Mass Analysis at the Advent of the 21st Century

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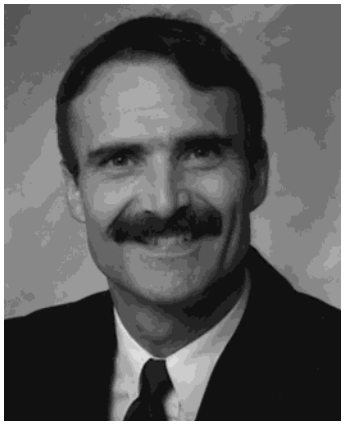
Received April 27, 2000

Contents



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I. Introduction

As we enter the 21st century, the practice of mass spectrometry has been making ever broadening contributions to our understanding of nature for slightly over 100 years. The origin can be traced back to the original measurement of mass-to-charge made just before the beginning of the 20th century with a device comprised of the basic elements found in all modern mass spectrometers. This measurement formed the basis for the discovery of the electron by Sir J. J. Thomson. This was certainly an impressive first contribution for mass spectrometry. An excellent description of the early days in Thomson's laboratory was recently given by Griffiths.[1] That measurement along with others made in the early part of the 20th century at the Cavendish Laboratories, using the

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10.1021/cr990087a CCC: $36.00
Published on Web 01/05/2001

famous parabola mass spectrograph and other early forms of mass spectrometers, were early indications of how powerful a tool mass spectrometry was to become. As the new century unfolds, mass spectrometry is an important tool in virtually all of the atomic and molecular sciences. In some fields, the practice of mass spectrometry can be described as mature. In others, both the technology and the basic science associated with the application of mass spectrometry are rapidly evolving. As the new century begins, this is certainly the case in the biological sciences, for example. Few would dispute that there is more research activity associated with making ions, probing their structures and stabilities, and measuring their masses taking place now than ever before.

Much of the emphasis in mass spectrometry in the past century was placed on volatile atomic and small polyatomic species. However, over the course of the century, matter in all forms (solid, liquid, gas) was subjected to scrutiny by mass spectrometry and the nature of the species of interest has come to include subatomic particles, elements, inorganic and organic polyatomic species, clusters, polymers (including biopolymers), noncovalently bound biocomplexes, and microparticles.[2] This issue of *Chemical Reviews* reflects the wide range of application areas to which mass spectrometry contributes. A wide variety of instruments and methodologies has proliferated to allow for the application of mass spectrometry to such a broad spectrum of species. In many ways, the measurements made for these various species bear little resemblance to one another. However, all mass spectrometry experiments share a common set of elements, which are listed as follows: (1) Question, (2) Measurement strategy, (3) Sample collection/preparation/separation, (4) Interface/ionizer or ionizer/interface, (5) Ion manipulation/mass-to-charge analysis, (6) Detection, (7) Data collection/reduction, (8) Interpretation.

All measurements, of course, are motivated by a scientific problem or question. For example, the issue can range from the isotopic composition of a uranium sample to the identity of a protein in regulatory flux. The answer might be derived from the mass or masses of ions in a mass spectrum, a ratio of ion abundances, the rate of change of an ion abundance, and so forth. The measurement strategy is driven by the nature of the material being investigated and the information required to answer the question. The remainder of the list given above summarizes the aspects of the analysis involving mass spectrometry that must be addressed for successful resolution of the scientific issue. They are articulated here simply to point out that while mass analysis is an essential component of any successful mass spectrometry experiment, it is but one component of several and that all components must be considered together in devising the optimum measurement strategy. Some of these areas are being explicitly reviewed in this issue, such as separations combined with mass spectrometry[3] and ionization methods.[4] This review is focused on ion manipulation/mass analysis. Emphasis is placed on the capabilities of laboratory-based instruments that have not necessarily been designed with constraints placed upon size, weight, and power requirements. Miniature and in situ instruments are designed with such constraints and constitute an important area of contemporary activity.[5]

By virtue of the large scope of activities that fall within the broad context of mass analysis, it is not feasible to provide a comprehensive review of each subtopic. The major objective of this review is to put into perspective the performance characteristics of the major mass analysis tools in use today in the broadly defined fields of chemistry and molecular biology. In striving to meet this objective, the major recent developments in each subtopic are identified and summarized. They are illustrated throughout with examples from the recent literature. While there are many possible means by which ions can be manipulated and analyzed, only a relatively few forms of mass spectrometry currently dominate the scene. It becomes clear why this is so when the various technologies are regarded in relation to a consistent set of figures of merit. No single mass analyzer type is superior to all others in all ways. Therefore, the mass analyzer of choice depends on the relative importance placed by the particular application on the various figures of merit. Mass spectrometers based on different mass analyzers can, therefore, provide complementary information. However, the various technologies can also be viewed as competitors for each application. For a variety of reasons, technology developers often have a favorite approach to mass analysis and are constantly working to improve its figures of merit in an effort to expand the range of applications for which it is best suited. Furthermore, novel approaches are also examined with an eye toward gaining a competitive edge for an important application. The review begins by presenting a set of figures of merit against which the various forms of mass analysis can be judged. The major forms of mass analysis are then discussed in turn within the context of these figures of merit. A few novel methods are also mentioned along with several ancillary techniques which, although not mass analysis tools in themselves, add significantly either to the performance of a mass analyzer or add to the information that can be obtained from a mass spectrometry experiment.

Tandem mass spectrometry or mass spectrometry/mass spectrometry[6−8] has, in addition to single-stage mass spectrometry, become a particularly important analytical methodology in many of the application areas reviewed in this issue. In addition to the figures of merit that apply to a single stage of mass spectrometry, further considerations are introduced with regard to coupling mass analysis steps. These considerations include, for example, matching characteristics of ions after the first stage of mass analysis with optimal acceptance conditions for the second stage of mass analysis. Perhaps a less widely appreciated consideration is the range of chemical reactions that can either be driven or observed in a particular form of tandem mass spectrometer. All forms of tandem mass spectrometers place limitations on the range of chemistry that can occur

between mass analysis stages. For this reason, the section devoted to tandem mass spectrometry begins with a brief overview of reactions in tandem mass spectrometry with emphasis on the energetics and kinetics of the reactions and how they can relate to the form of tandem mass spectrometric instrumentation. The section is then followed by a discussion of forms of tandem mass spectrometry with emphasis on the figures of merit of a tandem mass spectrometer. By presenting the material in this way, it is hoped that this review not only summarizes the most recent developments in instrumental methods for mass analysis, but also provides a context within which future developments can also be regarded. This review is largely focused on instrumentation devoted to application areas large enough to capture the interest of instrument manufacturers. While many of the most recent developments used as illustrative examples herein are not currently available in commercial systems, there is a strong likelihood that at least some will be features of future commercial offerings. It is recognized that there is a high degree of creativity and novelty associated with unique instrumentation developed for a specific research problem. However, to cover the enormous diversity of instrumentation in basic research that involves mass analysis is beyond the scope of this review.

II. Mass Analyzers

A series of mass analyzer characteristics, which are listed as follows, is used as the basis for discussion: mass resolving power, mass accuracy, mass range, linear dynamic range, abundance sensitivity, precision, efficiency (transmission × duty cycle), speed (time frame of experiment/spectra per second), compatibility with ionizer, cost, size/weight/utility requirements, reliability/ease-of-use/software.

The term mass resolving power, according to the recommendations of the Measurements and Standards Committee of the American Society for Mass Spectrometry,[9] is defined as $M/\Delta M_x$, where ΔM_x is the difference in mass-to-charge between two adjacent peaks in a mass spectrum that are of equal size and shape with a specified amount of overlap, the subscript "x" denotes the overlap criterion, and M is the average mass-to-charge ratio associated with the two peaks. Note that both the overlap criterion and the value of M should be specified when quoting a mass resolving power using this definition. Another commonly used measure of resolving power is based on a single well-resolved peak whereby the M in the $M/\Delta M_x$ ratio is the mass associated with the apex of the peak and ΔM_x is the width of the peak at a specified height x. The two definitions are not necessarily equivalent. For example, the phenomenon of peak coalescence for ions with very similar mass-to-charge ratios in ion-trapping instruments (see below) can significantly reduce resolving power as measured using the former definition but is not a factor in determining resolving power by the latter approach. Most reported values for resolving power have been determined from a single well-resolved peak, largely for convenience, and the values mentioned in this review are those using the full width at half-height criterion. The specification of M by practical necessity is not defined in this review. With some mass analyzers, conditions can be established whereby resolution is constant over a very large fraction of the accessible mass-to-charge scale. However, it is frequently the case that conditions can be tuned to optimize resolution over a particular mass-to-charge range, sometimes at the expense of resolving power for ions outside of this range. In other cases, resolving power is clearly a function of M. The ranges of resolving powers provided herein are qualified to the extent possible in the discussion of each analyzer type. Mass accuracy is the ratio of the mass-to-charge measurement error (i.e., the difference between the measured M and the true M) divided by the true mass-to-charge and is usually stated in terms of parts per million. Mass range is the range of mass-to-charge ratios amenable to analysis by a given analyzer. The term "mass-to-charge ratio range" is obviously more accurate terminology, but the less cumbersome historical term "mass range" is used here with the understanding that the "mass" spectrometer reports "mass-to-charge ratios". Normally only the upper limit is provided, with the implication that ions of all lesser mass-to-charge ratios can also be analyzed. In practice, this is frequently not the case. Measures used to achieve the highest possible mass-to-charge measurement may compromise the measurement of ions at the opposite end of the mass scale. It is beyond the scope of this discussion to provide detailed analysis of the possible tradeoffs associated with maximizing performance at either end of the nominal mass-to-charge range. Therefore, in keeping with common practice, the mass-to-charge range is discussed here in terms of the upper limit with the proviso that the reader should consult the references for the individual technologies for more information regarding performance at the margins of the mass-to-charge scale. Linear dynamic range is the range over which ion signal is linear with analyte concentration. This can be limited by the analyzer itself or, for many measurement scenarios, by one of the other elements of the mass spectrometry experiment (e.g., ionization method). Abundance sensitivity is the inverse of the ratio obtained by dividing the signal level associated with a large peak by the signal level of the background at one mass-to-charge unit lower or higher.[10] It is a particularly important figure of merit in the measurement of isotope ratios. Abundance sensitivity is related to dynamic range but differs in that peak shape plays a major role in determining abundance sensitivity (e.g., the tails of the more abundant ion ultimately determine the extent to which abundance sensitivity approaches dynamic range). Precision here refers to the reproducibility with which ion abundances can be determined. External precision refers to reproducibility observed for measurements of nominally identical samples whereas internal precision refers to repeated measurements of the same sample. Some questions, such as whether a material contains highly enriched uranium, can be answered with precisions of 5−10%, whereas some questions in the geosciences, for example, require external precisions of 0.01%.

Efficiency is defined here as the product of the transmission of the analyzer and its duty cycle, where duty cycle is defined as the fraction or percentage of the ions of interest formed in the ionization step that are subjected to mass analysis. The efficiency for a given type of analyzer can be highly measurement dependent. For example, the efficiency of a quadrupole mass filter can be relatively high in a single ion monitoring application at relatively low resolution, but it can be quite low in an application requiring relatively high resolution and scanning over a wide mass-to-charge range. Speed refers to the time frame of the experiment and ultimately is used to determine the number of spectra per unit time that can be generated. For a beam-type instrument using a continuous ionization source, wherein ionization, mass analysis, and detection are all occurring in parallel, scan speed is defined by the time frame needed to acquire a spectrum of the ions over the specified mass-to-charge range. For experiments involving ion-trapping instruments, in which various stages of the experiment occur in sequence, the time required to analyze the mass-to-charge ratios of the ions comprises only a fraction of the time required to generate a spectrum. To compare the various types of analyzers in a meaningful way, therefore, speed must be defined as the spectral generation rate in Hertz. Compatibility with ionizer is a criterion intended to encompass how well a mass analysis approach is suited to a particular ionization method. There is subjectivity in this category because "suitability" can only be defined well within the context of the relative priorities of a given measurement. Nevertheless, some generalizations can be made. For example, scanning analyzers are generally not optimally suited to pulsed ionization methods. Cost, size, weight, utility requirements are included together in this discussion as a set of practical criteria because performance alone is rarely the only consideration in the choice of a technology. While cost is almost always a factor, size, weight, and utility requirements tend to be most important in field applications.[5] Reliability, ease-of-use, and software are clearly important considerations in the purchase of a mass spectrometer system. However, this set of criteria is not considered further in this discussion because these factors in this category are not fundamentally linked to the various analyzer technologies.

For any given measurement scenario, the relative importance of each of these figures of merit tends to vary. Mass range is far less important for elemental isotope ratio measurements than it is for protein molecular weight measurements, for example, whereas the opposite can be said for precision or abundance sensitivity. The selection of an approach to the measurement challenge is, therefore, a balancing act that usually hinges on a few overriding factors. Any of the characteristics just listed can be such a factor, given the range of problems to which mass spectrometry is applied. It is the range of applications that makes mass spectrometry such a diverse field. However, while there are many possible combinations of technologies associated with the various elements of a mass spectrometry experiment, relatively few forms of mass analysis are predominant. They include time-of-flight mass spectrometry, the quadrupole mass filter, sector field mass spectrometry, and ion-trapping forms of mass spectrometry including Fourier transform ion cyclotron resonance (FTICR) and the quadrupole ion trap (or Paul trap). Each of these forms of mass spectrometry is discussed in turn using illustrative data from recent literature with a summary and brief discussion of the figures of merit of each technology. In some instances, the maturity level associated with some types of measurements is too low to make a clear assessment of the relative strength of the analyzer for a given figure of merit.

A. Time-of-Flight Mass Analysis

For detailed presentations of the fundamental factors associated with achieving optimum performance for the various mass analysis figures of merit by time-of-flight mass spectrometry, the reader is referred to several recent reviews that are tutorial in nature and/or provide recent summaries of the state-of-the-art.[11−20] It is often said, and rightly so, that time-of-flight mass spectrometry has undergone a renaissance over the course of the past two decades. The technique has had a remarkable impact upon the practice of mass spectrometry over the last 20 years, and its "footprint" continues to grow. While attention was re-focused on time-of-flight in the 1970s due to interest in new pulsed ionization methods involving lasers, pulsed primary ion beams, and the radioactive decay of ^{252}Cf, advances in digital electronics and other developments in time-of-flight have stimulated the use of the technique in a variety of applications beyond those limited to pulsed ionization techniques (see below).

Perhaps the single most important event to generate widespread attention to time-of-flight in the decade just passed was the introduction of matrix-assisted laser desorption ionization (MALDI).[21] Time-of-flight and MALDI are particularly well-matched. The pulsed nature of the ionization event and the fact that desorption from a flat surface removes the so-called "turn-around time" problem[17] are attractive characteristics for mass analysis via time-of-flight. Furthermore, the need for high mass range and the desirability of acquiring the entire mass spectrum from a single ionization event make time-of-flight an obvious choice as a mass analyzer for MALDI. However, the complex MALDI process[19,22−25] gives rise to relatively broad spatial and kinetic energy distributions which degrade resolution in continuous extraction linear time-of-flight instruments. Significant improvement in performance has been demonstrated by use of an ion mirror or reflectron,[26] which can compensate, at least in part, for a spread in kinetic energy. Further improvement has been demonstrated by use of "delayed extraction"[27−30] whereby MALDI is effected in the absence of an electric field (or in a weak electric field[27]) followed by pulsed extraction. Delayed extraction techniques have clear analogies with the so-called time-lag focusing technique described by Wiley and McLaren.[31] The utility of delayed extraction is illustrated in Figure 1, which was reproduced from ref 27. Nine spectra are shown

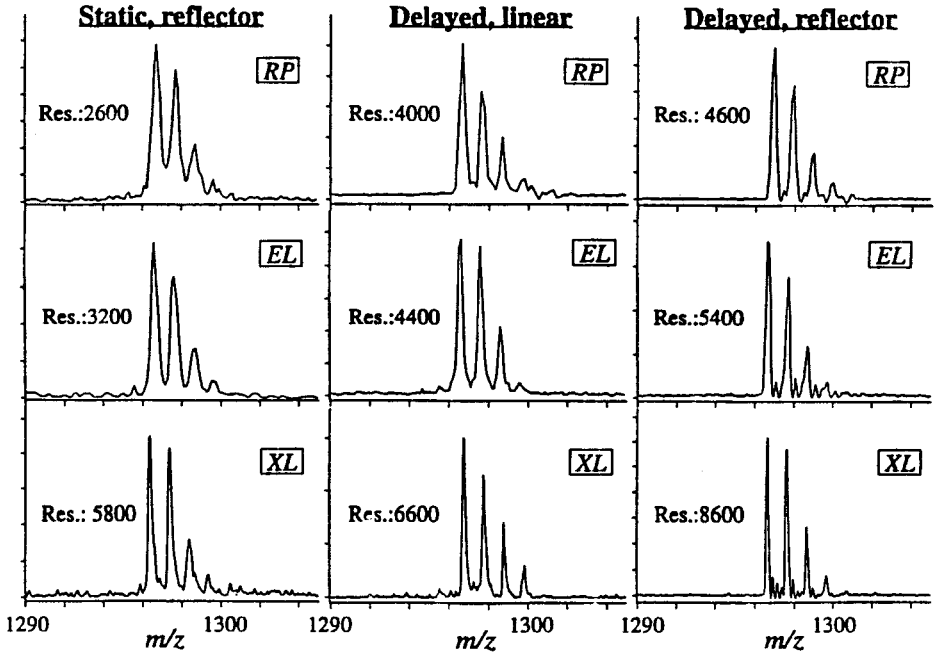

Figure 1. Nine time-of-flight mass spectra are shown of the molecular ion region of angiotensin I using normal acceleration in combination with a reflectron, delayed extraction used with a linear time-of-flight mode of operation and delayed extraction in combination with a reflectron. Data for each mode of operation are illustrated with three time-of -flight distances using instrument geometries labeled RP, EL, and XL, where flight distance follows the order RP < EL < XL. (Reprinted with permission from ref 27. Copyright 1995 John Wiley and Sons Limited.)

displaying the mass-to-charge region of singly protonated angiotensin I using normal acceleration in combination with a reflectron, delayed extraction used with a linear time-of-flight spectrometer, and delayed extraction in combination with a reflectron. Data for each mode of operation are illustrated with three time-of-flight distances indicated with the labels RP, EL, and XL where RP < EL < XL. The delayed extraction approach has been demonstrated to provide significant improvements in resolving power over limited ranges of mass up to several tens of kilodaltons.

The use of an ion mirror is commonplace in modern time-of-flight instruments. Such ion mirrors can employ linear electric fields with one,[32] two,[33,34] or three stages[35] or they can employ a nonlinear electric field.[36−46] In most cases, only a single ion reflection step is involved. The purpose is to compensate for the nonidealities associated with the starting conditions in order to improve mass resolving power. Another approach to improving resolving power is to increase the time-of-flight path length. This can be accomplished without significantly increasing the size of the spectrometer by use of a multiturn[47−52] or multipass[53−55] design. Figure 2 shows a schematic diagram of a recently described multiturn time-of-flight mass spectrometer.[51] For mass resolution to improve with each transit of the closed time-of-flight loop, 'perfect' focusing conditions must be achieved. The combination of four cylindrical electric sectors and eight electric quadrupole lenses has been shown to meet this condition.[51] Figure 3 shows mass spectra of the xenon isotopes recorded with the device of Figure 2 after 0.5, 3.5, 6.5, and 9.5 cycles, respectively. The mass resolving power increases from a few hundred to over 4000 with 30% of the ions detected

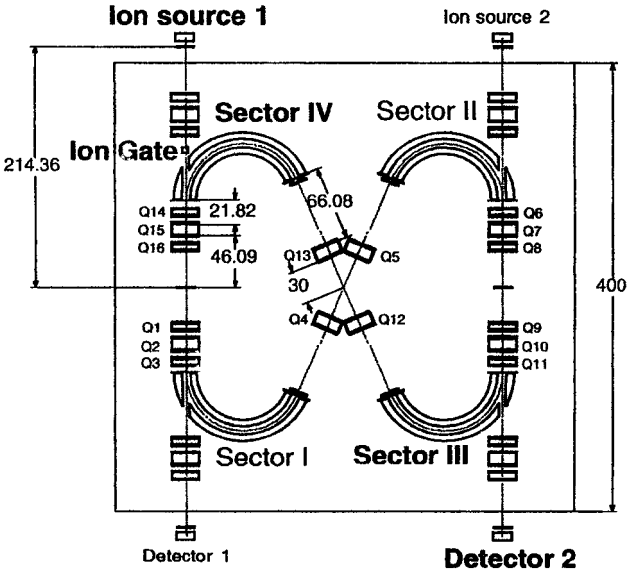

Figure 2. Schematic diagram of a recently described multiturn time-of-flight mass spectrometer. (Reprinted with permission from ref 51. Copyright 2000 John Wiley and Sons Limited.)

after 0.5 cycles remaining after 9.5 cycles. Most of the ion losses were observed in the first 5.5 cycles.

A major expansion in the range of applications to which time-of-flight mass spectrometry can be applied has resulted from the development of orthogonal acceleration time-of-flight for coupling with continuous ionization sources.[56−72] Dawson and Guilhaus described such an instrument coupled with electron ionization in 1989.[56] Also in the latter part of the 1980s, Dodonov et al. coupled electrospray ionization[73] with an orthogonal acceleration time-of-flight instrument that featured an ion mirror.[57] Figure 4

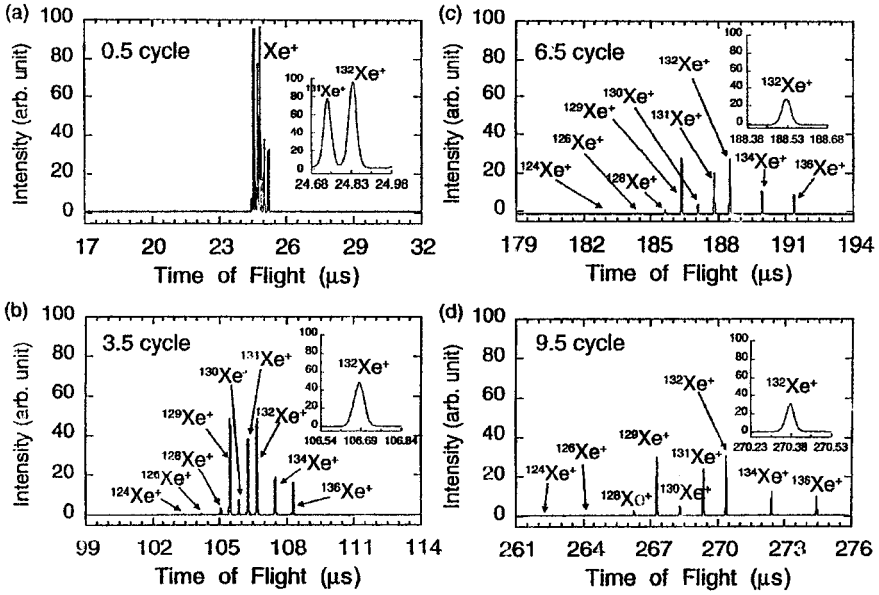

Figure 3. Time-of-flight mass spectra of the xenon isotopes obtained after (a) 0.5 cycles through the apparatus of Figure 2, (b) 3.5 cycles, (c) 6.5 cycles, and (d) 9.5 cycles. (Reprinted with permission from ref 51. Copyright 2000 John Wiley and Sons Limited.)

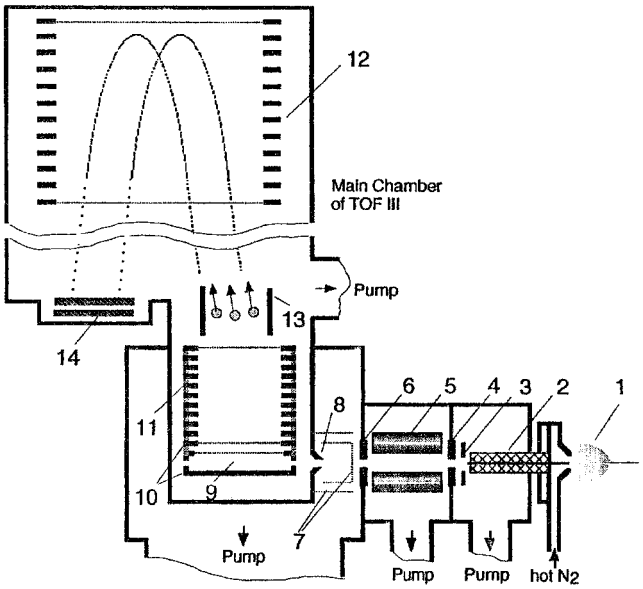

Figure 4. Schematic diagram of an orthogonal acceleration time-of-flight instrument coupled with electrospray. (Reprinted with permission from Elsevier Science from Elsevier Science, ref 65. Copyright 1998 American Society for Mass Spectrometry.)

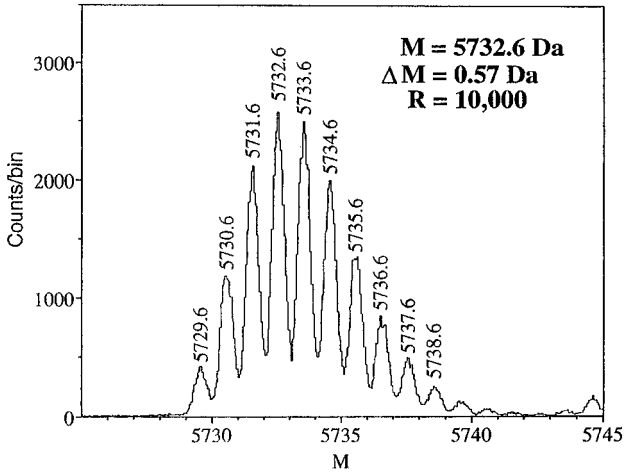

Figure 5. Zero-charge electrospray mass spectrum of bovine insulin obtained using the apparatus of Figure 4. (Reprinted with permission from Elsevier Science, ref 65. Copyright 1998 American Society for Mass Spectrometry.)

shows an instrument schematic published by Krutchinsky et al. showing an electrospray/time-of-flight mass spectrometer.[65] This instrument also features a radio frequency quadrupole intermediate between the electrospray interface and the ion accumulation region. The rf-only quadrupole serves both to transmit ions and to allow for collisional damping of the ions, which results in narrower spreads in ion position and energy. (The use of radio frequency multipoles for this and other purposes is mentioned again below in the ancillary device section.) Figure 5 shows the deconvolved mass spectrum obtained from electrospray of bovine insulin showing resolution of the isotopic distribution.[65] The spectrum reflects a mass resolving power of 10^4. Matching the time associated with filling the ion accumulation region with the flight time of the ions accelerated orthogonally into the reflectron time-of-flight analyzer can, in principle, allow for 100% duty cycle time-of-flight analysis. However, constant duty cycle across a wide mass-to-charge range is difficult to achieve due to mass-dependent residence times in the ion accumulation region. Duty cycle values of 5−50% are typical for orthogonal acceleration time-of-flight.

Orthogonal acceleration has allowed electrospray to enjoy the strong suits of time-of-flight, such as high mass range, high speed, high transmission, good resolution, and excellent mass accuracy. The inorganic mass spectrometry community has also taken an interest in the potential for time-of-flight coupled with plasma sources. For example, Hieftje et al. investigated the combination of the inductively coupled plasma[74] with orthogonal acceleration time-of-

flight.[66−69] The results show the capability for high-sensitivity measurements and potential for abundance sensitivities of 10^6 (10^4 demonstrated) for cases in which the isotope of lesser abundance arrives at the detector first. In the opposite scenario, detector ringing resulting from the signal generated by the ion of higher abundance can adversely affect the abundance ratio measurement.

Harrison et al. demonstrated potential for orthogonal acceleration time-of-flight combined with pulsed glow discharge ionization applied to solids analysis.[70,71] Pulsing the discharge provides far greater maximum power delivered to the surface than the conventional continuous plasma mode, thereby resulting in a much higher sputtering rate. Furthermore, ionization efficiency is higher because the degree of excitation in the pulsed plasma is higher than in the DC plasma. The pulse lengths for the pulsed discharge range from 5 to 20 μs, which is long for an axial time-of-flight system. Orthogonal acceleration readily accommodates such a long ionization period without deleterious effects on resolution. Figure 6 shows a schematic of the pulsed glow discharge/orthogonal acceleration time-of-flight instrument along with some illustrative data for both linear and reflectron versions of the time-of-flight analyzer.

The foregoing examples are given to provide some highlights in recent developments in time-of-flight mass spectrometry. A listing of the figures of merit of time-of-flight mass spectrometry are given below:

mass resolving power	10^3-10^4
mass accuracy	5−50 ppm
mass range	$>10^5$
linear dynamic range	10^2-10^6
precision	0.1−1%
abundance sensitivity	up to10^6
efficiency (transmission × duty cycle)	1−100%
speed	10^1-10^4 Hz
compatibility with ionizer	pulsed and continuous
cost	moderate to high
size/weight/utility requirements	benchtop

As with all of the analyzer technologies, it is important to recognize that it is either difficult or impossible to achieve the highest levels of performance in each figure of merit simultaneously. There are often tradeoffs between two or more levels of performance. In any case, it is clear that time-of-flight has a very attractive set of figures of merit for a wide range of applications. The advent of orthogonal acceleration time-of-flight adds to the already important role that time-of-flight has played with pulsed ionization methods by allowing for high efficiency in conjunction with continuous ionization. Mass resolving power has been demonstrated to be as high as 10^4 both for MALDI using delayed extraction and for orthogonal acceleration time-of-flight coupled with electrospray or MALDI.[16] Several groups demonstrated resolving powers well in excess of 10^4 at the 2000 American Society for Mass Spectrometry conference using multipass time-of-flight analyzers. Descriptions of these works in the literature including compromises in mass range and signal, for example, are anticipated. The technique provides very good mass accuracy, and the wide mass range that it can provide is widely recognized. The linear dynamic range is primarily determined by the detection electronics with a tradeoff between the speed of the electronics and their dynamic range. The point at which this tradeoff occurs, however, continues to improve with the state-of-the-art of high-speed digital electronics. The precision and abundance sensitivity values listed here were taken from the work of Hieftje et al.[66−69] They are based on the performance of a particular instrument and do not necessarily represent the ultimate potential performance in these areas. The efficiency of time-of-flight can certainly be a strong suit. In principle, it can be 100%, but in practice it is sometimes necessary to trade efficiency for resolution. Clearly, a major advantage of time-of-flight is its unparalleled potential for speed. Assuming a total flight time of 100 μs, spectra can in principle be collected at rates of up to 10 kHz. In practice, due to limited data transfer rates, limited data storage, and/or the need for averaging, data are output at far lower rates. Nevertheless, time-of-flight is the fastest means for recording full mass spectra.

B. Beam-Type Mass Analyzers

1. Mass Filters

The quadrupole mass filter[75] has been the most widely used mass analyzer for "low resolving power" applications for nearly 30 years. It continues to see extensive use both as a stand-alone mass analyzer, usually coupled with an on-line separation technique, and as an analyzer in multistage mass spectrometers (see below). Its characteristics are well-known, and while there have been evolutionary advances in fabrication and electronics, there have been no major changes in the mass analysis figures of merit of mass filters in recent years. Nevertheless, there have been some recent reports of quadrupoles being used in novel ways that show advantages in improving some types of mass spectrometry experiments. Some of these novel uses are made in conjunction with another form of mass analysis such that the quadrupole itself is not the mass spectrometer. Some developments of this type are mentioned below in the Ancillary Technologies section. A few studies involving novel methods of operation of quadrupoles in which mass filtering remains the main objective are discussed in this section along with a listing of the figures of merit of the quadrupole mass filter.

Virtually all quadrupole mass filters are operated in the so-called first stability region. Douglas et al. recently explored the operation of mass filters in higher stability regions[76−82] with the primary objective of improving elemental ion analysis via inductively coupled plasma ionization. By operating in higher stability regions, Douglas et al. noted that improvements over operation in the first stability region in mass resolving power, abundance sensitivity, and the ability to resolve high kinetic energy ions can be obtained. Operation in higher stability regions compromises mass range and transmission, however. These observations are complemented by theory.[83−87]

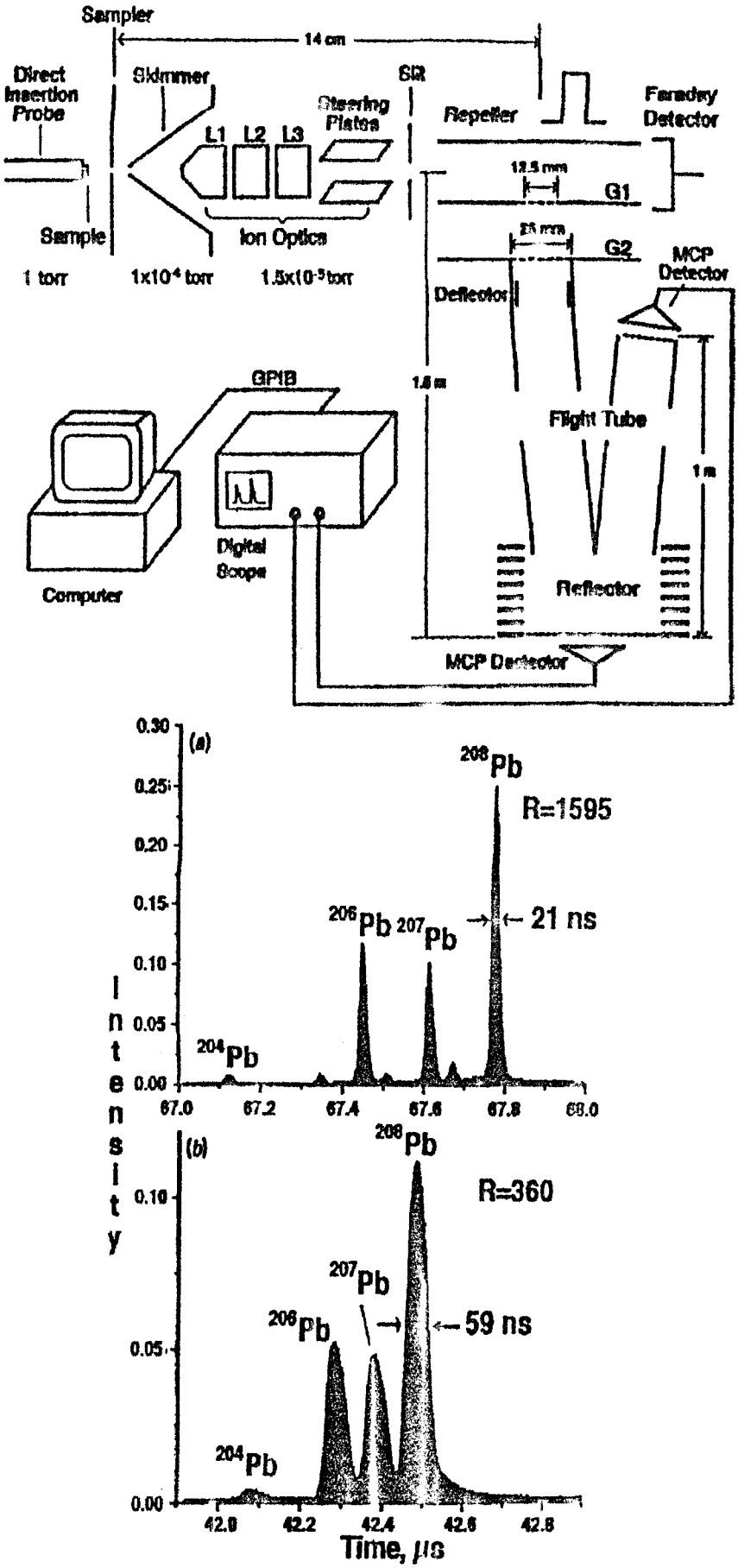

Figure 6. Schematic diagram of an orthogonal acceleration time-of-flight instrument coupled with a pulsed glow discharge source. Illustrative data for both linear and reflectron versions of the time-of-flight analyzer are also included. (Reprinted with permission from ref 70. Copyright 1996 The Royal Society of Chemistry.)

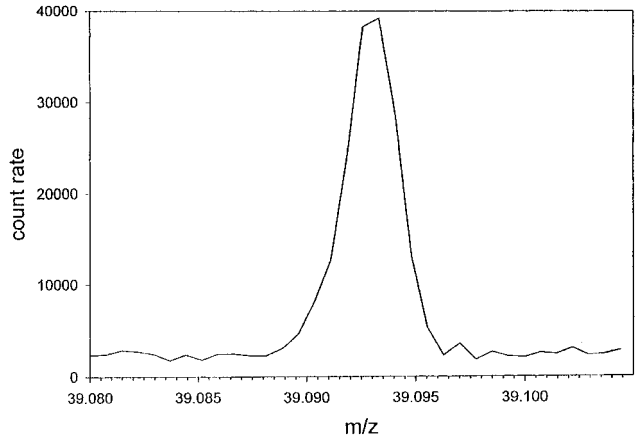

Figure 7. Peak shape obtained for 40 eV $^{39}K^+$ ions acquired by operating a quadrupole in the rf-only mode in the fourth stability region indicating a resolving power of 13 900. (Reprinted with permission from ref 81. Copyright 2000 American Chemical Society.)

Figure 7 shows the peak shape acquired for 40 eV $^{39}K^+$ ions obtained by operating a quadrupole in the rf-only mode in the fourth stability region.[82] A resolving power of 13 900 is achieved. It was also shown that a resolving power of 1000 could be obtained on 4800 eV ions. The latter observation is significant in that it suggests that a quadrupole could be used to analyze high-energy ions, which is desirable from the standpoint of minimizing deleterious space charge effects. Higher resolving powers are desirable because isobaric interferences pose problems in elemental analysis. Douglas' group recently demonstrated the baseline resolution of Fe^+ and ArO^+ ions using a mass filter operated in the second stability region[76] reflecting a resolving power of 9000. These results show that the mass filter can be used to resolve isobaric ions at the expense of sensitivity by operation in higher stability regions, in analogy to the transmission/resolution tradeoff with sector instruments. Improved performance in abundance sensitivity was noted with a tandem quadrupole mass analysis approach whereby two mass filters, each operated in the third stability region, were scanned in concert with a fixed mass off-set.[77]

Another approach to improving the mass resolving power of a mass filter was recently reported in a preliminary study by Amad and Houk[88] based on a "multipass" or ion storage approach. In some cases, the resolving power of a quadrupole mass filter is related to the square of the number of radio frequency cycles that the ion experiences.[75] Therefore, by reflecting the ions multiple times within the mass filter, resolving power is expected to increase. Figure 8 compares mass spectra obtained over the narrow mass range that encompasses CO^+ and N_2^+ using a single pass through the mass filter (i.e., conventional operation) and after multiple reflections within the mass filter. A resolving power of 5000 is reflected in the multipass data accompanied by a loss in signal by a factor of about 100. Furthermore, the scan rate of the experiment was about 5 u/s, which is much lower than a typical quadrupole scan rate. Not surprisingly, there is a compromise between resolving power on one hand and scan speed and transmission on the other.

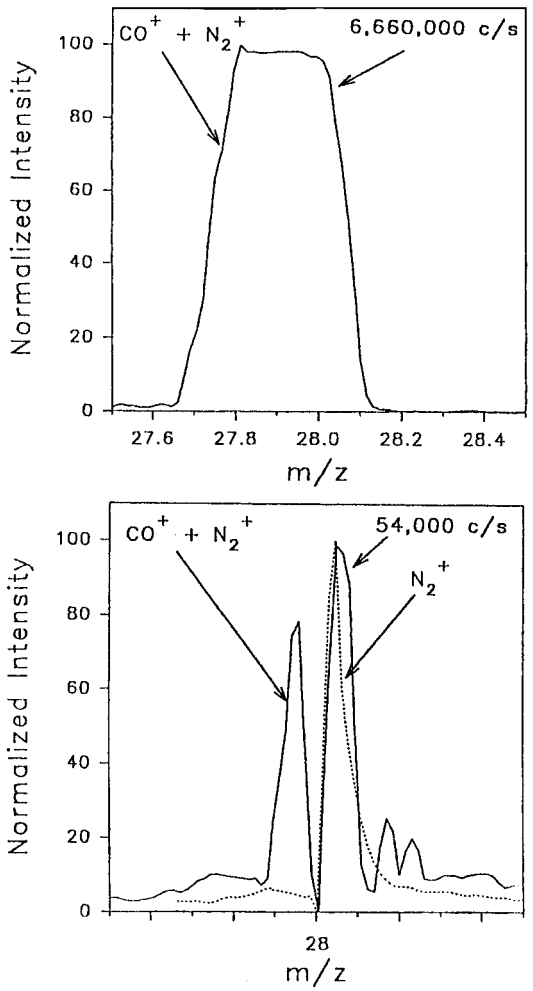

Figure 8. Comparison of mass spectra obtained over the narrow mass range that encompasses CO^+ and N_2^+ using a single pass through the mass filter (i.e., conventional operation) (top) and after multiple reflections within the mass filter (bottom). (Reprinted with permission from ref 88. Copyright 1998 American Chemical Society.)

The mass filters in most common use today are characterized by relatively modest ranges of mass-to-charge. They are, therefore, directly amenable for use with relatively low mass singly charged ions. The advent of electrospray with its tendency to form multiply charged ions from high-mass molecules has made possible the application of quadrupole mass spectrometry to biological problems because the ions typically fall within the usual mass-to-charge range of commercial devices. However, it has been demonstrated in numerous cases that relatively high mass-to-charge ions can be generated by electrospray and are common with noncovalently bound aggregates.[65] Such ions can easily exceed the typical upper mass-to-charge limit of 2000−4000 for most commercial quadrupoles. Several groups, therefore, have modified quadrupole mass filters by reducing the operating frequency to extend the quadrupole mass range. For example, Smith et al. recently extended the mass-to-charge range of a quadrupole mass filter to 45 000 by reducing the operating frequency from 1.2−1.5 MHz to 262 kHz.[89−91] The extension to higher mass-to-charge range came at the expense of resolving power and transmission. Collings and Douglas reported the modification of a mass filter for higher

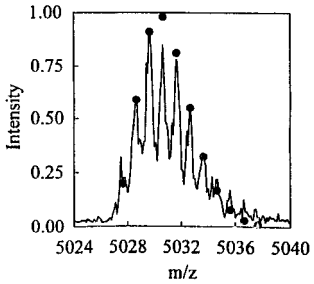

Figure 9. Example of a high-resolution mass filter scan of ions derived from electrospray of poly(propylene glycol) 4000 (*m/z* range of 5024−5040) indicating a resolving power of about 8500. (Reprinted with permission from ref 92. Copyright 1997 Elsevier Science.)

mass-to-charge range operation with significantly better resolution than that shown with the 262 kHz quadrupole and better transmission at low mass-to-charge but with a lower mass range extension (to *m/z* 8565).[92] The mass range extension was achieved by reducing the operating frequency from 1 MHz to 683 kHz. Figure 9 shows an example of a high-resolution scan of ions derived from electrospray of poly(propylene glycol) 4000 (*m/z* range of 5024−5040) indicating a resolving power of about 8500.[92] This apparatus also employed a relatively high-pressure collision quadrupole between the ion source and the mass filter to collisionally focus the ions to a relatively narrow beam for injection into the mass filter. The collisional focusing step proved to be key to obtaining this level of performance with relatively little loss in transmission as the operational frequency was reduced. Given the several decades of experience with the mass filter in a variety of applications (operated in the first stability region), its figures of merit, a representative set of which are given below, are fairly well-known.

mass resolving power	10^2-10^4
mass accuracy	100 ppm
mass range	10^4
linear dynamic range	10^7
precision	0.1−5%
abundance sensitivity	10^4-10^6
efficiency (transmission × duty cycle)	<1−95%
speed	1−20 Hz
compatibility with ionizer	continuous
cost	relatively low
size/weight/utility requirements	benchtop

As with the analyzers already described, the figures of merit for any particular device depend on a number of factors and there are compromises between some of these characteristics (such as scan range and duty cycle, resolution and efficiency, and so forth). The figures of merit of the mass filter continue to be attractive for many applications. A particularly useful feature not reflected in the figures of merit delineated in this discussion is the ready conversion between total-ion transmission (rf-only mode) and single-ion transmission (rf/dc or mass filtering mode). However, the requirement that the device must be scanned to acquire a mass spectrum is a characteristic that has motivated the exploration of other methods of mass analysis, particularly for pulsed ionization methods.

2. Sector Field Mass Spectrometers

Sector technology is the most mature of all forms of mass analysis and has been a mainstay in many areas for much of the last century. With respect to some of the analyzer figures of merit, sector mass spectrometry continues to enjoy the highest levels of performance. However, advances in some of the other technologies and the size and cost of sector instrumentation have inhibited the use of sector technology from growing in proportion to the overall growth of the field. In particular, the expansion of biological applications, which accounts for much of the growth of the field of mass spectrometry, has been largely accommodated by time-of-flight, quadrupole mass filter, and ion-trapping instruments. However, in many inorganic and elemental applications, sector field mass spectrometry remains the gold standard for performance,[93,94] particularly in the areas of dynamic range, abundance sensitivity, and precision. Furthermore, sector mass spectrometers are still widely used for exact mass measurements to identify or verify novel synthesis products. Recent instrumental innovations have involved the combination of multicollector detection schemes with ion sources that present either transient or fluctuating ion signals.[95−98] For example, Figure 10 shows an instrument schematic of a recently described sector mass spectrometer that has been designed for inductively coupled plasma ionization with multiple Faraday cup detection.[96] The multicollector approach corrects for many errors in the measurement of isotope abundance ratios, and this instrument has provided high-precision measurements using a plasma source. Coupled with laser ablation, this approach can allow for high-precision isotope ratio measurements of elements on solids using the inductively coupled plasma, in addition to normal measurement of elements in solution.

A set of typical figures of merit for sector mass spectrometers is given below.

mass resolving power	10^2-10^5
mass accuracy	1−5 ppm
mass range	10^4
linear dynamic range	10^9
precision	0.01−1%
abundance sensitivity	10^6-10^9
efficiency (transmission × duty cycle)	<1% (scanning)
speed	0.1−20 Hz
compatibility with ionizer	continuous
cost	moderate to high
size/weight/utility requirements	lab instruments

The characteristics for a given instrument vary with design and operational mode. In relation to the other forms of mass analysis described here, it is clear that sectors retain preeminence in linear dynamic range, precision, and abundance sensitivity. These factors are at a premium in elemental mass spectrometry, including accelerator mass spectrometry,[98] particularly when isotope ratio measurements are of interest. However, these factors are much less important in most current mass spectrometry applications to biological problems which involve high-mass polyatomic ions. Furthermore, these figures of

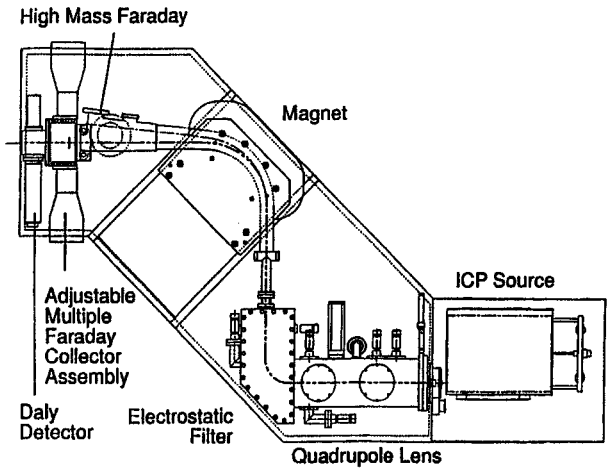

Figure 10. Schematic diagram of an ICP multicollector sector mass spectrometer. (Reprinted with permission from ref 96. Copyright 1995 Elsevier Science.)

merit do not include the criterion of translational energy measurement, which is a strong suit of sector instruments (see Tandem Mass Spectrometry section).

C. Ion-Trapping Mass Analyzers

The two most widely used forms of ion-trapping mass spectrometers, the ion cyclotron resonance instrument and the electrodynamic or Paul trap, have both seen expanded application in recent years. The application of Fourier transform methodologies to the ion cyclotron resonance instrument[99] underlies its prominence in the field of mass spectrometry. In the case of the Paul trap, recent interest was catalyzed by the introduction of the mass-selective instability method for acquiring mass spectra.[100] While there are many conceptual parallels between the FTICR and the Paul trap, their operational and performance characteristics are quite different and are discussed separately.

1. Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

Fourier transform ion cyclotron resonance mass spectrometry has been a very active area of research in mass spectrometry for over two decades. It has been the subject of several recent reviews that describe progress in the field, historical perspectives, and tutorial descriptions of the technique.[101−112] There have also been several books[113−115] and journal issues devoted to the subject.[116,117]

Modern FTICR enjoys the highest resolving power of any form of mass analysis, at least for mass-to-charge ratios less than about 10^4, and the highest mass measurement accuracy. There are, therefore, many applications for which the performance of FTICR is unparalleled. For example, high resolving powers and high mass accuracies are particularly valuable in the analysis of multiply charged macroions derived from electrospray. The multiple charging phenomenon is, in general, advantageous in that it relaxes mass-to-charge range requirements; however, it also forces all ions into a relatively narrow mass-to-charge range and, therefore, places a premium on resolving power. An example of the power of FTICR in the mass analysis of biologically derived ions is given in Figure 11, which shows electrospray FTICR mass spectra of bovine serum albumin obtained with an 11.5 T instrument.[118] Figure 11a shows a broad charge state distribution using broad-band FT, whereas Figure 11b shows the results after isolation of the 49^+ and 48^+ charge states via stored waveform inverse Fourier transform (SWIFT).[119,120] Figure 11c shows the data for the 49^+ charge state, which reflects a mass resolving power of about 350 000, after summing 50 time-domain acquisitions. The high-mass measurement accuracy of FTICR is illustrated in the data of Figure 12, which shows the electrospray mass spectrum derived from the tryptic digestion of bovine serum albumin.[121] A total of 123 distinct components were identified in the data, and 71 were assigned to expected tryptic digestion products. All components were measured with mass accuracies less than 2 ppm with the average error being 0.77 ppm.

The data just presented illustrate the remarkable resolving powers and mass accuracies afforded by FTICR to ions derived from electrospray and how these capabilities can be used. As with most forms of mass spectrometry, measurements are typically made on populations of ions. However, the multiple charging phenomenon of electrospray has also facilitated the development of FTICR techniques for the analysis of single highly charged ions.[122−126] The ability to form, trap, manipulate, analyze, and detect single high-mass ions constitutes a new capability for mass spectrometry. (See also the section on electrostatic trapping.)

The marriage of electrospray with FTICR is a happy one because electrospray tends to yield ions in the mass-to-charge range where FTICR performance is excellent. Less effort has been expended in developing FTICR for high-mass singly charged ions. However, significant progress has been made in recent years with the combination of MALDI and FTICR in trapping high-mass ions,[127−129] obtaining high resolution,[130,131] and achieving high-mass measurement accuracy.[132] For example, Figure 13 shows the FTICR mass spectrum of protonated cytochrome *c* formed by MALDI. This spectrum was derived from the Fourier transformation of a 52 s transient yielding a resolution of 81 000 (fwhm).[130] Easterling et al. recently demonstrated that mass measurement accuracies on the order of 2 ppm can be achieved routinely on ions derived from MALDI despite the relatively poor shot-to-shot reproducibility of the number of ions.[132]

The high resolving power of FTICR has also been demonstrated in inorganic mass spectrometry via the coupling of plasma ion sources, such as glow discharge[133−137] and inductively coupled plasma.[138] Figure 14, for example, shows a resolving power of 1.7×10^6 for $^{58}Fe^+$ formed via glow discharge ionization using a 7 T FTICR spectrometer.[137] Such a resolution easily allows for separation of any isobaric interferences that are problematic in much lower resolution instruments. Although relatively little effort has been directed toward optimizing FTICR for precision, or

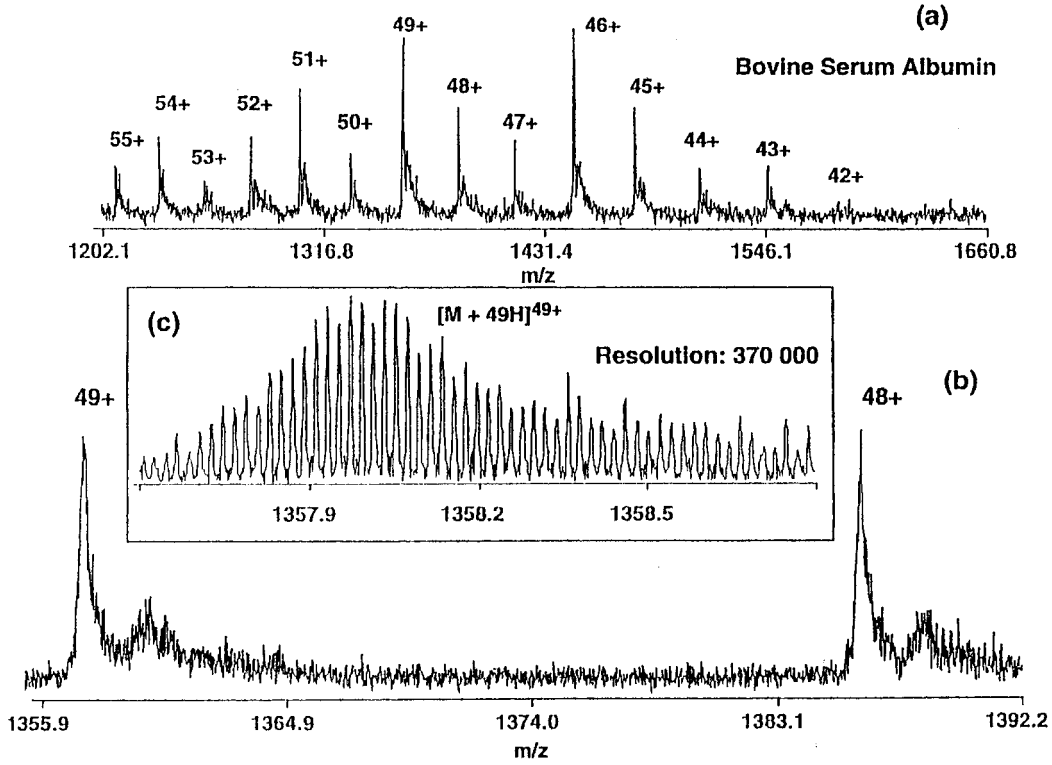

Figure 11. Electrospray FTICR mass spectra of bovine serum albumin obtained with an 11.5 T instrument: (a) a wide range of charge states obtained using broad-band FT, (b) spectrum acquired after isolation of the 49^+ and 48^+ charge states via stored waveform inverse Fourier transform, and (c) data for the 49^+ charge state after summing 50 time-domain acquisitions, which reflects a mass resolving power of about 350 000. (Reprinted with permission from ref 118. Copyright 1998 American Chemical Society.)

abundance sensitivity, a measurement precision of 0.32% has been noted with FTICR coupled with a glow discharge source.[136] The potential roles that ion-trapping instruments might play in elemental mass spectrometry has been discussed.[137,139]

The preceding discussion illustrates at least some of the important characteristics of FTICR. A listing of its figures of merit is given below.

mass resolving power	10^4-10^6
mass accuracy	1−5 ppm
mass range	$>10^4$
linear dynamic range	10^2-10^5
precision	0.3−5%
abundance sensitivity	10^2-10^5
efficiency (transmission × duty cycle)	<1−95%
speed	0.001−10 Hz
compatibility with ionizer	pulsed and continuous
cost	moderate to high
size/weight/utility requirements	lab instrument

The mass resolving power and mass accuracy strengths of FTICR have been mentioned. Coupled with the often high efficiency of the technique, which allows for high-quality measurements of very low sample quantities,[140−142] it is clearly the most powerful form of mass spectrometry for electrospray of high-mass biomolecules, in terms of resolution, mass accuracy, and sensitivity. The mass range now exceeds 10^4 (but not 10^5) with high resolution, which makes FTICR increasingly attractive for MALDI applications as well. Ion−ion interactions tend to limit dynamic range and abundance sensitivity,

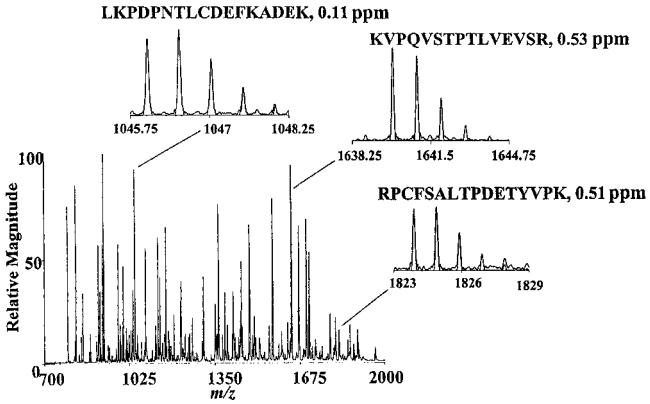

Figure 12. Electrospray FTICR mass spectrum of tryptic digestion products of bovine serum albumin using an 11.5 T system. (Reprinted with permission from Elsevier Science, ref 121. Copyright 1999 American Society for Mass Spectrometry.)

particularly when the ions of interest make up only a small fraction of the ion population. There has been relatively little attention paid to these characteristics, but tailored waveforms to control the identities and numbers of ions should allow for strategies to optimize abundance sensitivity and dynamic range. Furthermore, a common practice to maximize dynamic range by minimizing deleterious ion−ion interactions is to add data from many identical experiments, each of which involves a relatively small number of ions. It is important to recognize that the cost associated with the extremely high performance characteristics of FTICR, in addition to the price of a new instrument, is time. The mass measurement

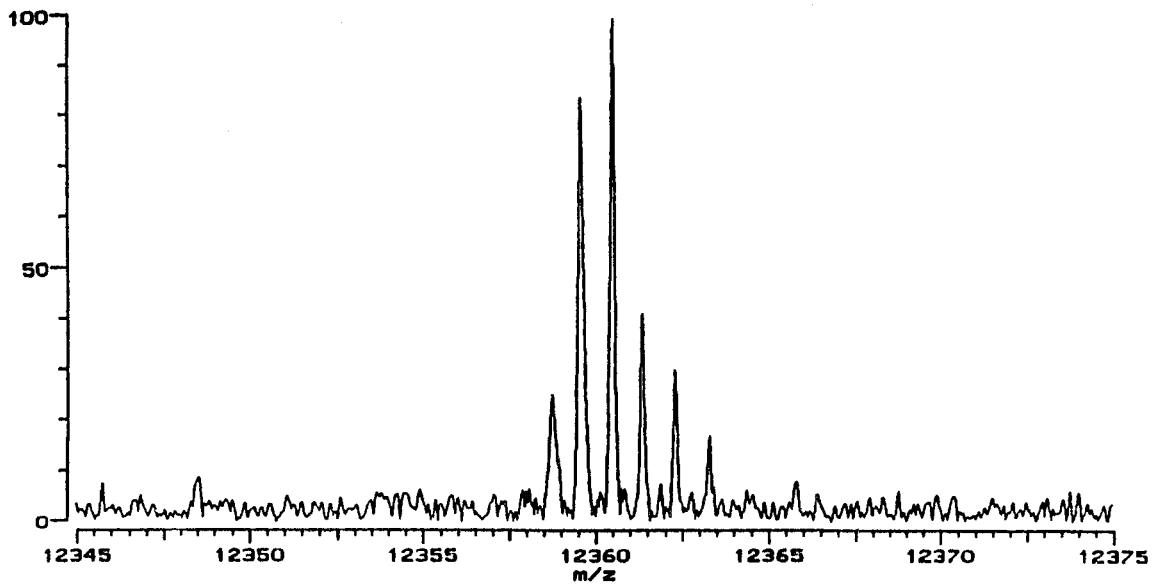

Figure 13. Matrix-assisted laser desorption ionization FTICR mass spectrum of cytochrome *c* displayed over the *m*/*z* region of the protonated molecule. (Reprinted with permission from ref 130. Copyright 1996 Elsevier Science.)

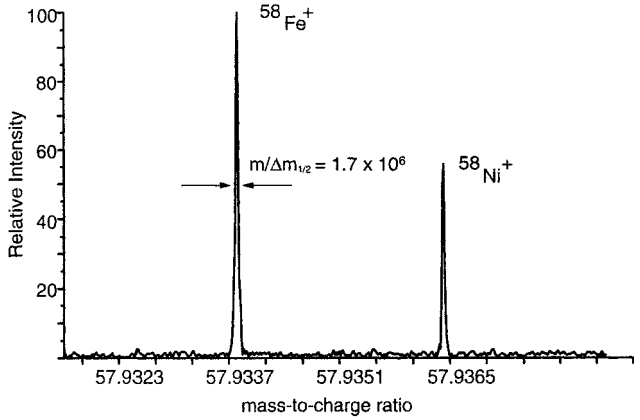

Figure 14. FTICR glow discharge mass spectrum of Fe and Ni using a 7 T FTICR spectrometer indicating a resolving power of 1.7×10^6 for $^{58}Fe^+$. (Reprinted with permission from ref 137. Copyright 2000 Marcel Dekker, Inc.)

in FTICR is based on a frequency measurement, and frequency and time are complementary properties. Therefore, the uncertainty in frequency can only be decreased by increasing the time of the measurement. High-performance FTICR is, therefore, a relatively slow form of mass spectrometry.

2. Quadrupole Ion-Trap Mass Spectrometry

It is convenient to divide the history of the quadrupole ion trap between events that transpired prior to the commercial introduction of quadrupole ion-trap mass spectrometers, which have recently been reviewed,[143] and those that have taken place since. Useful background information that spans both periods can be found in the book of March and Hughes.[144] Material that is largely focused on modern ion-trap mass spectrometry can be found in a recent three-volume series,[145] journal special issues,[146,147] and recent reviews.[148−154] While a two-dimensional quadrupole ion storage device has been used to acquire mass spectra,[155] by far most data to date have been acquired using the three-dimensional quadrupole ion trap. This review, therefore, concentrates on the performance of the three-dimensional ion trap.

Gas chromatography combined with the quadrupole ion trap has been a mainstream tool in mass spectrometry for over a decade in that there are several thousand in existence and they are used for routine analytical measurements. Liquid chromatography and capillary electrophoresis combined with electrospray/ion-trap mass spectrometry[152,153] can now also be regarded as a mainstream tool in that there are now several thousand in routine use as well. Figure 15 shows a side-view schematic of a commercially available electrospray/ion trap that incorporates two octopole ion guides for ion transport from the electrospray interface to the entrance endcap electrode of a Paul trap.[156] Many of the developments in quadrupole ion-trap mass spectrometry of the mid-1980s to early 1990s, such as mass range extension by resonance ejection (to roughly *m*/*z* 70 000), high resolution via slow scanning (to as high as 10^7 at *m*/*z* 600), MS^n (with *n* as high as 10), the use of tailored waveforms for ion manipulation, etc., are currently available, to varying degrees, in commercial instruments. While the figures of merit listed below pertain largely to the capabilities of currently available commercial instrumentation, the following discussion first emphasizes recent developments in quadrupole ion-trap instrumentation that are generally not yet available commercially.

The commonly used methods for acquiring mass spectra using a quadrupole ion trap, i.e., mass-selective instability and resonance ejection, involve scanning the applied voltages on the trap to sequentially eject trapped ions to an external detector and as such are inherently destructive. However, some of the earliest work with the Paul trap employed nondestructive ion detection, and several groups have revisited the use of nondestructive detection in quadrupole ion traps.[157−161] All such detection approaches involve an ion excitation step to induce ion oscillation close to one or more detection electrodes. Ion detec-

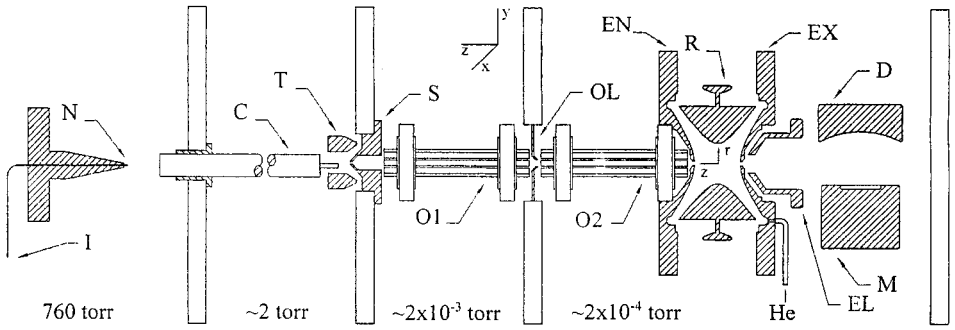

Figure 15. Side-view schematic diagram of a commercially available quadrupole ion-trap mass spectrometer. (Reprinted with permission from ref 156. Copyright 1999 Elsevier Science.)

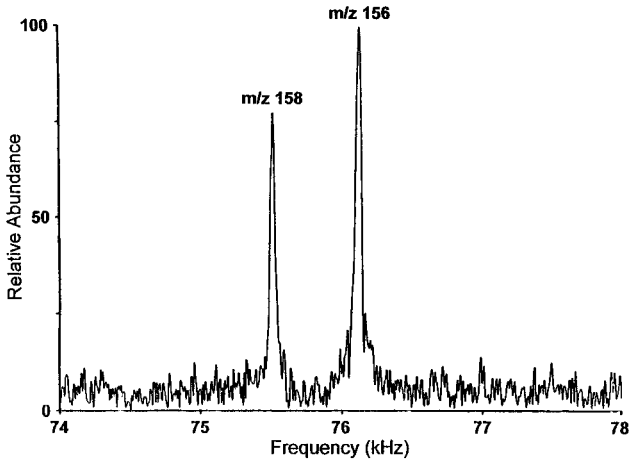

Figure 16. Fourier transform quadrupole ion-trap mass spectrum derived electron ionization of bromobenzene plotted over the molecular ion region. (Reprinted with permission from ref 160. Copyright 1999 John Wiley and Sons Limited.)

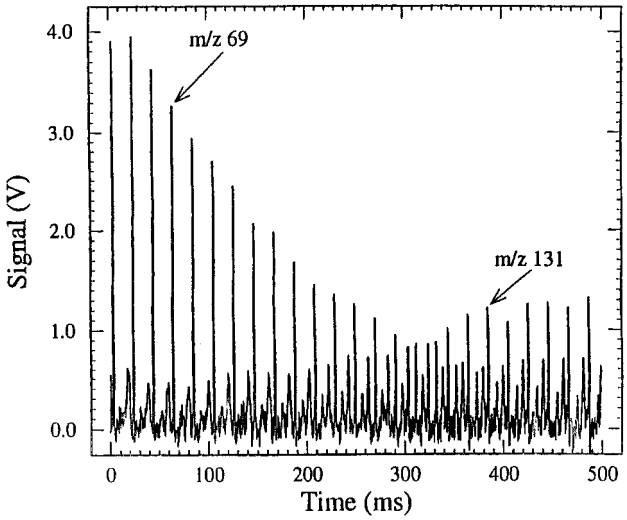

Figure 17. Series of 25 measurements of ions over an *m*/*z* range of 50−150 for ions derived from perfluorotributylamine and in the presence of helium bath gas. The ion population formed by the ionization step is transformed by ion molecule reactions over the time period of the 25 measurements. (Reprinted with permission from ref 158. Copyright 1995 American Chemical Society.)

tion can take place either during ion excitation or afterward, as in FTICR. The latter approach is not practical, however, using a bath gas pressure of 1 mTorr, as is usually present in most quadrupole ion-trap experiments, due to rapid collisional damping of the transient signal. As with FTICR, Fourier transformation of the time-domain transient image current signal obtained in a quadrupole ion trap is best performed at low pressures. Following up on initial studies by Syka and Fies,[162] Cooks and coworkers are developing the Fourier transform quadrupole ion-trap experiment.[159,160,163,164] This is a low-pressure nonscanning experiment employing broadband excitation and detection. Figure 16 shows the FT/Paul trap mass spectrum derived from electron ionization of bromobenzene[160] plotted over the molecular ion region. The in situ detection experiment in the Paul trap is complicated by the presence of the large radio frequency trapping potential applied to the ring electrode. Cross-talk from this voltage limits sensitivity. Cooks et al. dealt with this problem by use of pin electrodes inserted in apertures in the end-cap electrodes, which reduce the capacitance between the ring and the detection electrodes,[159] thereby reducing the cross-talk. Aliman and Glasmachers approached this problem by redesigning the ion-trap ring electrode.[161] At the 2000 American Society for Mass Spectrometry conference, Senko demonstrated a Fourier transform/linear ion-trap experiment with a resolving power of several thousands. A published account of this work is anticipated.

It is possible to perform in situ detection in the presence of relatively high bath gas pressure by detecting ions while they are undergoing excitation. To date, mass spectra obtained in this way have involved some sort of scanning experiment. The resolving power is not expected to be as high as that of an optimized Fourier transform experiment, but this approach does not compromise the advantages associated with the use of a bath gas. The combination of relatively high background pressures and the rapidly oscillating quadrupole electric field of the ion trap tends to collapse ion motion toward the center of the trapping volume. Therefore, no additional measures are required to effect "broad-band axialization" of ions undergoing large amplitude oscillations, as in FTICR (see section III.C.1). It is, therefore, possible, in principle, to remeasure ions relatively rapidly with a Paul trap using image current detection. Ion remeasurement in FTICR has also been described.[165] Figure 17 shows a series of 25 remeasurements over an *m*/*z* range of 50−150 for ions derived from perfluorotributylamine and in the presence of helium bath gas.[158] The ions were repeatedly

swept through resonance with a sine wave applied to the end-caps by increasing the amplitude of the radio frequency trapping potential. The CF_3^+ ion reacts with neutral perfluorotributylamine in the vacuum system to yield $C_2F_4^+$ (m/z 100) and $C_3F_5^+$ (m/z 131). The rate constant for this reaction is readily derived from these data. While the resolution in this experiment is relatively low, the remeasurement efficiency was observed to exceed 99%, suggesting that ion remeasurement in the Paul trap operated at high bath gas pressures is feasible as part of an overall ion-trap experiment.

Most ion-trap experiments have employed ionization methods in which ionization times are long relative to the frequencies of motions of the ions, thereby making them more or less continuous with respect to the time frame of ion capture. However, pulsed ion sources in which ions are formed or enter the ion trap over relatively short periods of time can benefit from strategies to improve ion capture efficiency over those obtained with continuous ion sources.[156] For example, several groups have recently reported that increasing the amplitude of the trapping potential on the time frame of ion entrance into the trapping volume can enhance trapping efficiency.[166−168] A recent report has discussed the role of phase angle on trapping efficiencies for laser-desorbed ions.[169] Trapping efficiencies as high as 39% have been reported for polypeptides produced in an external MALDI source,[168] suggesting that MALDI combined with an ion trap can play a role in a strategy for high-sensitivity sequencing of peptides. The use of an ion trap for the analysis of larger ions, such as those derived from MALDI of proteins, requires significant extension of the mass-to-charge range of the ion trap relative to those afforded by most commercial instruments. Mass-to-charge ranges of the order of 70 000 using resonance ejection with conventional ion traps has been demonstrated for cesium iodide cluster ions[170] and for proteins.[171] An ion trap using a reduced drive frequency was recently demonstrated to provide a mass-to-charge range of at least 150 000 for ions derived by MALDI.[172]

It is apparent that Paul traps are finding application in a variety of areas. This is due to one or more of several highly attractive figures of merit for ion traps operated in the mass-selective instability or resonance ejection modes, which are listed below.

mass resolving power	10^3-10^4
mass accuracy	50−100 ppm
mass range	1.5×10^5
linear dynamic range	10^2-10^5
precision	0.2−5%
abundance sensitivity	10^3
efficiency (transmission × duty cycle)	<1−95%
speed	1−30 Hz
compatibility with ionizer	pulsed and continuous
cost	low to moderate
size/weight/utility requirements	benchtop

While mass analysis procedures other than conventional mass-selective instability and resonance ejection involving ion ejection continue to be investigated,[173,174] the figures of merit listed here are based on mass-selective instability and/or resonance ejection. Mass resolving power at scan rates exceeding a few thousand m/z units per second is typically on the order of 1000 and, using resonance ejection, can be increased by reducing the scan speed. There is a tradeoff, therefore, between resolution and time, just as in FTICR, so that high-resolution scans are typically performed over narrow mass ranges. The mass-to-charge range cited here is that associated with the specialized low-frequency ion trap mentioned above. Commercial systems typically support an upper mass-to-charge limit of 650−6000. A recent report with a modified commercial instrument achieved a mass-to-charge range of 9000.[175] Mass accuracy is generally not as good with Paul traps as with FTICR, time-of-flight, or sectors, for example, but has been reported to be as good as 20−30 ppm with careful control of ion number and internal calibration.[176,177] The factors that affect mass accuracy in an ion trap have been discussed,[178] and progress has been made recently in understanding the origins of so-called "chemical mass shifts"[179,180] noted in early commercial development of quadrupole ion traps.[181] Linear dynamic range is ultimately limited by ion/ion interactions and can be quite low for low-abundance ions in the presence of much more abundant ions. However, selective ejection techniques devised to prevent accumulation of uninteresting ions have been demonstrated to ameliorate this problem. A common tactic to improve linear dynamic range is to control the number of ions in a systematic fashion, such as altering the ionization time. A combination of these tactics has shown that a linear dynamic range of at least as high as 10^5 can be achieved.[182] Precision and abundance sensitivity have received far less attention than some of the other ion-trap characteristics, so that the values reported here are derived from a relatively limited data set.[183] Efficiency is highly dependent upon how the ions are formed and/or injected into the ion trap, if the ionization is pulsed or continuous, and how long the ion manipulation/reaction/analysis steps take. In terms of speed, most quadrupole ion-trap experiments are completed well within 1 s, with the shortest being on the order of a few tens of milliseconds. This is slower than time-of-flight and faster than FTICR. The Paul trap has been interfaced successfully with a wide range of ionizers, but since it is typically operated in a pulsed mode, it is most compatible with pulsed ionization methods. Finally, much of the attraction to the Paul trap results from its practical advantages of relative low cost, size, weight, etc., and toleration of high background pressures. These characteristics lend the Paul trap to ready application as a benchtop or field instrument[5] with remarkable flexibility and performance.

3. Electrostatic Ion Traps

While the magnetic/electrostatic ion trap, as employed in FTICR, and the electrodynamic (Paul) ion trap are widely known and used, recent efforts on trapping ions in purely electrostatic fields for the purpose of mass spectrometry merit mention in this review. For example, Benner and co-workers[184−187] described a device for the purpose of trapping and

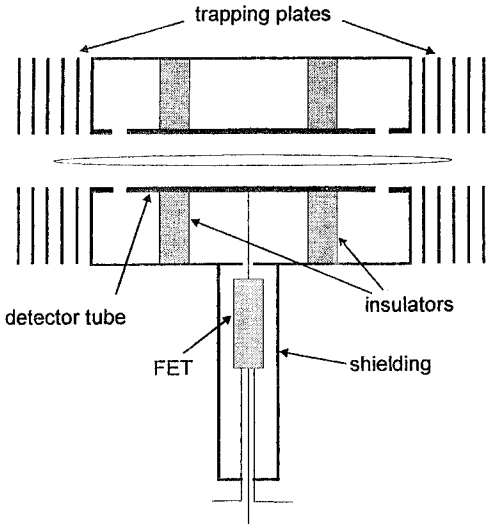

Figure 18. Side-view schematic of an electrostatic ion trap which is based on an opposing ion mirror design. (Reprinted with permission from ref 184. Copyright 1997 American Chemical Society.)

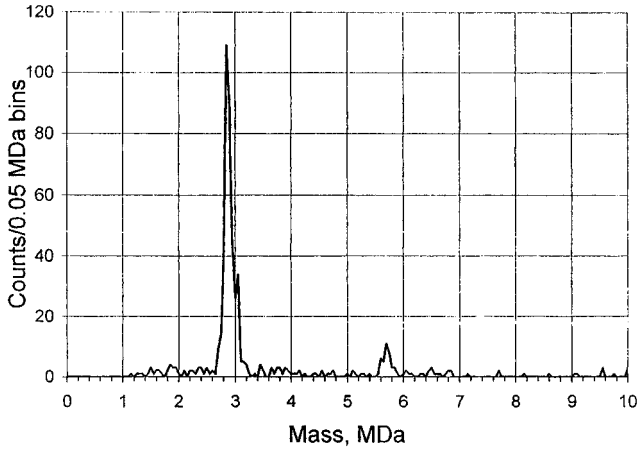

Figure 19. Histogram of signal versus mass from electrospray of pB*R*322, a 4.3 kilobase circular DNA molecule of a bacterial plasmid, acquired using the instrument depicted in Figure 18. (Reprinted with permission from ref 184. Copyright 1997 American Chemical Society.)

measuring the mass, charge, and velocity of individual high-mass multiply charged ions derived from electrospray. Figure 18 shows a side-view schematic of the analyzer/ion trap which is based on an opposing ion mirror design. A single high-mass multiply charged ion is admitted into the ion trap by temporarily lowering the potential on the ion entrance mirror. The trapped ion is repeatedly detected by the image current induced as it passes back-and-forth through a coaxial tube placed between the mirrors. The charge on the ion is determined by the amplitude of the induced voltage measured as it enters and exits the detection tube. The time between the signals observed upon entrance and exit are used to determine the ion velocity. Mass can then be determined from the measured charge and velocity along with the kinetic energy at which the ion was injected into the ion trap. Figure 19 shows illustrative data obtained using this approach. It shows a histogram of signal versus mass from electrospray of pB*R*322, a 4.3 kilobase circular DNA molecule of a bacterial plasmid. The major peak in the histogram falls at 2.88 MDa, which is the expected mass for an ion in which sodium is the counterion associated with the phosphodiester linkages. The peak at twice the mass is presumed to be the dimer ion. The mass resolving power of the device, which is expected to be somewhat better than 25, is comparable to or superior to gel separation methods, which are currently used to size such large molecules. The gas-phase measurement is, of course, much faster as the data of Figure 19 were collected in several minutes.

The electrostatic ion trap of Benner shares commonality with the multiturn time-of-flight devices discussed above in the time-of-flight section in that electrostatic deflection of ions is used to increase either path length or number of measurements and the measurement of ion flight time is central to determining mass. Another form of mass spectrometry involving electrostatic trapping, based on the Kingdon trap,[188−191] is more closely analogous to the magnetic and electrodynamic ion traps in that the measurement of ion frequency is central to mass measurement. (It must be recognized, of course, that ion velocity and frequency are related in the trap described by Benner.) Makarov recently described a mass spectrometer, referred to as the Orbitrap, wherein ions trapped in an electrostatic trapping cell can be mass analyzed by image current measurements followed by Fourier transformation of the time domain signal transient, as is commonly done in FTICR, or by a mass-selective instability scan, in analogy with a common Paul trap technique for mass analysis.[191] Both approaches represent an ion frequency measurement. Too little information has thus far been presented to provide a quantitative listing of the figures of merit of this mass spectrometry approach. However, the initial results are very promising with respect to resolution, mass accuracy, mass range, etc. Thus far, data have been described using laser ablation and MALDI.

D. Ancillary Technologies

1. Radio Frequency Transmission Devices

Radio frequency transmission devices (e.g., quadrupoles, hexapoles, octopoles,[192,193] and the "ion funnel"[194−196]) are playing increasingly important roles as transmission elements, reaction regions, and storage devices in modern mass spectrometers. Radio frequency quadrupoles originally found widespread use as collision regions in triple-quadrupole tandem mass spectrometers.[197] rf-multipoles, including hexapoles and octopoles as well as quadrupoles, continue to find widespread use as reaction regions in tandem mass spectrometers. A significant recent development in triple-quadrupole tandem mass spectrometry is the demonstration that relatively high target gas thicknesses in a collision quadrupole (e.g., 4−16 mTorr in a 15 cm set of rods) can yield improved MS/MS efficiency and resolution in the second quadrupole,[198] particularly at high mass-to-charge. This improvement stems from collisional focusing in the off-axis directions and a reduction in the axial energy spread of the ions.[199−202] These characteristics have proved to be particularly important in coupling

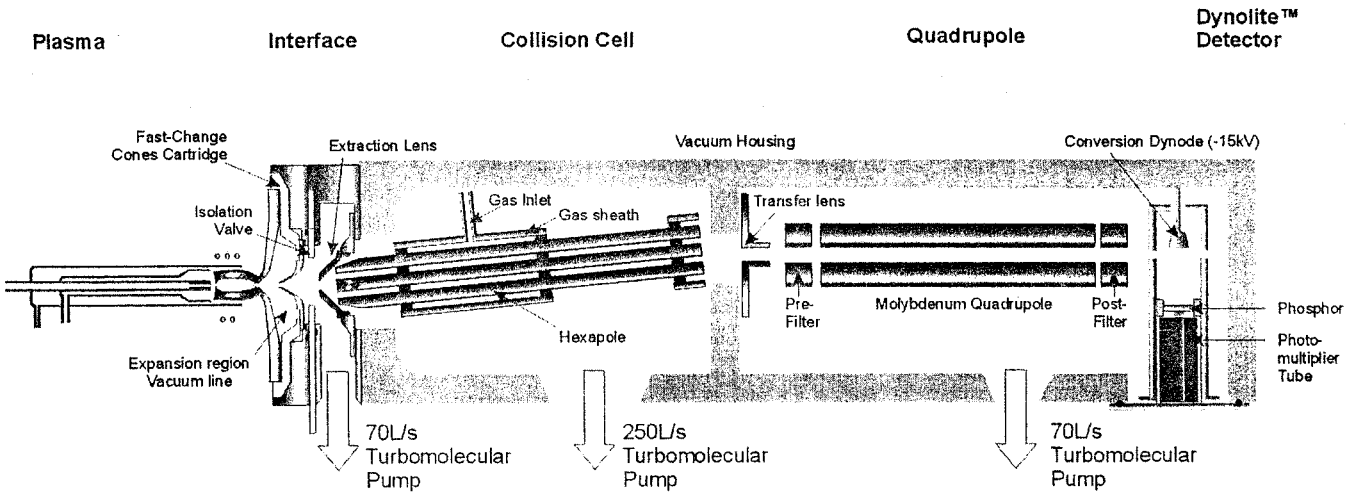

Figure 20. Side-view schematic diagram of an ICP mass spectrometer that employs an off-axis hexapole reaction/transmission region prior to a quadrupole mass filter. (Reprinted with permission from ref 210. Copyright 2000 Elsevier Science.)

electrospray and MALDI with orthogonal acceleration time-of-flight mass spectrometry.[16]

The use of relatively high pressures with rf-multipoles has led to the incorporation of an axial electric field to effect ion acceleration or drift in the axial dimension.[203−205] The incorporation of a linear drift field imposed along the direction of ion motion yields a device that allows for drift measurements with radial confinement.[203] Furthermore, the use of a drift field can eliminate deleterious hysteresis effects in triple quadrupole tandem mass spectrometry arising from slow moving ions in the collision region.[204] The selective activation of ions by placing them close to a stability boundary such that rf-heating effects come into play has also been demonstrated in an rf-only multipole with an axial drift field.[205]

In addition to use as transmission devices and collision regions for collisional activation, rf-only multipoles are increasingly seeing use as reaction regions for ion/molecule reaction studies.[206] An important analytical application has been in the use of reaction regions in ICP/MS.[207−209] rf-only Collision cells with reactive gases, such as hydrogen, have been placed between the mass analyzer and the atmosphere/vacuum interface of the inductively coupled plasma. Exoergic ion/molecule reactions, such as the charge-transfer reaction involving ionized argon with hydrogen, allows for removal of undesired ions prior to mass analysis. In the case of argon-based ICP/MS, for example, the benefits are 2-fold: (1) the reactions remove potential isobaric interferences and (2) the charge exchange reaction involving argon produces hydrogen ions that fall below the low mass-to-charge cutoff of the multipole thereby reducing space charge associated with the very bright argon beam. Figure 20 shows a side-view schematic diagram of an ICP mass spectrometer that employs an off-axis hexapole reaction/transmission region prior to a quadrupole mass filter.[210]

rf-"transmission" Devices are also being used as storage devices prior to ion injection into a mass analyzer. Ions are trapped in the radial plane by the radio frequency field and by an electrostatic potential in the axial dimension.[211−216] (For the purpose of this review, if the ion storage device is also used to effect mass selection it is considered a hybrid tandem mass spectrometer. These devices are discussed below under "hybrids".) Several groups now employ an ion storage period in ion guides leading to an FTICR instrument prior to admitting ions into the ICR cell.[217−224] The prestorage event is useful for collisionally cooling the ions prior to admission into the ICR cell and has been shown to enable improvement in FTICR duty cycle with electrospray ionization and the rate at which mass spectra can be recorded.[217] It has also been noted recently that dissociation can take place within these storage devices.[218−222] This phenomenon can be useful in structural studies but must be taken into consideration when choosing ion accumulation conditions for relatively weakly bound species.[221] Ion storage external to the ICR cell has also been used to study ion/molecule reactions, such as hydrogen/deuterium exchange.[223] These examples illustrate what has become a trend in mass spectrometry instrumentation development. A variety of technologies are being brought to bear in a single instrument to improve analytical performance and to expand the range of chemistry that can occur within the instrument.

2. Ion Mobility Devices

The measurement of the mobilities of ions in gases in analytical chemistry has a history that dates back into the 1960s.[225] The combination of an ion mobility device with a mass spectrometer was reported roughly 30 years ago.[226] The combination of ion mobility with mass spectrometry was largely devoted to studies directed at understanding the chemistry giving rise to the ions observed in the ion mobility spectra. In recent years, however, attention has been re-focused on the combination of ion mobility with mass spectrometry both for fundamental ion chemistry and structure studies[227−232] and for analytical applications.[233−237]

The separation of ions on the basis of ion mobility for subsequent mass spectrometry has significant

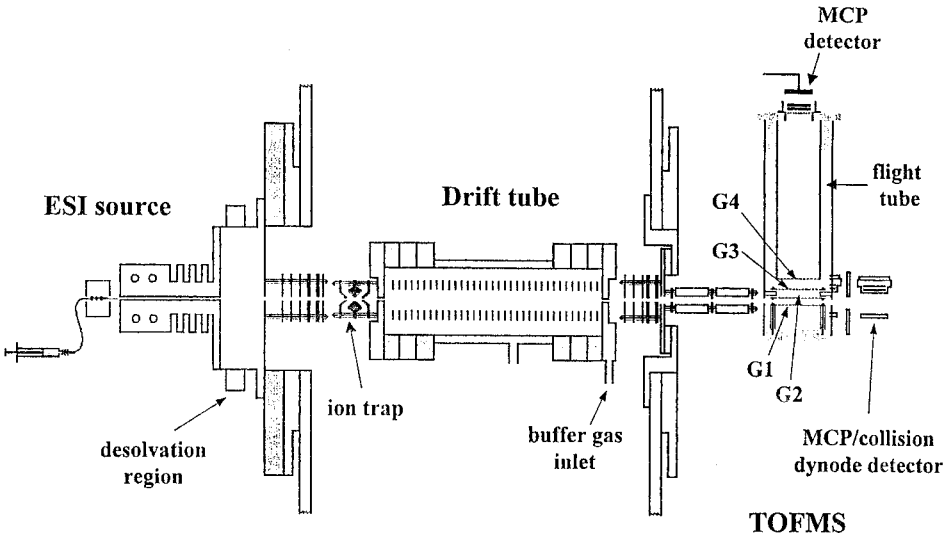

Figure 21. Schematic diagram of an instrument that combines an electrospray ionization source, a three-dimensional quadrupole ion trap for ion accumulation, an ion drift region, and an orthogonal acceleration time-of-flight mass spectrometer. (Reprinted with permission from ref 233. Copyright 1999 American Chemical Society.)

potential for the rapid analysis of complex mixtures of ions. Clemmer et al. demonstrated this potential using the instrument, and others like it, shown in Figure 21. This figure shows a schematic diagram of an instrument that combines an electrospray ionization source, a three-dimensional quadrupole ion trap for ion accumulation, an ion drift region, and an orthogonal acceleration time-of-flight mass spectrometer.[233] The ion drift times are relatively slow with respect to the spectral generation rate of the time-of-flight mass spectrometer such that mass spectra can be acquired at many points along the ion mobility spectrum. This experiment is analogous to other combined separation/mass spectrometry techniques with the exception that, in this case, the separation takes place much more quickly in the gas-phase than it does in, for example, capillary electrophoresis or liquid chromatography. Figure 22 shows illustrative data forthcoming from this apparatus in two-dimensional format (a third dimension of information, viz. abundance, is also acquired). It was derived from a mixture of ions produced by electrospray from the digestion of cytochrome *c*. Mobility-resolved mass spectra are obtained from this experiment, which significantly increases the informing power of the analysis over either ion mobility or mass spectrometry alone. Data for a much more complex peptide mixture have been reported more recently using a similar instrument but without the ion-trapping step.[234]

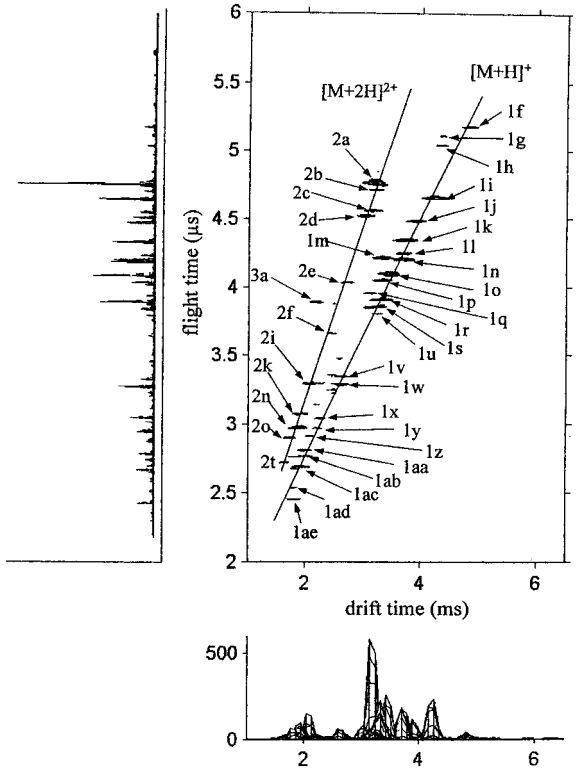

Figure 22. Data forthcoming from a mixture of ions produced by electrospray of the digestion products of cytochrome *c* using the apparatus of Figure 21. (Reprinted with permission from ref 233. Copyright 1999 American Chemical Society.)

Most ion mobility experiments are conducted under relatively low electric field conditions. However, it has been shown that ions can be separated and focused at atmospheric pressure on the basis of differences in ion mobilities at high field strengths. The technique is based upon the application of an asymmetric waveform transverse to the flow of ions. If an ion's mobility at high field strength differs from its mobility at low field strength, it will experience a net deflection during the course of the asymmetric wave cycle. A dc potential applied across the electrodes can compensate for this deflection but only for ions with a particular difference between the high and low field mobilities.[237−243] This forms the basis for dispersing ions at atmospheric pressure in a dimension orthogonal to the axial ion motion. A selected population of ions can then be admitted into a mass spectrometer. This represents a new capability, and several applications relevant to biological and environmental measurements have been reported.[239−242] It has also been demonstrated that selected mobility ions can also be stored at atmospheric pressure by using an appropriate electrode geometry.[243] It is likely that a number of new analytical

mass spectrometry applications will be developed to take advantage of these capabilities.

III. Tandem Mass Spectrometry (MS^n) Technologies

By definition, a true MS^2 or MS/MS experiment must be able to define the mass-to-charge relationship between a precursor ion and a product ion.[6−8] It is clear, therefore, that the mass analyzer characteristics discussed above must be considered for both the precursor ion(s) of an MS/MS experiment as well as for the product ions. However, there are several additional considerations that go into determining the relative strengths of the various forms of tandem mass spectrometers. Those highlighted herein include MS/MS efficiency, MS^n capability (where $n > 2$), and the range of chemistry that can occur between mass analysis stages. MS/MS efficiency is defined as the fraction of precursor ions that can be converted to measured product ions. MS/MS efficiency is determined by the extent to which a reaction can be driven, that is, the fraction of precursor ions that are converted to product ions, as well as the extent to which product ions are collected and transmitted to the detector. MS^n capability is an obvious reference to the capacity for performing experiments with multiple reactions steps interspersed with stages of mass analysis. There are a variety of scan types that can be executed to determine genealogical relationships between ions, and the number of possible scan types is a function of n. A systematic delineation of scan types in MS/MS and MS^n has been provided.[244] MS^n experiments are mostly associated with ion-trapping instruments but can also be performed with beam-type instruments with more than two discrete mass analyzers. By far the most complex issue associated with instrumentation for tandem mass spectrometry is the range of chemical reactions that can occur between stages of mass analysis. Because this topic is not addressed elsewhere in this issue, it is discussed here. The intent is not to exhaustively review recent studies associated with each reaction type. Rather, it is to provide a brief overview of the various reaction types and the conditions that a tandem mass spectrometer must be able to establish to allow for the reactions to occur between stages of mass analysis. No single form of tandem mass spectrometer is amenable to the study of all reaction types. Therefore, this issue can become an important consideration in choosing the most appropriate form of MS/MS for a particular application. After having presented the salient considerations associated with the various reaction types, work is reviewed for the various classes of tandem mass spectrometers within the context of the figures of merit already presented for the individual mass analyzers, as well as the additional figures of merit for tandem mass spectrometers introduced here.

A. Reactions in Tandem Mass Spectrometry

For a reaction to yield useful information, it must result in a readily measurable change in going from reactants to products. In MS/MS there is either a change in mass or charge (although some tandem mass spectrometers are sensitive to a change in kinetic energy). Analytically useful reactions that result in changes in mass and/or charge fall into one of two categories, viz., endoergic reactions and exoergic reactions. In the case of endoergic reactions, clearly there must be means for providing the energy necessary to drive the reaction. The nature of the instrumentation is a factor in determining which means for supplying the necessary energy are practical for a given tandem mass spectrometer. In the case of both reaction types, the kinetics of the reactions of interest are important considerations. The time frame associated with the instrument defines the lower limit to reaction rates that can be studied.

1. Endoergic Reactions

a. Dissociation. By far the most important reaction in MS/MS has been unimolecular dissociation. It is by this reaction that mass spectrometry has been able to provide information about the primary structures of polyatomic molecules. The energy needed to induce fragmentation is sometimes provided by the ionization reaction itself, as in the case of the MALDI 'post-source' decay experiment,[245−247] for example. Indeed, the early tandem mass spectrometry experiments of the late 1960s and 1970s were largely focused on 'metastable' ions,[248] viz., ions with sufficient energy to fragment but only at rates too low for the fragmentation to take place in the ion source. However, the vast majority of MS/MS experiments today employ some means for adding energy to a precursor ion after its formation for the purpose of inducing fragmentation. The major approaches for ion activation include energetic collisions with a neutral target gas[249−252] or surface,[253−255] referred to herein as collisional excitation approaches, and photoexcitation.[256,257]

For each of the collisional and photoexcitation categories, a range of conditions has been used that allows for a degree of flexibility in how fast energy is imparted into a precursor ion. Activation times can range from less than 10^{-15} s to greater than 1000 s depending upon the activation approach used and the tandem mass spectrometer time frame. It is instructive to consider the various activation techniques by listing them on an activation time scale, as shown in Figure 23.[258] In considering this figure, it is apparent that there are three broad categories. The fastest activation methods ($<10^{-10}$ s) involve a single activation event during which relatively little chemistry can occur. Most of the response of the ion to the rapid input of energy takes place after the activation event itself. In the intermediate case, as with the collisional excitation approaches using conditions in which multiple collisions are likely, unimolecular reactions can and do take place during the activation process. That is, fragmentation and/or rearrangement reactions can occur between activating collisions. However, the time frame over which the multiple activation process occurs is typically too short for ion deactivation processes to take place to an appreciable extent. In the case of activation methods that proceed over periods of tens of milliseconds and greater, it is

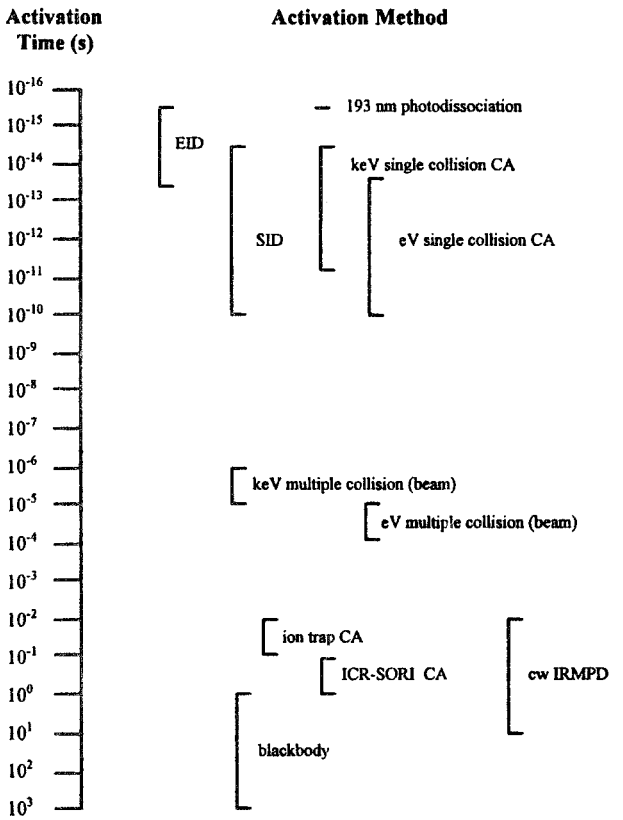

Figure 23. Chart of commonly used activation methods for tandem mass spectrometry listed according to the time frame of activation. (Reprinted with permission from ref 258. Copyright 1997 John Wiley and Sons Limited.)

possible for ion deactivation processes, such as infrared emission and collisional de-excitation to occur. In fact, with these activation methods it is possible to achieve a steady-state condition in which ion activation and deactivation rates are equal. Under these conditions it is frequently the case that ion dissociation only occurs while the activation process is ongoing.

To employ a particular form of activation method, the time frame of the tandem mass spectrometer must be at least as great as the time frame over which the activation process takes place. Furthermore, if the products resulting from the dissociation reactions that ensue from activation are to be observed, the observation window of the tandem mass spectrometer must be long enough to allow for the dissociation reactions to proceed. The observation window of the spectrometer, therefore, defines the unimolecular dissociation rate range amenable to study. This can be a very important consideration for a given application. For example, the dominant fragmentation processes can vary dramatically over a rate range of $10^{15}-10^{-3}$ s^{-1}. As a consequence, the value of the information obtained from an MS/MS experiment can hinge upon the reaction conditions used to induce fragmentation. In the case of collisional excitation, the tandem mass spectrometer must allow for the precursor ion to be accelerated to suprathermal energies. For some tandem mass spectrometers, as discussed below, it is most convenient to accelerate the ions to several kiloelectronvolts whereas for others only ion acceleration to some tens of electronvolts is practical.

b. Ionization. While the vast majority of MS/MS studies rely on inducing fragmentation, as reflected by a change in mass, some experiments rely on inducing a change in charge via an endoergic ionization reaction, usually induced by an energetic collision. These reactions involve the ejection of one or more electrons and have been used to study both positive and negative ions, as well as neutral species. In the case of positive ions, the process is sometimes referred to as charge stripping whereby a singly charged ion is converted to a doubly charged ion.[259] In the case of singly charged negative ions, information can be obtained only by removing two electrons to form a positive ion. (Removal of one electron results in a neutral molecule which, in general, is not amenable to mass spectrometry.) This process is sometimes referred to as charge inversion.[259] Application of collisional ionization to neutral species takes place in the so-called "neutralization−reionization" experiment[260,261] whereby a fast ion is first neutralized and the fast neutral species so formed is then subsequently re-ionized. In all cases, the collisional ionization reaction is expected to occur on the time frame of the collision and requires kiloelectronvolt energy ions to make the cross-section for the ionization reaction large enough to produce measurable yields.

2. Exoergic Reactions

While most tandem mass spectrometry experiments continue to rely on endothermic reactions to obtain the desired information regarding the precursor ion, the use of exoergic reactions continues to expand. These types of reactions are attractive in part because no external means for excitation, such as a laser, are needed and, as a rule, ion acceleration is not only unnecessary but undesirable. By far the most widely studied reaction type in this category is thermal energy ion/molecule reactions. However, relatively recent MS/MS studies involving the reactions of multiply charged ions derived from electrospray with oppositely charged ions or with electrons have been reported, and both have been shown to hold considerable analytical potential as reactions in MS/MS.

a. Ion/Molecule Reactions. The study of ion/molecule reactions has long been a major activity of the ion chemistry community, and the topic is being reviewed in this issue.[262] Tandem mass spectrometry is a major tool in this line of work, which is ordinarily devoted to the study of fundamental issues in the chemistry of gaseous ions. However, ion/molecule reactions are increasingly used as analytical probes within the context of a tandem mass spectrometry experiment, and their use can be expected to grow in the coming decade. In contrast with the conditions that must be established for the study of highly endoergic reactions discussed above, ion acceleration is generally not desirable for the observation of reactions that require the formation of a relatively long-lived complex, through which most exoergic ion/molecule reactions proceed. Furthermore, since by definition an ion/molecule reaction is a collisional process, the macroscopic reaction rate is a product

of the microscopic ion/molecule rate constant and the number density of the reactant. Therefore, the extent to which the precursor ion can be converted to the product ion(s) in a given tandem mass spectrometer depends on the number density of the neutral reactant that the instrument can tolerate without compromising another aspect of the experiment, such as mass resolving power, and the observation time window. (This is in direct analogy with collisional activation using a gaseous target with the exception that the bimolecular rate constants are for exoergic versus endoergic reactions.) As discussed below for the individual tandem mass spectrometer types, the utility of a tandem mass spectrometer for studying ion/molecule reactions is determined both by the facility with which ions of low translational energy can be delivered to the reaction region and the time window over which the reactions can proceed.

b. Ion/Ion Reactions. The tendency of electrospray to form multiply charged ions from large multifunctional macromolecules opens up the possibility for exoergic reactions involving multiply charged ions with ions of opposite polarity without resulting in mutual neutralization. A number of examples where such reactions have been used within the context of an analytical tandem mass spectrometry experiment have recently been reported.[263] Like exoergic ion/molecule reactions, the rate constants for exoergic ion/ion reactions are highest at low relative translational energies. Therefore, to maximize the rate of reaction, it is desirable to avoid acceleration of either ionic reactant. Furthermore, since the phenomenological reaction rate is a function of both the rate constant and the number densities of the ionic reactants, it is important to maximize the spatial overlap of the ionic reactants in addition to maximizing ion densities. This consideration, of course, is not usually important with *ion/molecule* reactions because the neutral reagent can ordinarily be flooded at constant number density over the entire volume of the reaction region.

c. Ion/Electron Reactions. In analogy with the ion/ion reactions just mentioned, electrospray allows for the possibility for studying ion/electron reactions involving slow ions and electrons. Several examples of such studies have recently been reported[264−269] which have important analytical implications. Like exoergic ion/ion reactions, the kinetics of exoergic ion/electron reactions are maximized at low relative translational energies. Furthermore, it is critical that the instrumentation allow for spatial overlap between the positive multiply charged ions and the low-energy electrons.

B. Tandem-in-Space Devices

1. Sector Tandem Mass Spectrometry

The first analytical tandem mass spectrometers were two-sector instruments comprised of a magnetic sector and an electric sector arranged in either forward or reverse geometry. MS/MS data were collected either via linked scanning techniques,[270] for dissociation reactions that preceded the sectors, or by scanning the electric sector in reverse geometry instruments, thereby analyzing products formed between the sectors from mass-selected precursor ions. The product ions arose from dissociation of metastable precursor ions or precursor ions rendered unstable via kiloelectronvolt energy collisions with a target gas admitted into the relevant reaction region. Relative to most of the tandem mass spectrometers in use today, these instruments suffered from poor MS/MS efficiency, which was often less than 0.1%, and either poor precursor ion resolution or poor product ion resolution. Nevertheless, many of the basic concepts that underlie MS/MS were first demonstrated with these instruments. As the power of tandem mass spectrometry became apparent, efforts went into developing instruments specifically designed for tandem mass spectrometry, such as the triple-quadrupole instrument discussed below. Further development of sector-only instrumentation was also pursued with the intent to improve precursor and/or product ion resolution, extend the MS^n capability beyond $n = 2$, or improve duty cycle. These developments were manifested in three-, four-, and five-sector instruments and instruments with array detectors. Despite the improvements that resulted from these developments, the rapid growth of MS/MS has largely been accommodated by competing technologies. For many of the most widely practiced applications of MS/MS, other technologies are either competitive or superior in the performance figures of merit and are often less expensive. The MS/MS efficiency of most sector tandem mass spectrometers is poorer than most other MS/MS technologies. This is in part due to the fact that the sectors have relatively narrow ion acceptance characteristics. Reactions between sectors must be driven at rates that exceed 10^5 s^{-1}. Target gas pressures necessary to dissociate a large fraction of precursor ions typically lead to sufficient scattering and charge transfer to preclude high MS/MS efficiency. Furthermore, instruments that require scanning to record the product ion spectrum, as is the case with most sector tandem mass spectrometers, suffer from poor duty cycle. Nevertheless, the collisional ionization reactions mentioned above are only observed at kiloelectronvolt collision energies. Despite this unique capability, the fraction of applications in analytical tandem mass spectrometry that are addressed by multisector tandem mass spectrometers is relatively small.

For most analytical applications of sector tandem instruments, ion activation is limited to kiloelectronvolt collisional activation using gaseous targets. However, relatively recent reports have described modifications designed to enhance MS/MS flexibility for sector instruments. For example, Schey et al. described the modification of the collision region of a four-sector tandem mass spectrometer to allow for surface-induced dissociation studies.[271] The "in-line" SID collision region provides an alternative to high-energy collisional activation with a gaseous target, which has limitations in the activation of high-mass ions due to low center-of-mass collision energies. A different modification to the collision region of a four-sector tandem mass spectrometer has been described

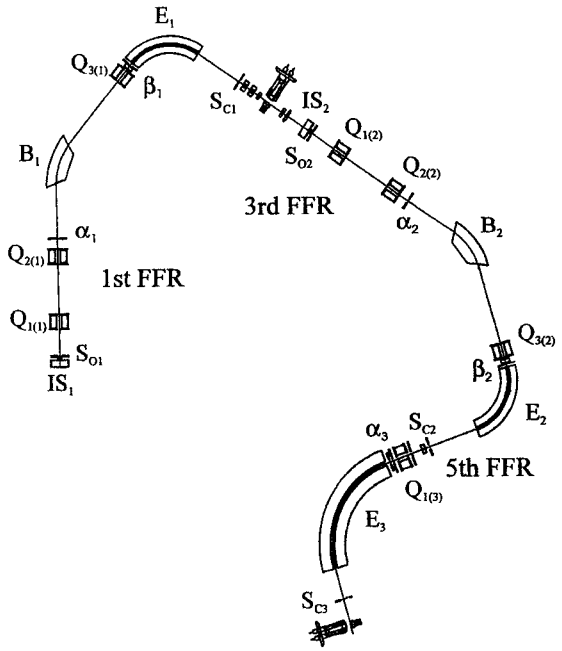

Figure 24. Side-view schematic diagram of a five-sector instrument designed for MS^n studies. (Reprinted with permission from Elsevier Science, ref 276. Copyright 2000 American Society for Mass Spectrometry.)

by Cheng et al. which was designed to allow for collision energies as low as a few electronvolts.[272] An electron ionization filament to allow for ionization of neutral species present in the collision region was also incorporated. These modifications expanded the range of reactions amenable to study to include ion/molecule reactions, low- and high-energy collisional activation, and ionization of neutral fragments formed via dissociation.

While most mass spectrometry experiments are devoted to the measurement of ion mass and/or abundance, the answer to some of the important scientific questions that a mass spectrometry experiment is intended to address is not manifest in mass and/or abundance alone. The angles and energies of collision products or dissociation products can provide important information on the energetics and dynamics of a reaction, for example. The measurement of such parameters is often the primary objective of beam experiments, and sectors are important tools in this type of work. For example, translational energy spectroscopy[273,274] is an area in which sector instrumentation continues to play a central role. The measurement of ion translational energy is important in atomic and molecular physics research and continues to play an important role in the measurement of kinetic energy release distributions upon ion dissociation,[2] which provide information about the potential energy surface of the dissociation reaction. The importance of translational energy release measurements is not restricted to small systems, however, as they can provide useful insights into fundamental aspects of the dissociation of ions of biological origin as well.[275−277] Figure 24 shows a schematic diagram of a five-sector instrument that has been used to study the dissociation reactions of several multiply charged polypeptide ions.[276] Figure 25 compares spectra obtained from the dissociations of metastable doubly protonated angiotensin II in the third field-free region using a constant B/E linked scan (Figure 25a) and in the fifth field-free region using a mass-analyzed ion kinetic energy scan (Figure 25b). The linked scan shows superior resolving power for the product ions, while the electric sector scan provides kinetic energy release information.

Because sector tandem mass spectrometers are comprised of at least two scanning mass analyzers, sector instruments can perform all combinations of "scan types". These include, for example, the precursor ion scan, the neutral loss scan, and the product ion scan. The performance characteristics of the first and second stages of mass analysis in a sector tandem mass spectrometer depend, in part, upon the instrument geometry and the scan mode. Scans at constant B/E ratio in a two-sector instrument comprised of a magnetic sector and an electric sector, for example, sacrifice precursor ion resolution for product ion resolution. When the product ions are analyzed by an electric sector scan, on the other hand, the resolving power for the product ions is generally much poorer than the resolution at which the precursor ion was selected. The MS/MS efficiency of most sector instruments is relatively low due to difficulty in driving reactions to completion without severely reducing the collection efficiency of the product ions. The capability for conducting MS^n experiments is ultimately limited by the number of reaction regions (also referred to as field-free regions in most sector instruments). In general, the range of chemistry that can occur in sector tandem mass spectrometry is limited to kiloelectronvolt energy collisional activation, UV photodissociation, and metastable ion decompositions. Reaction rates must exceed roughly 10^5 s^{-1}. While many reactions in tandem mass spectrometry proceed at lower rates, sector instruments are among a minority of MS/MS tools capable of collisional activation at kiloelectronvolt collision energies and of studying the endoergic ionization reactions discussed above.

2. Multiple Quadrupole Devices

The triple-quadrupole tandem mass spectrometer,[197] which is comprised of a mass filter for precursor ion mass selection, an rf-only quadrupole collision cell, and a mass filter for product ion mass analysis, has been the workhorse tandem mass spectrometer for the past two decades. Since the initial introduction of commercial systems, there have been several evolutionary changes that make these devices well-suited to many analytical applications. In some cases, the quadrupole collision cell has been replaced by either a hexapole or octopole collision cell making the term "tandem quadrupole instrument" more generally applicable to this class of instruments. In fact, most of the major developments in tandem quadrupole mass spectrometry, aside from improvements in ion sources and interfaces, have been made with collision regions. Developments in this area are discussed above in the radio frequency transmission devices section.

A relatively recent development has been in the application of triple-quadrupole instruments to the

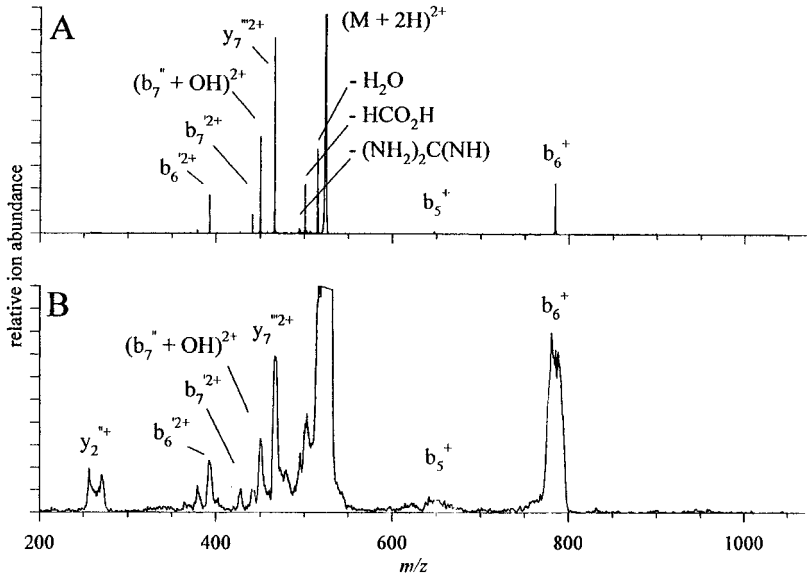

Figure 25. Comparison of spectra obtained from the dissociations of metastable doubly protonated angiotensin II (a) in the third field-free region using a constant B/E linked scan and (b) in the fifth field-free region using a mass analyzed ion kinetic energy scan. (Reprinted with permission from Elsevier Science, ref 276. Copyright 2000 American Society for Mass Spectrometry.)

measurement of collision cross-sections.[278−283] The approach works on the basis of differential energy loss in the collision quadrupole as a result of different collision cross-sections. While this measurement does not necessarily involve a chemical reaction, in the sense that there is no fragmentation or other reaction resulting in a change in mass or charge, it does involve a process that results in a loss of kinetic energy. This information can then be modeled to yield a collision cross-section. The triple-quadrupole approach and ion mobility measurements are the two main methods now used to obtain information about the shapes of gaseous ions.

True MS^3 experiments have been conducted with multiquadrupole instruments that involve at least three mass filters. These devices, which typically include quadrupole collision regions between mass filters and are, therefore, referred to as pentaquadrupole instruments,[284,285] have been used primarily for fundamental ion chemistry studies. Like the triple-quadrupole instrument, the pentaquadrupole is amenable to computer control and can be scanned at high rates without hysteresis problems common to magnetic sector instruments. Therefore, the MS^3 scan types can be readily implemented[286] thereby providing a flexible tool for mixture analysis and ion chemistry studies.

Tandem quadrupole instruments are particularly well-suited to linked scanning to produce, for example, precursor ion spectra and neutral gain/loss spectra in addition to the common product ion spectra. A procedure for identifying multiply charged ions in electrospray mass spectra has also been described recently.[287] In general, it is more facile to produce data requiring the coordinated scanning of two analyzers with a tandem quadrupole device than with a sector tandem mass spectrometer. The MS/MS efficiencies of modern tandem quadrupole instruments can be very high, with very little ion loss taking place either in the collision region or in transmission between multipole devices. The duty cycle for full-scan product ion spectra is relatively low but, of course, can be 100% for single reaction monitoring where a single precursor to product transition is monitored. As with sector instruments, the number of stages of mass spectrometry is generally limited to the number of collision regions, although pseudo-MS^3 experiments can be performed with triple-quadrupole instruments (or any instrument with only one collision region between mass analyzers) by inducing fragmentation prior to the first mass filter.[288] Furthermore, the mass-selective dissociation of an ion as it passes through an rf-only quadrupole via acceleration at its secular frequency of motion orthogonal to the axial direction has been demonstrated[289] in a tandem quadrupole time-of-flight instrument. The mass-selective nature of the acceleration makes accessible genealogical information from dissociation reactions that might not otherwise be obtained in a conventional beam-type tandem mass spectrometer. Assuming that the collision region is not used to store ions, that is, the collision region is used in the usual transmission mode, the range of chemistry is limited to reactions with rates that exceed roughly 10^4 s^{-1}. Ion kinetic energies are typically less than 100 eV for singly charged ions, which limits collisional activation to low energies. Conditions that make multiple collisions likely are usually employed. Therefore, the period over which activation can occur is equal to the transit time through the collision region. The use of low precursor ion kinetic energies and reactive neutral species in the collision region makes the study of low-energy ion/molecule reactions possible, in contrast with most sector tandem mass spectrometers. However, the cross-sections for the endoergic collisional ionization reactions are too low for these reactions to be amenable to study at typical tandem quadrupole collision energies. Furthermore, low-energy collisional activation may not be able to access some

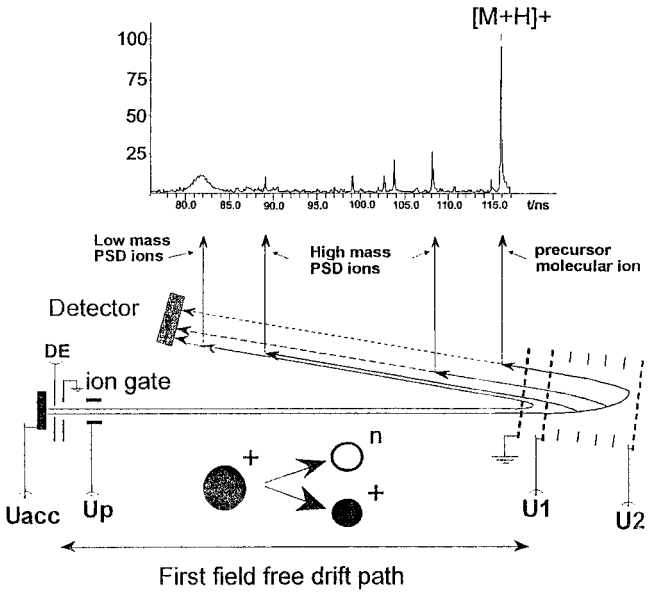

Figure 26. Schematic representation of the post-source decay process associated with matrix-assisted laser desorption ionization. (Reprinted with permission from ref 246. Copyright 1999 American Chemical Society.)

desirable structurally useful fragmentation reactions that can be accessed with kiloelectronvolt energy collisions. In this case, UV photodissociation might be employed but the efficiency of this approach is low in tandem quadrupole instruments of conventional geometry. On the other hand, the collisional energy transfer distribution at low collision energies can be highly sensitive to the magnitude of the collision energy. This has given rise to experiments referred to as "energy resolved mass spectrometry" wherein MS/MS data are collected systematically as a function of collision energy.[290] Tandem quadrupole devices are also amenable to use of surface-induced dissociation. In fact, many SID studies have been conducted using specially built tandem quadrupole instruments.[254,255]

3. Tandem Mass Spectrometry with Time-of-Flight

As time-of-flight continues to expand into new areas as a tool for mass analysis of atomic and molecular ions, it has also been developed further for use as an ion structural tool. For example, the phenomenon of post-source decay (PSD) in MALDI has been exploited for polypeptide structural information by taking advantage of the reflectron, as described by Kaufmann et al.[245] The principle of MALDI/PSD/TOF is illustrated in Figure 26[246] and involves fragmentation of ions in the flight tube due to energy imparted by the MALDI process and focusing of the product ions by the reflectron. This technique has recently been reviewed by Chaurand et al.[246] and Spengler.[247] The linear-field reflectron brings regions of the product ion spectrum to a focus at the detector, thereby requiring the full product ion spectrum to be pieced together by acquiring data using several voltage combinations in the reflectron. Cornish and Cotter recently showed, however, that a reflectron employing an appropriate nonlinear field, referred to as a curved-field reflectron, can bring essentially the entire product spectrum to focus at the detector using a single set of reflectron potentials.[42,43] Figure 27 shows illustrative data for the MALDI/post-source decay of angiotensin II using a curved-field reflectron.[43] An advantage of the PSD/TOF approach is that the tandem mass spectrum can be obtained using a single mass analyzer. Precursor ion selection can be performed by ion gating or beam blanking procedures.[246,247] A "high resolving power" ion selector for post-source decay measurements in a reflecting time-of-flight mass spectrometer using deflection plates judiciously positioned after the ion source was recently described.[291] However, the resolution with which the precursor ions can be selected is poorer than the mass resolving power afforded to the product ions. Furthermore, the precision with which product ion masses can be determined in PSD are reported to be less than those achievable for stable ions.[247]

The PSD experiment is unusual from the standpoint of most modern MS/MS experiments in the sense that it does not involve a discrete activation step divorced from the energy associated with the ionization process. Several approaches, however, have been taken in recent years to provide for precursor ion selection and discrete ion activation in time-of-flight, thereby defining a typical MS/MS experiment whereby a mass-selected precursor ion is subjected to an ion activation step separate from the ionization step. Well over a dozen tandem time-of-flight instruments have been described in the literature. A subset of these instruments is identified here.[292−303] In most cases, fast activation methods, such as high-energy collisional activation with a gaseous target, photodissociation, and surface-induced dissociation, have been used in these instruments. An exception is the use of an octopole collision cell intermediate to a linear time-of-flight for precursor ion selection and a reflectron time-of-flight for product ion analysis.[303] In this apparatus, precursor ions are decelerated to interact with a target gas at relatively low collision energies (tens of electronvolts) and the product ions are re-accelerated for analysis by the second TOF analyzer.

It is noteworthy that MS/MS data involving fragmentation have been demonstrated in instruments that rely solely on time-of-flight for both precursor ion selection and product ion analysis. MS/MS in such instruments, with the exception of the instrument that employs an octopole reaction region,[303] is largely restricted to fast activation methods and fragmentation as the reaction between stages of MS/MS. Tandem time-of flight instruments are not directly amenable to executing experiments to yield precursor ion or neutral loss spectra. They are primarily devoted to acquiring product ion spectra. To retain the characteristic of high speed, the reactions between stages of mass spectrometry must proceed on the microsecond time scale. This requirement can make it difficult to achieve high efficiency. Like the beam-type instruments, MS^n studies are limited to the number of reaction regions. No purely time-of-flight instruments designed for MS^n for $n > 2$ have been reported.

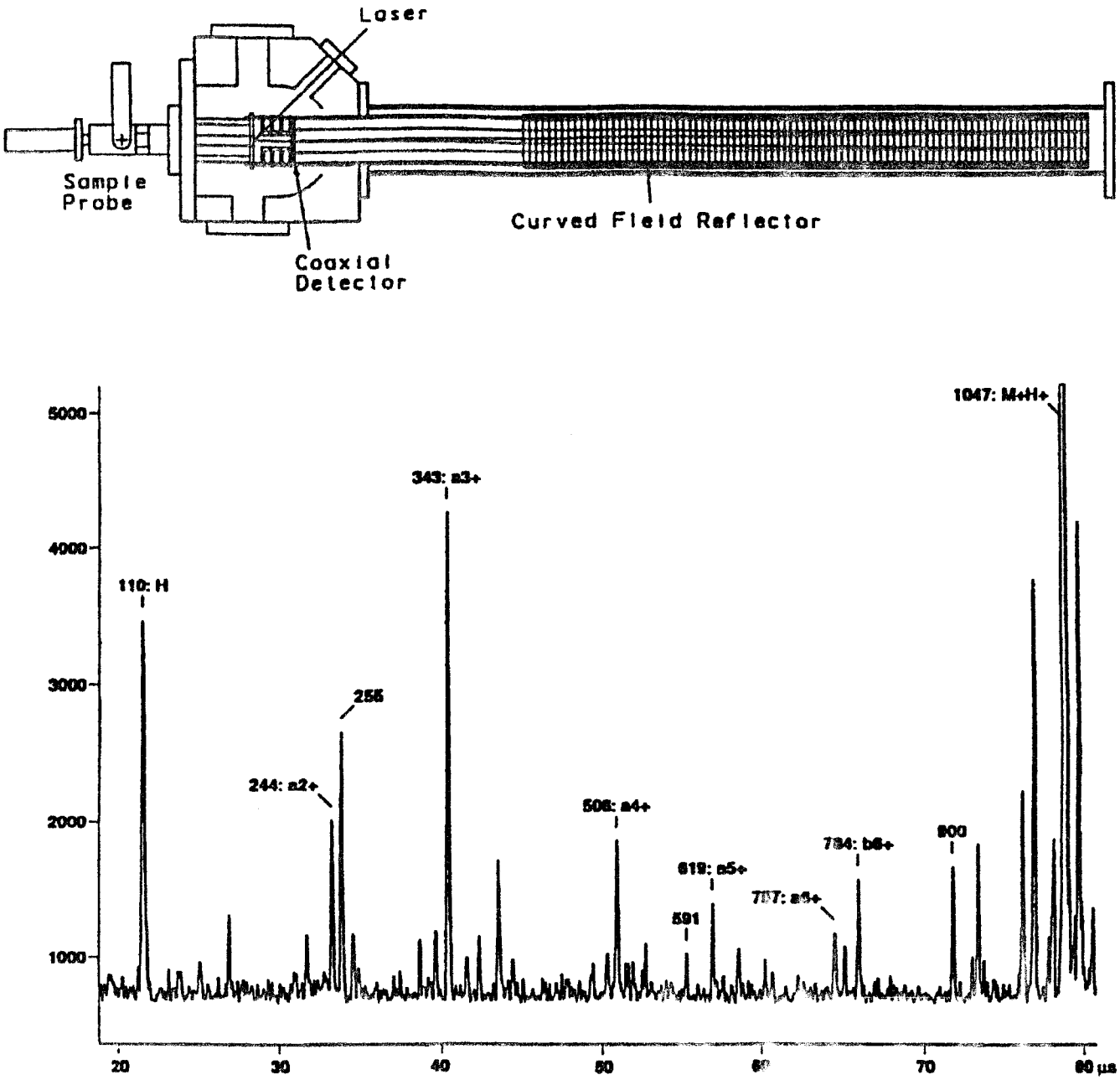

Figure 27. Time-of-flight mass spectrum resulting from the MALDI/post-source decay of angiotensin II obtained using a curved-field reflectron. (Reprinted with permission from ref 42. Copyright 1994 John Wiley and Sons Limited.)

4. Hybrid Tandem Mass Spectrometers

Hybrid tandem mass spectrometers are intended to refer to combinations of two analyzer types. The term was originally applied to combinations of sectors with quadrupoles[304−308] but has since been used in a more broad context to include ion-trapping instrumentation and time-of-flight. In principle, a hybrid combines strengths of each analyzer type while minimizing comprises that might arise from interfacing the two technologies. While attention in the 1980s and early 1990s was largely directed to combinations of sectors and quadrupole mass filters, many recently reported hybrids include either time-of-flight or an ion trap or both. Time-of-flight serves as the final mass analyzer in most of the hybrids that include time-of-flight. The quadrupole ion trap, on the other hand, has seen use both as the first-stage analyzer and as a final mass analyzer. A few illustrative examples are mentioned here.

The advantages of orthogonal acceleration time-of-flight combined with continuous beam ion sources have recently been exploited in hybrids that employ the time-of-flight as the second mass analyzer in MS/MS. For example, Figure 28 shows a schematic of a triple-sector mass spectrometer followed by an orthogonal acceleration time-of-flight analyzer.[309] This instrument combines the high resolving power of the sector instrument for precursor ion selection with the high efficiency, resolution, and mass accuracy of orthogonal acceleration time-of-flight.[309−311] This hybrid combination has clear advantages over sector tandem mass spectrometers and sector/quadrupole hybrids in terms of speed and efficiency. Hybrids that replace the sector as MS−I with a quadrupole mass

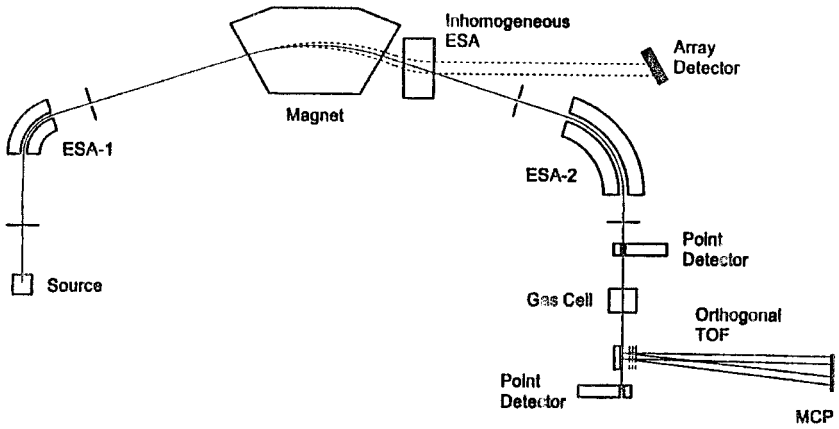

Figure 28. Schematic diagram of a sector mass spectrometer followed by an orthogonal acceleration time-of-flight analyzer. (Reprinted with permission from ref 309. Copyright 1995 John Wiley and Sons Limited.)

filter have also been reported[312,313] and show impressive performance in terms of sensitivity, resolution, and mass accuracy. This combination is particularly interesting in that the mass filter can be operated in the rf-only mode so that the time-of-flight can also be used to acquire mass spectra, thereby taking advantage of the high speed and high efficiency of time-of-flight in both MS and MS/MS modes. Both of the hybrid types just described are commercially available. The quadrupole mass filter/orthogonal acceleration time-of-flight hybrid, in particular, is seeing increasingly widespread application.

The flexibility of the quadrupole time-of-flight combination for tandem mass spectrometry can be expanded via the use of supplementary frequencies applied to the collision quadrupole. For example, Cousins and Thomson described the use of supplemental frequencies applied to opposing rods in the collision quadrupole of a hybrid quadrupole/time-of-flight instrument for radially accelerating selected ions.[314] Fragmentation can be induced by accelerating a precursor ion of a particular frequency in the radial dimension, in analogy with the technique used in three-dimensional quadrupole ion traps, or by accelerating the ion axially into the collision region, as in the conventional triple-quadrupole experiment. When both techniques are used simultaneously, genealogical information normally accessible only via an MS^n experiment can be extracted from the data.

Several groups have combined the Paul trap with time-of-flight for a variety of reasons.[315−323] Lubman et al., for example, pursued biological applications using both electrospray and MALDI in conjunction with a Paul trap interfaced with a reflectron time-of-flight mass analyzer.[315] In this instrument, time-of-flight mass analysis is used in lieu of either mass-selective instability or resonance ejection, thereby reducing mass analysis time and enjoying the figures of merit of the reflectron time-of-flight. The ion trap is used to accumulate ions and to perform mass selection and ion activation in MS/MS experiments. Aicher et al. described an ion-trap/time-of-flight instrument designed for the analysis of nonvolatile materials that are desorbed into a supersonic molecular beam.[316] In this case, the ion trap serves as the ionization and ion accumulation region. Ion activation is effected in the higher vacuum of the time-of-flight analyzer via photodissociation at the space focus of the time-of-flight. Ji et al. described a segmented ring ion-trap/time-of-flight instrument[317] whereby a radio frequency potential is applied to two of the rings during ionization to simulate the field of a cylindrical ion trap within the ionization volume of an electron ionization source. After a defined ion accumulation period, the potentials are switched to provide a well-defined ion acceleration potential for time-of-flight analysis. The aim of this work is to use trapping to store ions between time-of-flight analyses to maximize duty cycle for gas chromatography/mass spectrometry without compromising speed. This work is part of a strategy to develop high-sensitivity/high-speed mass spectrometry for fast chromatography using time-of-flight. This group recognized very early the potential for mass spectrometry coupled with high-speed separations and articulated the requirements for the mass spectrometer.[324]

Campbell et al. recently described the combination of a linear ion trap with a time-of-flight mass analyzer for tandem mass spectrometry studies.[325] In this work, ions were accumulated and stored in the rf-only quadrupole using electrostatic trapping in the axial dimension. The use of supplementary signals applied to one set of opposing rods allowed for mass selection and ion activation, in analogy with the three-dimensional quadrupole ion trap. This approach has an important advantage over the three-dimensional ion trap, however, because the trapping efficiency for ions injected from an external ion source can be 1−2 orders of magnitude higher with the linear ion trap.

Wang et al. recently described the use of an octopole ion guide as a mass-selective ion accumulation device for subsequent injection of ions into an FTICR instrument.[326] With the use of combined radio frequency and DC potentials applied to the octopole electrodes, in analogy with the mass filter, a resolving power of roughly 10 has been demonstrated with an octopole. The main purpose for using the octopole in a mass-resolving mode is to allow for mass-selective injection of ions into the FTICR to mitigate linear dynamic range limitations associated with the finite charge storing capacity of the ICR cell. However, under appropriate conditions, fragmentation of polypeptide ions has also been observed thereby providing

the option of inducing fragmentation prior to ion injection into the FTICR.

Beam-type mass analyzers have also been coupled with quadrupole ion traps for various purposes. For example, mass filters have been used to mass analyze ions ejected from an ion trap.[180,327] In these instances, the mass filter was used instead of or in addition to mass-selective instability for mass analysis. The most recently reported instrument of this type was used to study chemical mass shifts associated with mass-selective instability.[180] Most hybrid tandem mass spectrometers comprised of beam-type analyzers and ion traps have employed a beam-type analyzer as MS−I. These have included mass filter/ion trap,[328,329,207] mass filter/ion-trap/mass filter,[330] mass filter/collision quadrupole/ion-trap,[331] and sector/ion-trap[332−334] combinations. The use of a beam type analyzer for MS−I is particularly advantageous for minimizing dynamic range limitations imposed by the ion trap. Furthermore, the beam-type analyzer is usually capable of providing superior precursor ion resolution to that normally obtained using the ion trap for precursor ion isolation.

Hybrid tandem mass spectrometers clearly cannot be summarized with a single set of figures of merit. Each hybrid system has a unique set of strengths and weaknesses. In some cases, the advantages of a particular hybrid system limit its unique strengths to a relatively specialized set of measurements. However, some hybrid systems, such as the quadrupole/time-of-flight tandem mass spectrometer, are widely applicable and competitive in cost and performance with virtually any other form of tandem mass spectrometry. It is clear that the combination of various ion transmission, ion storage, and mass analysis technologies for improved sensitivity, specificity, and range of chemistry applications has been a widespread activity in the development of instrumentation for tandem mass spectrometry in the past decade. The many successes associated with combining various mass spectrometry technologies will encourage further development along these lines in the coming years.

C. Tandem-in-Time Devices

1. FTICR

A great strength of FTICR is its ability to perform MS/MS and MS^n experiments with high resolution and mass accuracy in the final product ion spectrum and demonstrated high-resolution ion selection. A number of examples of how ultrahigh performance MS/MS can be used to obtain extensive information on the structures of high-mass biomolecules have recently been described.[335−342,264−269] The nature of the information that can be obtained via MS/MS, of course, is determined by the reactions that take place between stages of mass analysis. The identities and abundances of product ions arising from dissociation of high-mass biomolecules provides important primary structure information. The dissociation channels that contribute to the product ion spectrum are determined in part by the nature of the activation method. A strength of FTICR is that both fast and very slow activation methods can be effected during the course of an MS^n experiment. Very slow activation methods include collisional techniques, such as sustained off-resonance irradiation (SORI),[343] very low energy (VLE)[344] collisional activation, and multiple excitation collisional activation (MECA),[345] infrared multiphoton dissociation (IRMPD)[346] using continuous wave lasers, and blackbody infrared dissociation (BIRD)[347−350] arising from ambient blackbody photons. Fast activation methods include ultraviolet energy photodissociation[351] and surface-induced dissociation.[352−354] An example of data acquired using a fast activation method, vacuum UV photodissociation of the 10+ charge state of bovine ubiquitin, is shown in Figure 29.[351] The top of the figure includes a summary of the structural information obtained from the same precursor ion using SORI and IRMPD. The slow heating methods (viz., SORI and IRMPD) give rise predominantly to b- and y-type ions, whereas UV photodissociation gives rise predominantly to c- and z-type ions. Complementary information is obtained from the two types of activation methods. The compatibility of FTICR with a variety of activation methods adds further dimensionality to the MS^n capability.

The ICR has long been recognized as a tool for the study of thermal energy ion/molecule reactions which have now been extended to ions derived from peptides and proteins.[355] An interesting new development has been the observation of capture of low-energy electrons by high-mass multiply charged ions.[264−269] The ion/electron reaction has proved to be particularly valuable in deriving primary structural information from protein ions, for example. The electron capture process has been shown to be capable of inducing extensive fragmentation in polypeptide ions. The overall process involving electron capture and subsequent dissociation has been termed "electron capture dissociation" (ECD). The fragmentation is usually more extensive than is observed with activation of multiply protonated polypeptides formed directly via electrospray using the activation techniques mentioned above. Furthermore, the dissociation channels for the radical cation species formed via electron capture differ from those observed from dissociation of even-electron ions of the same charge state. Figure 30 shows the spectrum following ECD of the 11+ ion of ubiquitin.[264] A remarkably rich array of structurally informative fragmentation is observed. The interested reader is referred to the original work for a discussion of mechanistic aspects of ECD and the product ion designations listed in the figure. Another remarkable observation associated with ECD is that there is a particular tendency to induce fragmentation at disulfide linkages.[266] Cleavages at disulfide linkages for many multiply charged ions activated via conventional means is often not a favored process.[266,356]

The MS^n and ion remeasurement capabilities of the FTICR have been significantly enhanced in recent years by axialization techniques[357] that bring ions excited to relatively large cyclotron radii back to the center of the ICR for subsequent excitation. Quadrupolar axialization, for example, allows for highly

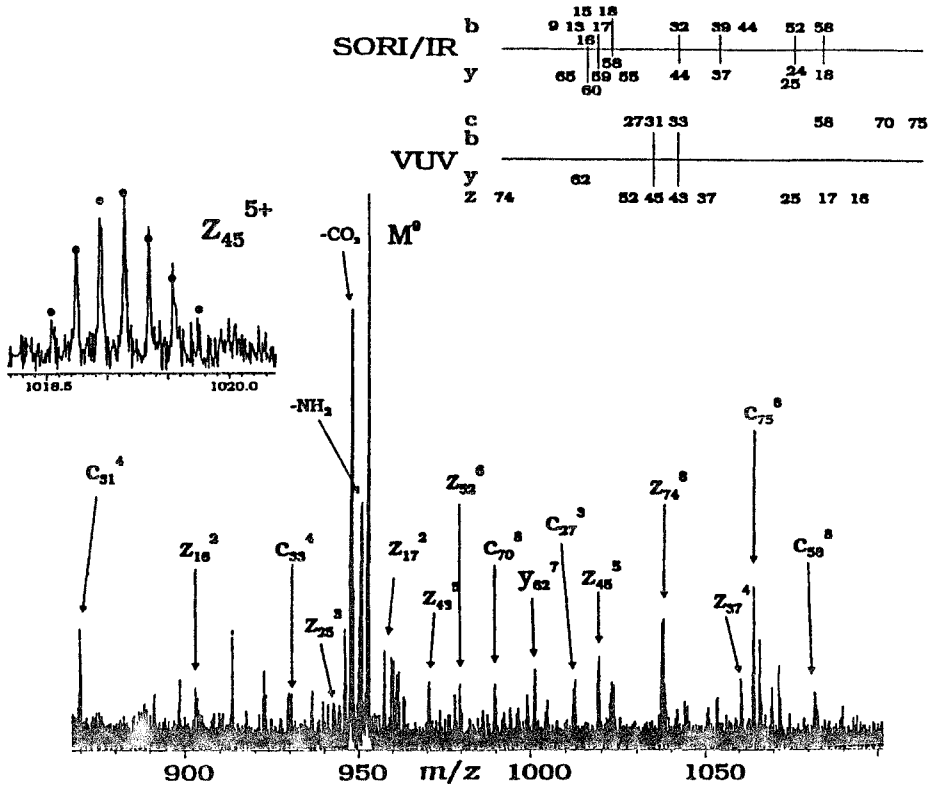

Figure 29. Results of an FTICR MS/MS experiment involving the 10+ charge state of bovine ubiquitin using a fast activation method, vacuum UV photodissociation. (Reprinted with permission from ref 351. Copyright 1996 Elsevier Science.)

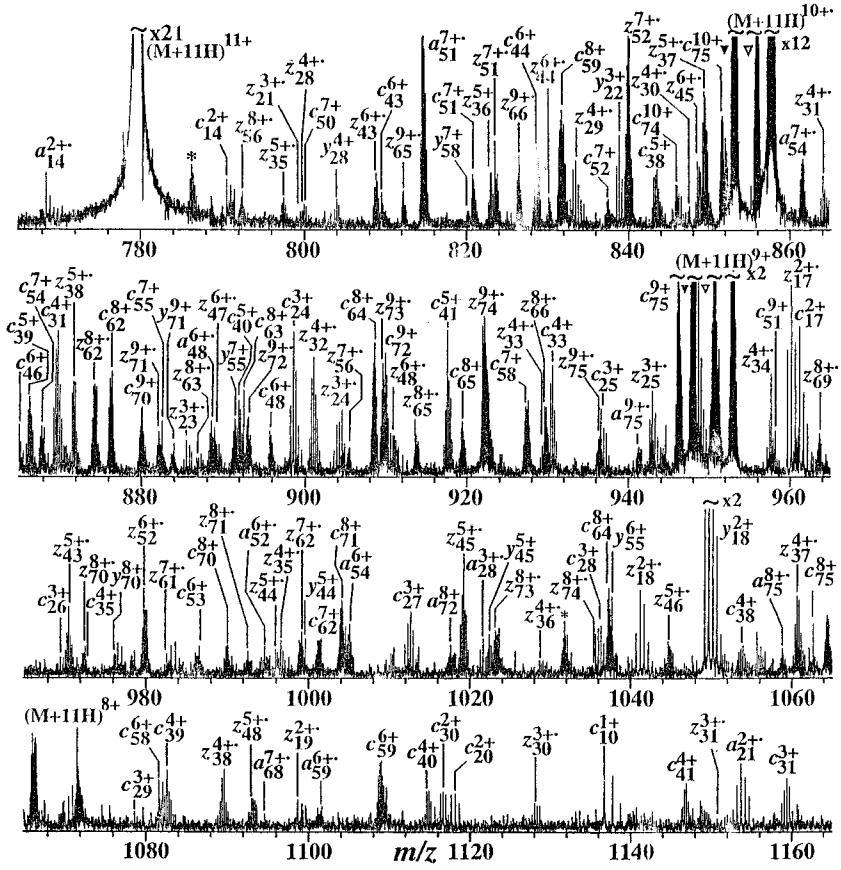

Figure 30. Product ions produced by electron capture dissociation of the 11+ ion derived from ubiquitin in an FTICR instrument. (Reprinted with permission from ref 264. Copyright 2000 American Chemical Society.)

efficient ion remeasurement giving the theoretical improvement in signal-to-noise ratio of the square root of the number of measurements.[358−362] Axialization works both for repeated measurements of the same ions and for product ions formed between stages of mass analysis. Thus, the nondestructive nature of

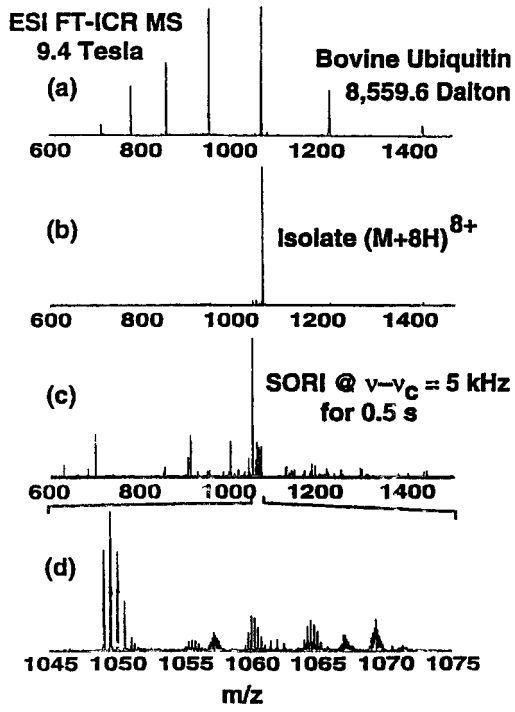

Figure 31. Results obtained during the course of an interactive mass spectrometry experiment involving ions derived from electrospray of ubiquitin. See text for a description of each step. (Reprinted with permission from ref 363. Copyright 1997 American Chemical Society.)

detection in FTICR combined with efficient axialization allows for "interactive" mass spectrometry, whereby the operator can choose the next step in an MS^n experiment without generating a new ion population between steps.[363] Such an experiment was recently described, and the results are shown in Figure 31.[363] The first measurement involved the acquisition of the electrospray mass spectrum of bovine ubiquitin. The 8+ charge state was then selected and a spectrum collected. The selected ion was then subjected to SORI for 0.5 s, and the product ion spectrum was recorded. The definition of each step leading up to the final spectrum was made on the original set of ubiquitin ions. Such an experiment is an elegant demonstration of MS^n and the first example wherein the experiment was both defined and conducted on a single population of ions.

Tandem mass spectrometry in the FTICR is often characterized by very high efficiencies. High efficiency combined with the ion-trapping nature of the experiment facilitates MS^n experiments, particularly when ion axialization techniques are employed. Although reactions that require kiloelectronvolt collision energies are generally not readily studied in ICR instruments, the range of chemical reactions that can be studied is remarkably wide providing the experimentalist with many options for interrogating gaseous ions. Tandem-in-time instruments are well-suited to conventional product ions scans. Other types of scans, such as neutral loss and precursor ion scans, are difficult to implement. Nevertheless, combined with its exceptional mass analysis figures of merit, the FTICR instrument is, in many ways, the most powerful tandem mass spectrometer.

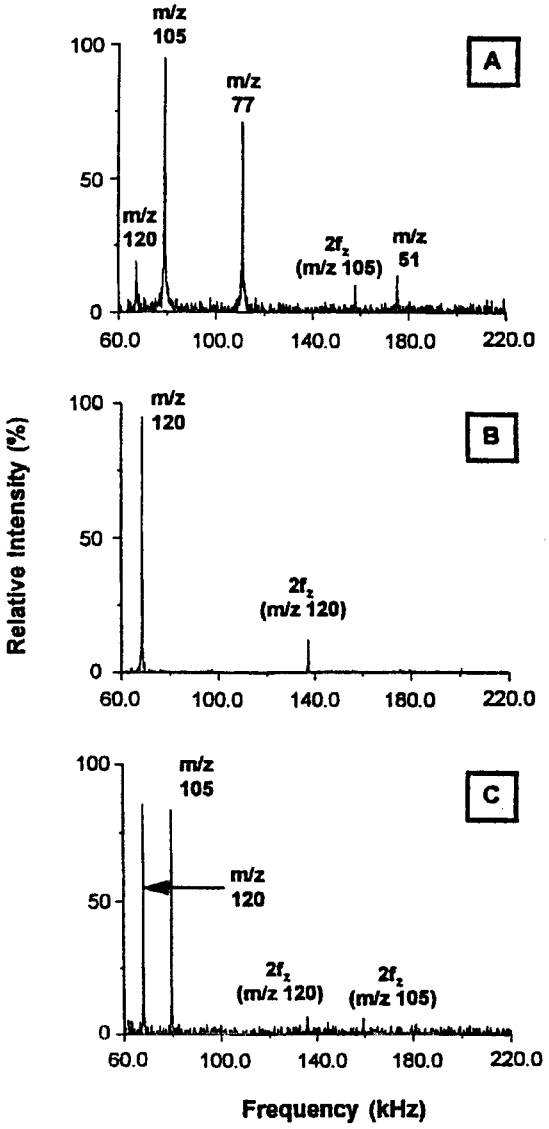

Figure 32. Results from an ion-trap MS/MS experiment wherein all steps are performed with the same population of precursor ions. (Reprinted with permission from ref 159. Copyright 1996 American Chemical Society.)

2. Quadrupole Ion Trap

Like the FTICR, the quadrupole ion trap is well-known for its ability to perform MS^n experiments.[364−366] Ion axialization between stages of mass analysis, however, is generally unnecessary due to the inherent collisional cooling that occurs when a bath gas is present in the ion-trapping volume. Partly for this reason the efficiencies associated with converting precursor ions to measured product ions often approach 100%. While most MS^n experiments conducted with quadrupole ion traps involve no true mass analysis steps until the destructive mass analysis step at the end of the experiment, in-situ detection in the ion trap allows for true MS^n experiments in which mass spectra can be obtained after each step of the process on the same original ion population. This experiment has recently been demonstrated,[159] and Figure 32 provides an example of illustrative data. Figure 32a shows the electron impact mass spectrum of acetophenone, Figure 32b shows a re-measurement of the precursor ion after its isolation,

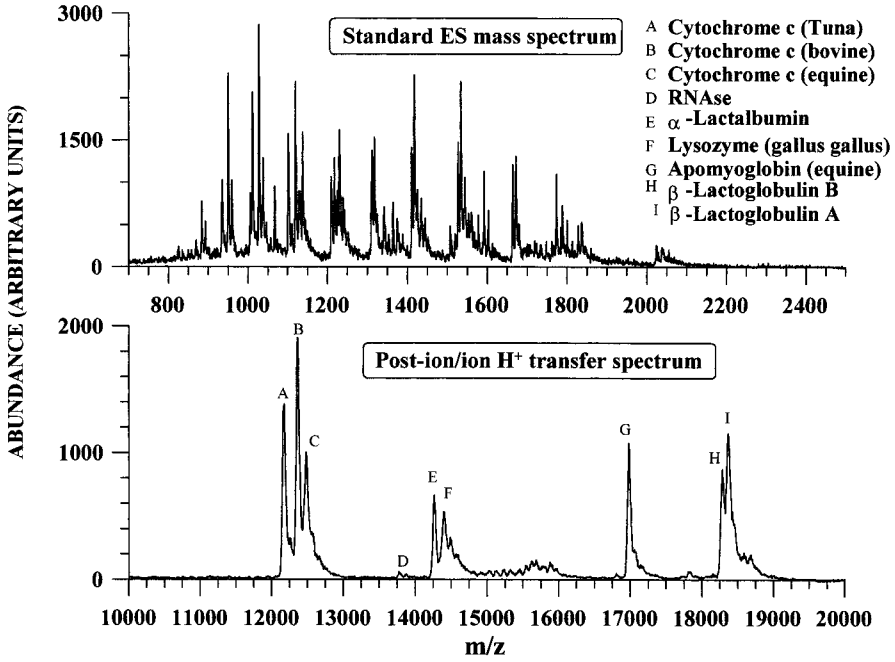

Figure 33. Electrospray mass spectra of a mixture of positively charged protein ions before (top) and after (bottom) reaction with anions derived from glow discharge ionization of perfluoro-1,3-dimethylcyclohexane for a period sufficient to reduce charge states to relatively low values. (Adapted with permission from Elsevier Science, ref 386. Copyright 1998 American Society for Mass Spectrometry.)

and Figure 32c shows the product ion spectrum obtained after collisional activation of the precursor ion.

A wide range of reactions can be studied in the ion trap, once again in close analogy with the ICR instrument. In terms of ion activation reactions, various means are available for accelerating ions in the presence of the bath gas to effect collisional activation. These include, for example, conventional resonance excitation,[367] broad-band excitation,[368−370] short dc pulses,[371] low-frequency ac ion acceleration,[372] ion activation resulting from placing an ion near a stability boundary,[373−376] and red-shifted off-resonance large amplitude excitation.[377] Ion activation via bath gas heating has also been described recently as another collisional activation technique.[378,379] The use of IRMPD has also been described[380−382] as has UV photodissociation in the Paul trap.[383] While IRMPD and heated bath gas activation are analogous to conventional ion-trap collisional activation in that they are slow heating methods, they do not suffer as much from the low mass cutoff restriction. Conventional ion-trap collisional activation involves a competition between ion excitation and ion ejection. Poor efficiencies are obtained at low trapping levels due to precursor ion ejection. This is avoided by using higher trapping levels during excitation, but this can result in the loss of information if some of the product ions fall below the low mass cutoff. Laser irradiation and bath gas heating, of course, involve no ion acceleration, thereby allowing for lower trapping levels during the activation period. It has also been demonstrated in collisional activation experiments in which the precursor ion is accelerated that lower trapping levels can be used in conjunction with the pulsed introduction of heavier bath gases.[384,385] The higher center-of-mass collision energies afforded by the use of heavier targets at constant resonance excitation amplitude make the use of lower excitation amplitudes and, hence, lower trapping potentials possible.

As with the FTICR, the Paul trap is also well-suited to the study of ion/molecule reactions between stages of mass analysis, and a number of groups now use the Paul trap to study ion/neutral chemistry. The Paul trap also has the interesting characteristic that it can store ions of opposite polarity simultaneously and in overlapping regions of space. This allows for the study of ion/ion reactions. A number of studies have recently been reported discussing the reactions of multiply charged ions derived by electrospray with singly charged ions of opposite polarity.[263,386−390] Such reactions proceed at high rates in the Paul trap. Figure 33 shows the spectrum of a mixture of positively charged protein ions before and after reaction with anions derived from glow discharge ionization of perfluoro-1,3-dimethylcyclohexane for a period sufficient to reduce charge states to relatively low values.[386] Peaks associated with each protein component in the mixture are clearly resolved at these low charge states. These data demonstrate that ion/ion reactions have potential for protein mixture analysis using electrospray as the ionization technique. Ion/ion proton-transfer reactions can also simplify the interpretation of product ion spectra derived from multiply charged precursor ions.[388,389] Figure 34 compares ion-trap collisional activation product ion spectra for the 16+ ion derived from electrospray of human hemoglobin β-chain before and after ion/ion proton-transfer reactions.[389] The pre-ion/ion spectrum cannot be interpreted due to the limited resolving power of the ion trap. The post-ion/ion product ion spectrum, on the other hand, is readily interpretable. These results represent a significant increase in the size of precursor ions amenable to MS/MS in the quadrupole ion trap.

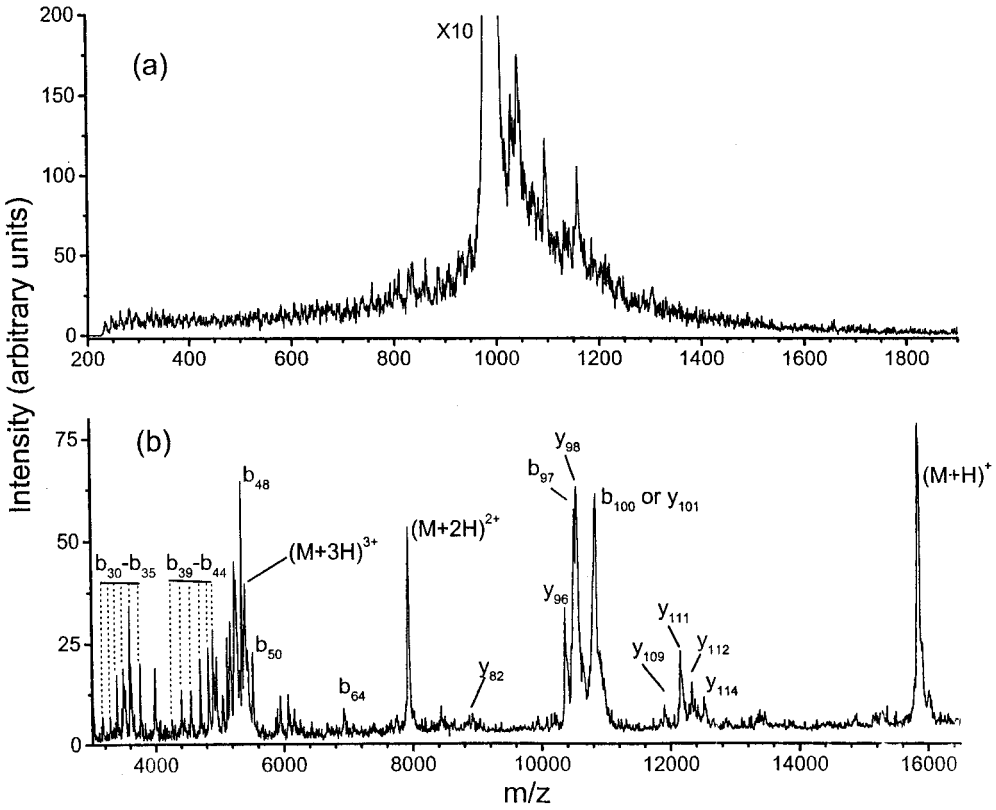

Figure 34. Comparison of ion-trap collisional activation product ion spectra for the 16+ ion derived from electrospray of human hemoglobin β-chain before (top) and after (bottom) ion/ion proton-transfer reactions. (Reprinted with permission from ref 389. Copyright 2000 American Chemical Society.)

While most applications of the Paul trap in the chemistry community have been with organic and biological species, the elemental mass spectrometry community has taken an interest in the technology for its MS/MS capabilities and potential for field use. Laser ablation,[400,392] glow discharge,[393,394] secondary ion,[395,396] and inductively coupled plasma ion sources[329] have been coupled with the ion trap. While the issues of abundance sensitivity and precision are still being explored, it is already apparent that ion chemistry can be helpful in addressing the problem of isobaric interferences. For example, advantage has been taken of ion/molecule chemistry to remove ions derived from the support gases from plasma sources[393,329] as well as to separate elemental ions on the basis of different reactivity with species, such as oxygen.[207] Destruction of polyatomic interferences has also been explored,[393] and species as strongly bound as TaO^+ (bond-strength = 8.2 eV) have been dissociated with efficiencies approaching 100% using neon as the bath gas.[397]

The quadrupole ion trap is a remarkably flexible device for conducting MS^n experiments and is among the most powerful forms of tandem mass spectrometry. However, as with the FTICR, it is difficult to implement neutral loss and precursor ion scans. It can access a wide range of chemistries, including unimolecular dissociation, ion/molecule reactions, and low-energy ion/ion reactions. It is most amenable to relatively slow activation methods, with the exception of UV photodissociation, and generally cannot access the collisional ionization reactions associated with kiloelectronvolt energy collisions. Furthermore, efficient surface-induced dissociation has not yet been demonstrated. The use of a light bath gas, such as helium, at 1 mTorr makes the ion trap somewhat unique relative to other tandem mass spectrometers. The bath gas serves as the target gas in collisional activation experiments and also serves as the conduit for collisional cooling both of translational motion and internal excitation. Dissipation of kinetic energy via momentum transfer collisions is a key element in the relatively high efficiency of ion-trap MS^n experiments. Furthermore, the relatively high collisional cooling rate in the ion trap can play a significant role in determining the product ions observed from reactions in the ion trap.[398,399]

IV. Summary

There have been many new and exciting developments in mass spectrometer systems in recent years. Many of these developments are being driven by challenges presented by molecular biology. The activity is fueled by resources being devoted to drug development, for example, and other medically and biologically related activities. Progress in these applications will be accelerated by improved sensitivity, specificity, and speed. In mass spectrometry, this translates to greater mass resolving power, mass accuracy, mass-to-charge range, efficiency, and speed. It is safe to say that the demands resulting from current analytical needs are likely to be met to varying degrees but probably not by a single analyzer technology or hybrid instrument. On-line and/or off-line separations and manipulations combined with

mass spectrometry will also play increasingly important roles.

For any analyzer, or combination of analyzers, to become widely used it must have an important application for which its figures of merit are best suited, relative to competing approaches. The relative cost of competing technologies is also an important factor. The mass filter has seen so much use in the past 30 years because its characteristics best fit a wide range of applications. As an example, biological applications, which are currently driving many instrument development activities in mass spectrometry, demand more information, of higher quality, from less material, faster, and at lower cost. Which technologies will dominate biological applications in the coming years is open to speculation. However, in considering the relative merits of today's dominant mass analyzers, areas of opportunity for improvement are apparent. Furthermore, new and more demanding measurement needs are constantly being recognized that will continue to exercise the creativity of the mass spectrometry community.

V. Acknowledgments

S.A.M. acknowledges support of his research program by the U.S. Department of Energy, Office of Science, the National General Medical Sciences Institute, and the Purdue Research Foundation.

VI. References

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CR990087A