trapping volume. The strength of the restoring force linearly increases as the ion trajectory deviates from the central axis, focusing the ion back to the center of the trapping volume. This is demonstrated in Fig. 2, a simulation of ion trajectories created using SIMION 3D version 6.0 (36). A population of trapped
ions is therefore observed to occupy only the space near the center of the trap due to the focusing effect of the oscillating electric fields. Assuming a cylindrically symmetric system, the potential an ion experiences at any point in the ion trap is given by

$$\Phi(r, z) = \frac{(U - V \cos \omega t)}{2} \left[ \frac{r^2 - 2z^2}{r_o^2} \right]$$

where \(U\) is the amplitude of a dc potential applied to the endcap electrodes with reference to the ring electrode, \(V\) is the amplitude of the fundamental rf applied to the ring electrode, \(\omega\) is the angular frequency of the rf potential, and \(r_o\) is the closest distance between the center of the trap and the ring electrode. The closest distance between the center of the trap and the endcap electrode is given by \(z_o\). To obtain an ideal quadrupolar field, \(r_o\) is equal to the square root of \(2z_o\). The actual geometry of the commercial ITMS is "stretched" and \(r_o\) is equal to 0.781\(z_o\), leading to the presence of higher-order fields within the trapping volume. The effects of nonlinear resonances produced in the stretched trap have been actively studied for the past few years and have led to many new insights regarding the fundamental performance characteristics of the ion trap mass spectrometer (37-43).

The force on an ion, given by the electric field, is obtained by

$$F(r, z) = \vec{E}(r, z) = -e\nabla\Phi(r, z) = ma(r, z)$$

and, using Newton's law, is proportional to the acceleration an ion of charge \(e\) experiences due to that force. Equation [2] may be placed in the form of the Mathieu equation (44) in the radial and axial directions when the substitutions

$$a_r = -\frac{8eU}{mr_o^2 \omega^2}, \quad a_z = \frac{4eV}{mr_o^2 \omega^2},$$

$$q_r = -q/2, \quad q_z = q/2, \quad \xi = \frac{\omega t}{2}$$

are made. Ion trajectories are determined by solutions to the Mathieu equation and are oscillating functions with regions of stability described by the parameters \(a_r\) and \(a_z\). Thus, the stability of ion motion depends upon the mass and charge of the ion (\(m\)), the size of the ion trap (\(r_o\)), the oscillating frequency of the funda-
QUADRUPOLE ION TRAP MASS SPECTROMETRY

The ion trap spectrometer is operated on the line \( a_z = 0 \). This corresponds to the case of maximizing the range of \( m/z \) values that may be stably trapped. Ion trajectories become unstable in the axial direction (between the endcap electrodes) but remain stable in the radial direction when \( q_z = 0.908 \). Ions are ejected through holes in the endcap electrode and are typically detected using an electron multiplier.

Trapped ions of a given \( m/z \) oscillate at a frequency known as the secular frequency that is proportional to the angular frequency of the applied signal, \( \omega \). The constant of proportionality is given by \( b_z \). For values of \( q_z < 0.4 \), \( b_z \) may be approximated by (45)

\[
\beta_z^2 = a_z + q_z^2/2
\]

which reduces to \( \beta_z = q_z \sqrt{2} \) for the mass-selective instability mode of operation. Resonance conditions are induced by matching the frequency of a supplementary potential applied to the endcap electrodes to the secular frequency of the ion. The ion will absorb energy from the applied field and the trajectory will linearly increase toward the endcap electrodes until the ion becomes unstable and is ejected (24).

**FIG. 3.** Diagram showing the regions of stability in the quadrupole ion trap parameterized in terms of the operating voltages and frequencies.

**FIG. 4.** Selected working points for an ion of \( m/z = 1500 \). The applied dc and rf potentials are shown in parentheses \((U, V)\). The corresponding \((a_z, q_z)\) values are as follows: \((-100 \text{ V, } 1000 \text{ V}) \rightarrow (0.0108, 0.0539)\), similarly \((-1000 \text{ V, } 3000 \text{ V}) \rightarrow (0.108, 0.162)\) and \((-100 \text{ V, } 6000 \text{ V}) \rightarrow (0.0108, 0.323)\). A judicious choice of conditions is required to ensure trajectory stability for a wide range of \( m/z \) values.

The secular rf \((\omega)\), and the amplitudes of the applied dc \((U)\) and rf \((V)\) voltages. One region of stability in which radial and axial stability overlap is shown in Fig. 3. An ion of a given mass-to-charge ratio will be stably trapped anywhere within that region. The position of the ion within the stability region can be moved by changing the amplitude of the applied dc and rf voltages to change the values of \( a_z \) and \( q_z \), termed the “working points” of the ion. Values of the working points are chosen to ensure stability or instability of an ion trajectory of interest. For the case of the commercial Finnigan ion traps (ITD, ITIMS), \( r_o = 1 \text{ cm, } \omega/2\pi = 1.1 \text{ MHz, and V ranges from 0 to 7500 V_0}\).

As an example, consider three working points for an ion of \( m/z = 1500 \), shown in Fig. 4. Values of the amplitudes for the applied dc and rf potentials are shown in parentheses. The corresponding \( a_z \) and \( q_z \) values are delineated in the figure legend. It is clear that a judicious choice for the amplitude of the applied potentials is required to ensure stability for all ions within a mass range of interest. The mass-selective instability mode of operation utilizes no dc voltage; thus, the mass-selective instability mode utilizes no dc voltage and the mass range of interest is not limited by the choice of this parameter.
able ionization techniques, i.e., electrospray ionization and matrix-assisted laser desorption ionization, to the ion trap. These externally created ions need to be injected into the ion trap and efficiently trapped. Ions are focused by an einzel lens system and allowed into the ion trap during the ionization period. A gating lens pulses from positive to negative voltages to repel or attract ions toward the entrance endcap aperture. The time during which ions are allowed into the trap is set to maximize the signal while minimizing “space-charge” effects, resulting from too many ions in the trap, that lead to an overall reduction in performance. The ion trap is typically filled with helium to a pressure of \( \sim 1 \) mtorr. Collisions with helium reduce the kinetic energy of the ions and serve to quickly contract trajectories toward the center of the ion trap, enabling trapping of injected ions. This cooling effect is demonstrated in Fig. 6 where the ion population forms a “packet” near the center of the trap.

Ion trapping. Ions of different \( m/z \) values may have stable orbits at the same time, as shown in Fig. 7. From the expression for \( q_z \) in Eq. [3], we see that

\[
\frac{m}{z} \propto \frac{V}{q_z}. \tag{5}
\]

Larger values of \( m/z \) will have smaller values of \( q_z \) and smaller values of \( m/z \) will have larger \( q_z \) values. Since ion trajectories become unstable when \( q_z = 0.908 \), a well-defined low-mass cutoff is created for a given value of the amplitude of the applied rf voltage, \( V \). No ions below that mass will be trapped, but ions above that mass will be trapped with trapping efficiency decreasing for larger \( m/z \) values (35). Low-mass cutoffs for various amplitudes of the applied fundamental rf potential are listed in Fig. 7. The trapping efficiency for an ion of interest depends, in part, upon the value of the low-mass cutoff, or the so-called exclusion limit (39). This can be a problem when using ionization methods that generate many low-mass matrix ions since the ion trap can accommodate on the order of \( 10^5 \) ions before space-charge seriously impairs the performance of the instrument. For example, the model peptide human angiotensin I (Mr 1296) may be most efficiently trapped at a low-mass cutoff of 85 u. Matrix-assisted laser desorption ionization (MALDI) generates matrix ions above this cutoff in a ratio of \( \sim 1:10^6 \). In this situation, the high sensitivity of the ion trap can be most effectively utilized if the ion of interest is selectively injected into the ion trap. Current efforts revolve around selective injection utilizing shaped excitation waveforms (46) or filtered noise fields (47) to cause all ions but the ion of interest to have unstable trajectories. Other

The Practice

To measure the \( m/z \) value of a molecule in an ion trap the molecule must be ionized, focused into the ion trap, trapped, ejected, and detected. Structural information is obtained by collision-induced dissociation with a helium damping gas and a mass spectrum is generated by sequentially ejecting fragment ions from low \( m/z \) to high \( m/z \). The mass-selective instability mode is utilized for ion ejection. The mass-selective instability line is the locus of \( q_z \) values where \( a_z \) is set to zero and maximizes the mass range that may be stably trapped. Operation of the ion trap consists of the construction of a scan function used to manipulate the working points of ions of interest. The scan function sets the amplitude of the fundamental and supplementary potentials and sets the time taken for each step. Typical scan functions for molecular weight analyses and MS/MS experiments are shown in Fig. 5.

Ion injection. Ion traps were initially utilized to analyze volatile samples by electron impact or chemical ionization. In this case, ions were created inside the trapping volume. An interest in the analysis of biological molecules led to the need to interface suit-
Simulations show that collisions with the helium damping gas lead to the creation of an ion packet near the center of the trap. Approaches include ramping the amplitude of the fundamental rf during injection to increase trapping efficiency, even at low pressures of the helium damping gas (48), as well as the addition of a quadrupole mass filter to afford selective injection of ions of interest into the ion trap (49).

**FIG. 7.** Relative positions of ions with three different mass-to-charge ratios along the mass-selective instability line, $a_z = 0$. The effect of increasing the amplitude of the fundamental rf voltage is shown in (a) through (c).

Ion ejection. Shown in Fig. 7 is an example of the relative positions of three ions of differing m/z ratios on the mass-selective instability line, $a_z = 0$. Three different values for the amplitude of the fundamental rf signal are given. As the voltage is increased, the $q_z$ value for the ion also increases. Figure 7c shows that...
at 6000 V, the ion of m/z 500 has been ejected from the ion trap. At the maximum amplitude of 7500 V, at m/z 1500, the \( q_z \) value has only reached 0.404; thus, that ion cannot be ejected from the ion trap and detected.

As noted above, a resonance condition may be induced by matching the frequency of an applied oscillating signal to the secular frequency of an ion in the trap (17). This will cause the ion to gain energy and the amplitude of the trajectory to linearly approach the endcap electrodes until the ion is ejected from the trap. Ejection can therefore be made to occur at voltages lower than those required for ejection at \( q_z \) of 0.908, extending the nominal mass range of the ion trap. Conceptually, this may be viewed as creating a "hole" in the stability diagram. The position of the hole is dependent upon the frequency of the supplementary potential while the size of the hole depends upon the amplitude of the signal. This effect is illustrated in Fig. 8 where an ellipse represents a resonance point that extends the mass range by a factor of 4. At 1000 V none of the ions have \( q_z \) values approaching that of the resonance point; thus, none will be detected. At 3000 V, m/z 500 has been ejected and m/z 1000 is in the process of being ejected. The \( q_z \) value for m/z 1500 is smaller than 0.227; thus, that ion will not be ejected. At 6000 V, the \( q_z \) values for all of the ions are greater than 0.227, the \( q_z \) value of the resonance point. This example shows that when resonance ejection is used and the amplitude of the voltage is ramped from low to high amplitudes, all of the ions "fall through the hole" and are ejected from the trap and detected.

**Ion isolation.** In a typical multiple-stage mass spectrometry experiment, the ion of interest is isolated before undergoing resonance excitation or charge state determination using high resolution. Isolation in the Finnigan ITMS may be accomplished in two ways, depicted in Fig. 9. One method, illustrated in Fig. 9a, includes the combined use of dc and rf potentials to bring the \( q_z \) and \( a_z \) values of the ion to an apex of the stability diagram; all other ions will be unstable (50, 51). The other method is shown in Fig. 9b and consists of scanning the amplitude of the fundamental rf voltage in a reverse-then-forward manner while applying a resonance signal (32, 34). This allows ejection of ions with m/z greater than the ion of interest followed by ejection of ions having m/z smaller than the ion of interest. Both isolation methods are used; however, the effects of space-charge and field nonlinearities on the shape of the stability diagram may degrade performance when the dc/rf isolation method is employed. A recent refinement includes the use of the stored waveform inverse Fourier transform (SWIFT) technique (46, 52) and filtered noise fields (47) to isolate ions using notched waveforms.

**Ion dissociation.** As discussed above, when an ion approaches a region of instability in the axial direction,
and the frequency and amplitude of the tickle voltage must be carefully tuned to optimize fragmentation. The auxiliary frequency generator outputs a single-frequency sinusoidal signal that is not sufficient to excite the envelope of ion signals resulting from isotopic abundances for ions with large m/z values. Stored waveform inverse Fourier transform techniques (52) and the application of random noise (55) have been successfully used to excite a broad range of ion secular frequencies. In addition, shifting the qz value of the ion and increasing the amplitude of the tickle pulse have substantially increased the amount of fragmentation observed for large peptides (56).

High resolution. The mass resolution of the ion trap mass spectrometer is a function of the number of rf cycles that the ion spends interacting with the trapping field (57). Resolution is increased by reducing the amplitude of the resonance ejection signal and reducing the ejection scan speed, nominally 5555 u/s for the Finnigan ITMS. The scan speed is attenuated utilizing a network of resistors placed in series with the digital-to-analog converter (DAC) that controls the amplitude of the rf voltage applied to the ring electrode (17). The fixed scanning rate of the DAC is applied to smaller “windows” of rf voltages with a concomitant gain in the number of data points taken per unit mass. A dc potential is used as an offset to position the rf voltage, or mass window. This is schematically illustrated in Fig. 10. Figure 10a shows the unattenuated mass window resulting from scanning the amplitude of the rf voltage from 346 to 7500 V while applying a supplementary frequency at 120 kHz to extend the mass range by a factor of 3. This increases the mass scan speed to 16665 u/s. Attenuation of the scan speed by a factor of 10 reduces the size of the mass window by the same factor; thus, Figs. 10b–10d represent a 186-u mass window created by the attenuation. The different dc offset voltages serve to position the mass window in different regions within the mass window. In Fig. 10b, the mass window is positioned at 267 u, in 10c it is 800 u, and in 10d it is 1600 u; therefore, different regions of the mass window are accessed. Attenuation by a factor of 100–300 is typically required to resolve the isotopes for singly to triply charged peptide ions to achieve resolutions of 10,000–30,000 at m/z values ranging between 500 and 2000.

An Example

The pulsed nature of the quadrupole ion trap makes it particularly well suited to pulsed ionization techniques such as MALDI. A MALDI ion trap that has been described previously (58) was used for mapping the tryptic peptides from tPA. A 1/2-µl aliquot of the digest, corresponding to ~1 pmol, was loaded onto a probe tip and cocrystallized with 1 µl of a saturated
cally used to obtain both MS and MS/MS spectra (61, 63), similar to results obtained by triple quadrupole and TOF mass spectrometry. Lower levels are possible, but are not routine at present. Sensitivity is improved by varying the ion collection time and selectively injecting the ion of interest. The ion trap, with MALDI, has shown equivalent performance to TOF mass spectrometers at low-mass range with the added advantage of exact precursor ion selection and MS<sup>n</sup>. It is unlikely the ion trap will be as suitable for ultrahigh mass analysis as TOF mass spectrometers due to hardware limitations of the auxiliary frequency generator used to extend the mass range. High-molecular-weight spectra obtained for singly charged proteins (30- to 50-kDa range) have shown results comparable to those obtained using a linear TOF mass spectrometer without the implementation of delayed extraction techniques. There are several limitations of the performance of quadrupole ion trap mass spectrometers. The alternate solution of α-cyano-4-hydroxycinnamic acid in 1:1 0.1% trifluoroacetic acid:acetonitrile. The probe tip was irradiated using a nitrogen laser (337 nm). The resulting mass spectrum of the digest is shown in Fig. 11. Several of the peaks corresponding to tryptic peptides are labeled. Approximately 75% of the expected peptides falling within the measurement mass range were detected. Extensive fragmentation of peptide ions generated by MALDI has been observed (59, 60) and many of the signals in the mass spectrum in Fig. 11 result from fragmentation upon injection into the mass spectrometer. The analytical potential of MALDI–ITMS continues to be explored and shows great promise for application to biological molecules (61, 62).

**Comparison with Other Methods**

As an ion storage device, an ion trap has the capability for high mass resolution, mass range, sensitivity, and MS<sup>n</sup> that translates into versatile performance as a mass spectrometer. In comparison to triple quadrupole and TOF mass spectrometers the ion trap is unique in its ability to perform MS<sup>n</sup>. All three techniques are about equal in terms of mass accuracy and sensitivity. When utilizing electrospray ionization or MALDI, mid-femtomole to low-picomole levels of sample are typi-
scan modes of triple quadrupole mass spectrometers such as precursor ion and neutral loss scans are currently not possible. Furthermore, the number of ions injected into the ion trap must be carefully controlled since space-charging can degrade the performance of the instrument. This problem is solved through the rapid prescan that assesses the ion current injected into the trap for \( \sim 50 \mu s \) then sets the ionization time to maximize the signal while minimizing space-charge. Finally, when MS/MS is performed, all ions with \( q_z \) values below that of the resonance point will be ejected from the ion trap; therefore, a complete sequence of complementary b- and y-type ions typically cannot be obtained. Cotter et al. have recently shown that using low \( q_z \) values in conjunction with a heavier target gas affords full tandem mass spectra; consequently, the ejection of low \( m/z \) fragment ions during CID is not a fundamental limitation of the ion trap (64). Perhaps one major advantage of the ion trap not easily overlooked is the size of the instrument. As lab space becomes tighter, the size of the ion trap and ease of maintenance become a considerable advantage.

**THE NEW GENERATION OF ION TRAPS**

In the past, the ITMS has not been an instrument well suited to the robust and routine analyses required by biochemists and biologists. High-performance innovations to the ITMS developed over the past several years have been used to build a new generation of ion trap mass spectrometer, the Finnigan MAT LCQ. This instrument has been carefully designed to interface with atmospheric pressure ionization techniques that are optimal for the analysis of biomolecules. The operating characteristics of the instrument have been changed by using a fundamental rf of 760 kHz instead of 1.1 MHz, an electrode spacing of 0.707 cm instead of 1.0 cm, and a \( q_z \) value of 0.83 instead of 0.908 for resonance ejection of ions (65). Ion injection into the ion trap has been optimized using a lensing system that consists of two rf-only octopoles, resulting in a narrow spatial and energy distribution of the injected ions (49, 66). Selective injection, trapping, and excitation of ions are performed using tailored waveforms, analogous to the SWIFT technique (67). Unit mass resolution, or the ability to separate an \( m/z \) value of 1500 from 1501, is maintained over the 2000-dalton mass range with a mass accuracy of 0.015% (68). These figures of merit are comparable to the performance of current triple quadrupoles. It is expected that the mass range of the LCQ will increase to 5000 daltons in the next year.

The most striking feature of the new ion trap is the software control of instrument operation. Ion traps are operated through the use of a scan function that sets the ion injection time, trapping voltages, cooling time, tickle voltages, and voltage ramping for acquisition of the mass spectrum (see Fig. 5). Once a scan function is established for a given experiment it can be used again but some parameters may need to be changed based on the \( m/z \) value of the ion of interest. For example, MS, MS/MS, MS\(^4\), and high-resolution experiments all require the construction of unique scan functions. Significant interaction and expertise with the software were required with the older ion traps. The LCQ was developed with ion trap instrument control language (ITCL), a computer language that controls all of the elements of the scan function. For example, the hypothetical ITCL command “\( \text{ hires } 1200 \)” would set up the scan function to isolate the ion at \( m/z \) 1200, then slow the scan rate to achieve high mass resolution. All parameters required are automatically set with the one ITCL command, compared with the necessity to manually set a number of parameters using the ITMS software.

ITCL also enables the user to perform data-dependent experiments. A mass scan can return to the computer program all the information it acquires during the scan. For example, a command such as “\( \text{ hires } \text{ mass(1)} \)” would perform a high-resolution mass scan on the most intense ion returned from the previous mass scan. Very complicated data-dependent routines such as “on-the-fly” tandem mass spectrometry can be performed by stringing together commands in the form of a computer program. A graphical user interface is employed to simplify the use of ITCL and to edit the type of experiment desired during the course of an analysis. An \( m/z \) measurement, followed by a high-resolution scan to separate the isotopes of the desired ion for charge state determination, followed by tandem mass spectrometry, is achieved by selecting the experiment through the user interface. The software can automatically select precursor ions based on some predefined criteria such as abundance, presence, or absence of an ion in a predefined list. No user intervention in the process is required except for the initial setup of the analysis. This level of control is unprecedented in mass spectrometry. In fact, the reliance on embedded software control is so great that instrument upgrades will essentially require downloading software from a CD-ROM to change operational parameters, obviating the need for expensive additions of hardware.

A number of different automated, data-dependent experiments are possible including full-range MS at unit resolution, MS\(^n\) with \( n = 1 \) to 10, single-ion monitoring (SIM), and single-reaction monitoring (SRM), charge-state determination (utilizing the “ZoomScan”) of up to +4 ions, and unit resolution isolation up to \( m/z \) 1200. An example of the application of these experiments is illustrated in Figs. 12 and 13.

The benefit of performing multiple stages of mass spectrometry is demonstrated in Fig. 12 where MS\(^4\)
FIG. 12. MS^4 on the doubly charged ion of m/z 880 from a tryptic digest of the model protein α-casein. A 0.5 pmol/μl sample solution was infused into a home-built microspray ionization source at a flow rate of 200 nl/min. The ionization time was automatically set using automated gain control for the first three stages of mass spectrometry. The AGC was disabled for the fourth stage and the ionization time was set to 400 ms to compensate for the loss in sensitivity due to the performance of multiple stages of mass spectrometry. Ten scans were summed for the MS, MS^2, and MS^3 experiments. Fifteen scans were summed for the MS^4 experiment. (a) Mass assignments for sequence ions corresponding to MS^2 of m/z 880, MS^3 of m/z 436, and MS^4 of m/z 266. (b) The precursor at m/z 880 displayed in the top panel was chosen for fragmentation. The b_{14} fragment ion at m/z 436, shown in the second panel, was chosen for a further stage of fragmentation and the resulting mass spectrum is exhibited in the third panel. The b_{12} fragment ion at m/z 266 was chosen to obtain the very low-mass end of the fragmentation spectrum. Results are displayed in the bottom panel.

was performed on an ion from a trypsin-generated peptide from the model protein α-casein. The top panel shows the unit resolution full mass-range spectrum of the entire digest. The ion of m/z 880 was chosen for further investigation. MS/MS on that ion provided the mass spectrum shown in the second panel. Ions below 245 u were ejected upon the application of the resonance excitation pulse. A third stage of mass spectrometry, depicted in the third panel, provides additional low-mass sequence ions while the sequence is completed using a fourth stage of mass spectrometry. The amino acid sequence was deduced to be HQGLPQEVL-NENLLR.

A final example demonstrates the automated application of the instrument to the analysis of components in a complex biological mixture. Cellular proteins from H. influenzae were fractionated using ion-exchange chromatography (MonoQ). One of the fractions was digested using trypsin then concentrated and buffer-exchanged using Centricon filters. An aliquot was loaded onto a 500-μm POROS R2 packed column and separated by reverse-phase high-performance liquid chromatography using a 50-min gradient from 0–40% B followed by 40–60% B in 10 min at a flow rate of 50 μl/min. Solvent A was H_2O/ACOH in a ratio of 100:0.5 and solvent B was ACN/H_2O/ACOH in a ratio of 80:20:0.5. Shown in the top panel of Fig. 13 is the unit resolution full-range mass spectrum corresponding to scan number 1083 in the ion chromatogram (data not shown). A number of coeluting species are observed.
The quadrupole ion trap is an extremely versatile instrument capable of performing multiple stages of mass spectrometry with one mass analyzer. High-resolution techniques afford easy charge-state determination, facilitating the interpretation of data generated by electrospray ionization. The sensitivity and performance characteristics of the instrument, especially the automated experiments developed for the newly commercialized ion traps, make quadrupole ion trap mass spectrometry an attractive technique to apply to the analysis of biological and biochemical problems.

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The authors thank John Stults of Genentech for graciously providing the tPA sample. Jon DeGnore and Richard Yost from the University of Florida provided the wonderful ion trap renderings. These can be accessed on their web page at http://analytic15.chem.ufl.edu/anim1.html. Edwin Carmack provided the ribosomal protein data.

APPENDIX

Ion Trap Jargon

ac voltage: also called supplementary or auxiliary potential, is a voltage placed on the endcap electrodes.

Bath gas, damping gas, target gas: helium gas in the trapping volume at a pressure of \( \frac{1}{10} \) mtorr.

High resolution: an experiment in which peaks corresponding to carbon isotopes may be resolved.

Fundamental rf: a (typically) 1.1-MHz potential applied to the ring electrode.

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Resonance: an ac voltage is applied to the endcap and the \( q_z \) value of an ion of interest is changed until the secular frequency of the ion matches the frequency of the applied ac voltage. A high-amplitude ac voltage will cause resonance ejection, while a low-amplitude ac voltage will cause resonance excitation.

Secular frequency: the frequency, dependent upon the \( q_z \) value, with which an ion oscillates in the trap.

Space-charge: too many ions in the trap distort the electric fields, leading to significantly impaired performance.

Tickle voltage: an ac voltage applied to the endcap electrodes during an excitation period. The amplitude of the voltage is generally small so as to enable fragmentation of the ions by collisions with the helium damping gas rather than ejection.

Working points: the values of the Mathieu parameters \( a_z \) and \( q_z \). For an ion of a given \( m/z \), the position of the working points depends upon the amplitudes of rf and dc potentials applied to the ring electrode.

FIG. 13. On-line data-dependent analysis of tryptic peptides from an H. influenzae cellular protein extract. The full-range mass spectrum from scan number 1083 is shown in the top panel. The ion at \( m/z \) 839 was automatically chosen for further analysis. Charge-state determination, shown in the middle panel, was accomplished by slowing the mass scan speed and enhancing the resolution. The \( \approx 0.5 \mu \) distance between the isotopic peaks indicates the ion is doubly charged. MS/MS was performed and the resulting fragmentation mass spectrum is illustrated in the bottom panel. Computer-aided identification of the peptide indicated it was a fragment from ribosomal protein homologous to the L7/L12 protein found in E. coli.

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