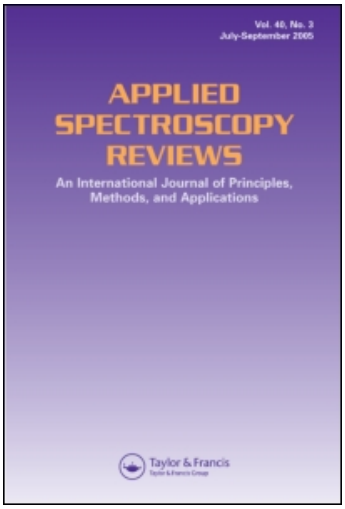

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Mass Spectrometry, Review of the Basics: Electrospray, MALDI, and Commonly Used Mass Analyzers

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Mass Spectrometry, Review of the Basics: Electrospray, MALDI, and Commonly Used Mass Analyzers

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Abstract: Mass spectrometry (MS) has progressed to become a powerful analytical tool for both quantitative and qualitative applications. The first mass spectrometer was constructed in 1912 and since then it has developed from only analyzing small inorganic molecules to biological macromolecules, practically with no mass limitations. Proteomics research, in particular, increasingly depends on MS technologies. The ability of mass spectrometry analyzing proteins and other biological extracts is due to the advances gained through the development of soft ionization techniques such as electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) that can transform biomolecules into ions. ESI can efficiently be interfaced with separation techniques enhancing its role in the life and health sciences. MALDI, however, has the advantage of producing singly charges ions of peptides and proteins, minimizing spectral complexity. Regardless of the ionization source, the sensitivity of a mass spectrometer is related to the mass analyzer where ion separation occurs. Both quadrupole and time of flight (ToF) mass analyzers are commonly used and they can be configured together as QToF tandem mass spectrometric instruments. Tandem mass spectrometry (MS/MS), as the name indicates, is the result of performing two or more sequential separations of ions usually coupling two or more mass analyzers. Coupling a quadrupole and time of flight resulted in the production of high-resolution mass spectrometers (i.e., Q-ToF). This article will historically introduce mass spectrometry and summarizes the

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advantages and disadvantages of ESI and MALDI along with quadrupole and ToF mass analyzers, including the technical marriage between the two analyzers. This article is educational in nature and intended for graduate students and senior biochemistry students as well as chemists and biochemists who are not familiar with mass spectrometry and would like to learn the basics; it is not intended for mass spectrometry experts.

Keywords: Electrospray ionization, MALDI, quadrupole, time of flight, MS/MS, Q-Tof

MASS SPECTROMETRY; A HISTORIC PERSPECTIVE

Mass spectrometry (MS) has rapidly evolved during the past 20 years with its various applications invading every discipline within the life and health sciences (1–4). Mass spectrometry relies on the formation of gas-phase ions (positively or negatively charged) that can be isolated electrically (or magnetically) based on their mass-to-charge ratio (m/z). In an MS spectrum, the x-coordinate represents m/z values, whereas the y-axis indicates total ion counts. Strictly speaking, mass spectrometers should really be called mass-to-charge spectrometers. Mass spectrometric analysis can provide important information about the analytes, including their structure, purity, and composition.

Mass spectrometry was first described by physicists in the late 1880s. Wilhelm Wien, for example, was the first to demonstrate in 1898 that superimposed electric and magnetic fields can deflect positive ions and his work was preceded by the work of physicist Eugen Goldstein, who in 1886 discovered a new kind of radiation, "*Kanalstrahlen*" and reached the conclusion that these "new" rays were merely positively charged particles (5, 6). Inspired by these findings, Sir Joseph John Thomson, a professor of experimental physics at Cambridge University, invented the first mass spectrometry instrumentation and in 1913 illustrated the value of his novel discovery within the field of analytical chemistry (7). Mass spectrometry was critical for stable isotope/radionucleotide studies (8) and, eventually, commercial mass spectrometric instrumentation, that can diagnostically detect organic substances, appeared on the market in the 1940s, as a response to the demands from the oil industry (5, 6). The "marriage" between gas chromatography and mass spectrometry expanded the interest in this powerful technique to include biochemistry. Gas chromatography is capable of separating thermally stable biological compounds, such as fatty acids, steroids, and carbohydrates. This analytical tool was invented in 1952 (9) and coupling it to a mass spectrometer (i.e., GC-MS) was pioneered by Holmes and Morrell in 1957 (10). The use of early mass spectrometric instruments was limited to volatile compounds with a low molecule weight range ($>$1000 Da). In addition, a conventional ionization technique, namely, electron impact (EI), is harsh and will result in the destruction of complex biomolecules (e.g., proteins, nucleotides, complex carbohydrates). In addition, the usage of mass spectrometry within biochemistry laboratories was limited to GC-MS instruments that

separate volatile mixtures. Under such conditions, complex biological substances, such as proteins, cannot be transmitted to the gas phase without significant destruction and degradation. It was only after the introduction of soft ionization techniques that mass spectrometry was used for proteomics studies. Mass spectrometry can now be interfaced with other separation techniques, such as high-pressure liquid chromatography (HPLC-MS) and capillary electrophoresis (CE-MS). It is also common to perform MS analysis for purified materials or synthetic conjugates, without the use of a separation procedure.

SOFT IONIZATION TECHNIQUES

Regardless of the method by which a sample is introduced into a mass spectrometer (i.e., GC, HPLC, direct injection), the ion source is the compartment where charged species are produced and is the "gate" to other sections of the instrument, namely, the analyzer and the detector. Many ionization techniques are currently used with mass spectrometric instrumentations. The traditional ionization method, namely, EI, utilizes energetic electron beams during the ionization process and operates only under vacuum while the analytes are already in the gas phase. A beam of electrons (negative charges) are formed from a heated metallic filament (e.g., tungsten) and these electrons are electrically accelerated and directed to collide with a vaporized sample, causing electron expulsion from the analytes and subsequent formation of positively charged radical cations. These conditions are not suitable for large molecules or many biological materials. EI, along with chemical ionization (introduced below), is, however, still the method of choice for GC-MS equipment (11). Figure 1 illustrates the process by which ions are formed during EI ionization and it should be noted that with specific compounds, negatively charged species can be observed (12) (Figure 1b).

Due to the limitations associated with EI ionization, chemical ionization (CI) and plasma desorption (PD) methods were introduced in 1966 (13) and 1974 (14), respectively. Both techniques will result predominantly in the formation of protonated (or deprotonated) ions, which are more stable than the radical ions formed during EI-MS analysis. CI depends on the interaction between energetic electrons and neutral molecules, such as methane, resulting in the formation of charged ions that will interact with the analytes, producing protonated species. Similar to EI ionization, this method poses some limitations in terms of mass range (<1000) and requires specific sample characteristics with regard to thermal stability and volatility. CI is, however, better than EI with respect to the production of the molecular ion. Nevertheless, both EI and CI were not capable of ionizing the most valuable, thermally instable, polar biological compounds.

PD ionization, on the other hand, is one of the early "soft" ionization techniques capable of analyzing biomolecules, up to a molecular weight (MW) of

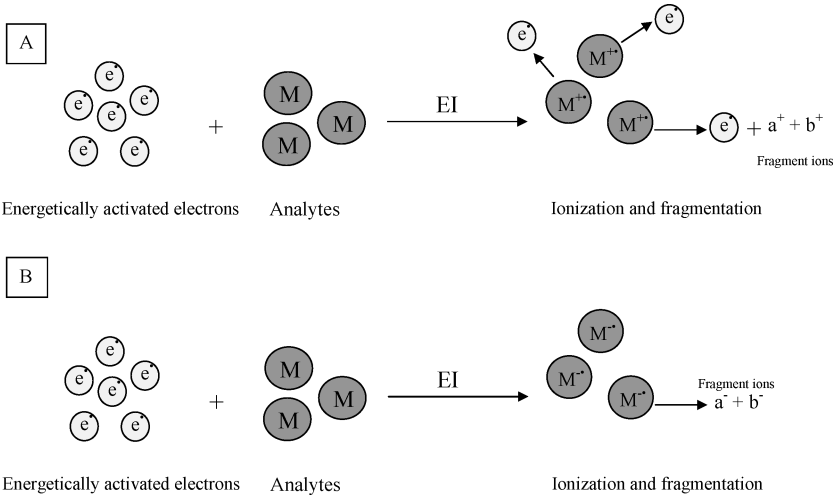

Figure 1. Conventional electron impact (EI) ionization process. (A) Formation of positively charged ions. (B) Formation of negatively charged ions.

100,000 Daltons (Da). The term *soft* indicates that minimum internal energy is transmitted to the analytes during the ionization process. PD ionization was introduced after the design of field ionization and field desorption methods (15, 16). Subsequently, additional soft ionization methods were developed and replaced older techniques. These include fast atom bombardment (FAB) (17, 18), liquid secondary ion mass spectrometry (LSIMS) (19), matrix-assisted laser desorption ionization (MALDI) (20, 21), and electrospray ionization (ESI) (22, 23). The last two ionization techniques, in particular, have revolutionized the usage of mass spectrometers and enabled researchers to easily study biological substances, such as glycoconjugates, proteins, and DNA. The significance of the development of MALDI and ESI was globally recognized when the "inventors," Koichi Tanaka of the Shimadzu Corp in Kyoto, Japan, and John Fenn of the Virginia Commonwealth University, Richmond, shared the 2002 Nobel Prize in chemistry. Nonetheless, much of the credit given to the development of MALDI should also go to Michael Karas and Franz Hillenkamp, who developed the ideas of laser desorption techniques (21, 24, 25). Table 1 compares different commonly used ionization methods, summarizing their various characteristics; information in Table 1 is extracted from Herbert and Johnstone (12) and Henderson and McIndoe (1).

Electrospray Ionization-Mass Spectrometry (ESI-MS)

Development of electrospray ionization started in the late 1960s with the work of Dole and coworkers, who successfully introduced a polystyrene polymer

Table 1. Summary of the various features of current ionization methods used in mass spectrometry

Ionization technique	Nature of analytes	Sample introduction	Mass range	Brief description
Electron impact (EI)	Volatile; thermally stable	Gas chromatography solid or liquid probe	<1000 Da	Hard method; mainly fragment ions
Chemical ionization (CI)	Volatile; thermally stable	Gas chromatography solid or liquid probe	<1000 Da	Soft method; Molecular ion
Fast atom bombardment FAB	Organometallic compounds	Liquid chromatography, direct injection	<5000 Da; optimal range 200–2000	Soft method; require matrix
Matrix-assisted laser desorption ionization (MALDI)	Biomolecules (proteins, DNA, glycoconjugates)	Sample is cocrystallized with a matrix	Can go beyond 500,000 Da	Very soft method singly charged ions
Electrospray ionization (ESI)	Organic and inorganic compounds	Sample in solution	From very low masses to extraordinarily high	Very soft method; multiply charged ions

(average MW = 51,000 Da) into the gas phase as a charged species (26). Surprisingly, this ionization technique is by far one of the simplest to understand. Samples are usually dissolved in a buffer or solvent that is introduced into the mass spectrometer in the form of a spray. This technique was linked to a quadrupole mass analyzer and was significantly optimized in the early 1980s (22, 23). ESI-MS is currently used for qualitative and quantitative studies of a wide variety of nonvolatile and thermally labile simple inorganic chemicals as well as complex biological structures (27).

In ESI-MS, the sample should be soluble in a preferably polar solvent, which can be infused, under atmospheric pressure, into the ionization source via a thin needle. As the sample is being constantly sprayed, a high electrical potential is applied at the needle (3–4 Kv), resulting in the formation of highly charged droplets (i.e., nebulization). These droplets are then driven electrically and are vaporized with the aid of a warm neutral gas (usually nitrogen). Under these conditions, the droplets break down and, while shifting inside the source, their size is continuously being reduced. Eventually, the repulsive forces, also termed the *coulombic* forces, among the ions on the surface of the shrinking droplets become very high. These forces will ultimately exceed the surface tension of the solvent, resulting in ions that desorb into the gas phase. This theory of ESI ion formation is termed the *ion evaporation method* (28, 29) and

is believed to favor ions with relatively low m/z values (30). An alternative theory, which is supposed to be dominant in the case of ions with very high m/z (30), is the charge residue model, which involves continuous evaporation of the solvent accompanied by droplet fragmentation so that a single ion (probably multiply charged) is formed at the end of this process (i.e., solvent is completely evaporated) (26, 29, 31). Figure 2 illustrates the different proposed mechanisms of ion formation during the ESI process.

ESI has obviously resolved the problems inherited in studying large proteins using traditional mass spectrometry and its performance can be further enhanced by linking it to liquid chromatography (i.e., LCMS). In conventional ES pioneered by Fenn's group, samples were injected at a flow rate of 2–20 μL/min (32), which posed some problems when dealing with limited biological samples. Therefore, additional research efforts (33, 34) resulted in the development of nanospray technology, which operates at a very low flow rate in the order of nanoliters per minute (nL/min). The advantage of nanospray technology is not limited to minimized flow rates but also to the mechanism of ion formation, improving, for example, glycoconjugate analysis (35). ESI continues to attract researchers from various disciplines who investigate both new applications as well as possible new improvements at the technical level.

Matrix-Assisted Laser Desorption Ionization (MALDI)

MALDI is a leading ionization source currently being used for protein sequencing and proteomic research and is commonly utilized together with ESI technology. Banoub's group, for example, have successfully characterized vitellogenin protein, a fish biomarker, by both ESI and MALDI (36, 37). Similar to ESI, however, MALDI is also powerful for studying DNA (38), lipids (39), and glycoconjugates (40).

In MALDI, ions are desorbed from the solid phase. A sample is first dissolved in a suitable solvent and mixed with an excessive amount of an appropriate matrix. Subsequently, it is spotted on a MALDI plate and air-dried (or under a stream of nitrogen gas). Under these circumstances, the sample is cocrystallized with the matrix. The components in the mixture are brought into the gas phase via a laser beam (usually a nitrogen laser at a wavelength of 337 nm) that hits the sample-matrix crystal, leading to absorption of the laser energy by the matrix and subsequent desorption and ionization of the analytes in the sample. Figure 3 represents the process by which ions are formed during MALDI-MS.

MALDI was initially operated under vacuum; however, atmospheric pressure (AP) MALDI was developed in 2000 (41). This development has reduced cost, enhanced ease of operation, and improved the commercial production of mass spectrometers with interchangeable MALDI and ESI sources. The mechanisms by which ions are formed in MADLI are still not fully understood

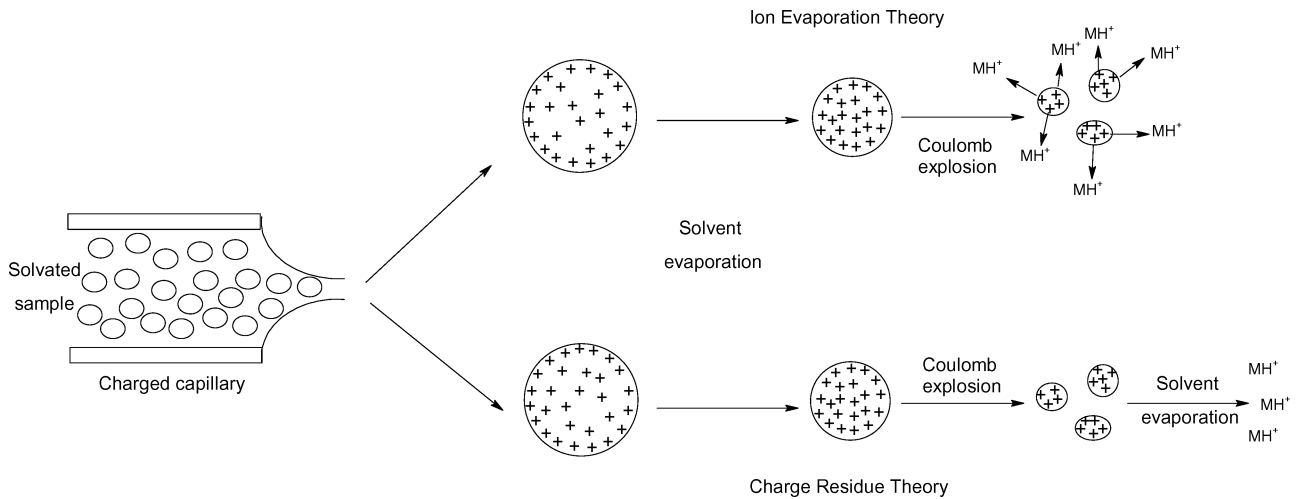

Figure 2. The formation of ions during electrospray ionization. The sample solution is passed through a charged capillary, resulting in the creation of charged droplets that evaporate with the aid of a stream of nitrogen. Coulomb explosion will break the highly charged droplets into smaller ones and eventually ions are desorbed from the surface according to the ion evaporation theory or solvent is completely evaporated as speculated based on the charge residue theory. This scheme represents ions as positively charged; the same mechanism applies in the case of negative ion mode.

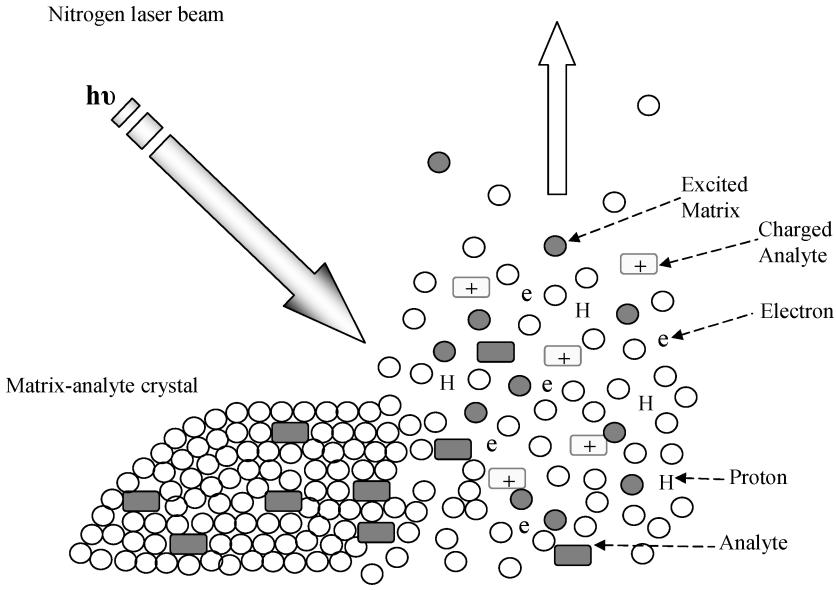

Figure 3. MALDI ionization process. The matrix-analyte crystal is bombarded with a UV laser beam that excites the matrix, which, in turn, transfers the energy to the analytes. This results in the ionization and desorption of the analytes, mainly as singly charged species.

and the choice of a specific matrix is mainly experimental. In addition to its dependency on the nature of the analytes, the choice of the matrix can also be influenced by the ionization mode, whether positive or negative. Basic matrices are favored in the case of the latter, whereas acidic ones are more efficient in the case of the former (i.e., proton donor) (1, 30).

Comparison between MALDI and ESI

Both ESI and MALDI are very sensitive analytical techniques utilizing analyte concentrations that are as low as picomolar. One of the main differences, however, between MALDI and ESI is the state in which the sample is introduced to the ion source. ESI uses solvated sample that is infused into the instrument, whereas MALDI uses the solid state. Therefore, when interfaced with LC, it is possible to efficiently utilize ESI for quantitative measurements (42). Despite the fact that ESI is capable of reproducing data better than MALDI, it should be noted that relative abundance of various ions in an ESI spectrum is not a real representation of the sample concentration. Hence, a standard solution (when the objective is quantification) should be prepared with suitable calibration,

preferably with the use of an isotopic analogue of the analyte (42). There were some attempts to expand the usage of MALDI from merely qualitative toward quantitative measurements (43, 44) and it has been recently interfaced with LC (45). However, the heterogeneity of a MALDI crystal sample poses a major difficulty for such an application. In fact, the quality of MALDI spectra can be significantly influenced by the position of the laser beam and the operator should identify a "sweet spot" within the sample crystal that can produce the most informative spectrum.

Attempts to interface MALDI to LC are, to some degree, related to the robustness of MALDI to the modest presence of salts and/or detergents (46, 47). In contrast, when using ESI, contaminants, adsorbed to the tubes through which a sample is infused into the ESI needle, can compete with the analytes and may influence the results. Such a problem is not encountered in MADLI because analytes can escape impurities during ionization in the same fashion they escape the bulk of the matrix. Finally, ESI tends to produce multiply charged species for biomolecules, such as proteins and peptides. This is the reason why ESI can, theoretically, have unlimited mass range, because very large proteins can appear at lower m/z values. MALDI, however, tends to produce singly charged species and this phenomenon is of great importance for identifying the molecular ion of proteins, carbohydrates and lipids. Karas and coworkers hypothesized that in MALDI-MS, neutralization of multiple charged species occurs due to the production of neutralizing electrons during the photoionization process and, hence, singly charged ions are "the lucky survivors" (48).

MALDI and ESI applications continue to grow within the scientific community and are the basis for newer ionization sources that can be used for specific purposes. Surface-enhanced laser desorption ionization (SELDI), for example, was a development of MALDI (49) and it combines the power of MALDI with the selectivity of a protein chip technique. It is currently being optimized for cancer diagnosis (50) and biomarker discovery (51). SELDI applications are not limited to human health; a recent investigation showed the usefulness of SELDI-MS profiling as a diagnostic tool in Atlantic salmon (52). More recently, a desorption electrospray ionization (DESI) source was developed and mass spectra can now be recorded on a sample in its native environment (53, 54).

MASS ANALYZERS

A mass analyzer is the part of the instrument in which ions are separated based on their m/z values. In a mass spectrometer, the isolation of ions is usually electrically driven, although traditional analyzers, namely, magnetic sectors, employ a magnetic field that influences ion separation. Similar to the ionization process in terms of the available methodologies, there are numerous systems

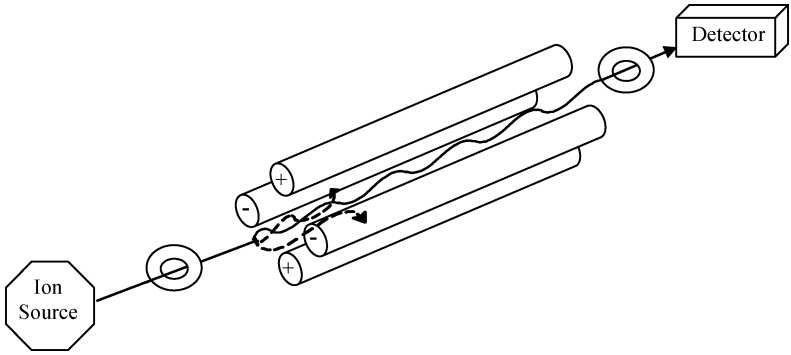

Figure 4. Representation of a quadrupole mass analyzer. Four parallel electrical rods with varying direct current and alternating radio-frequency potentials. Only one m/z value will possess the "right" trajectory and survive the path to reach the detector (solid line). The rest will collide with rods and will be ejected (dotted line).

that can isolate ions based on their m/z values. Currently, four main analyzers are widely used by mass spectroscopists, namely, quadrupole (Q), quadrupole ion trap (QIT), time of flight (ToF), and Fourier transform ion cyclotrone resonance (FT-ICR). These analyzers vary in terms of size, price, resolution, mass range, and the ability to perform tandem mass spectrometry experiments (MS/MS). Though QIT is capable of multiple mass spectrometric experiments (MS^n), FT-ICR is very powerful in terms of accurate mass measurements (55).

The following sections will focus on quadrupole and time of flight analyzers and illustrate the development of a hybrid instrument that link a quadrupole to a ToF analyzer with MS/MS capability (i.e., Q-ToF instrument).

Quadrupole Analyzer

The principle of a quadrupole mass analyzer was first described in the 1950s by Nobel Prize–winning physicist Paul Wolfgang and this ion filter was well suited for GC-MS instrumentation (5). This analyzer is composed of four parallel electrical rods (with a circular cross section), as shown in Figure 4. A direct current (DC) potential (U) is applied to two of these rods, and the other two are linked to an alternating radio-frequency (rf) potential (the potential is termed V, and the frequency is termed ω). Ions, formed in the ionization chamber, are pulsed toward a quadrupole by an electrical field in the range of 5 Kv. A positively charged ion, for example, will move in the direction of the negatively charged rod. However, once the polarity is changed, the ion will switch its movement path before striking the rod. In such a situation, ions will undergo complex oscillation (trajectory) and with the appropriate values of V, U, and ω, only ions within a narrow range of m/z will survive the path

toward the detector. The remaining ions will possess the "wrong" trajectory and will eventually collide with one of the rods. The ramping of V, U, and ω values can result in the transmission of various ions (with different m/z) toward the detector (1, 56). This simplified illustration of a quadrupole is based on a complicated second-order differential equation, known as the Mathieu equation (57).

The major advantages of quadrupole analyzers are the low cost, relatively small size, robustness, and ease of maintenance. A quadrupole possesses, however, limited capability in terms of mass range (usually <4000 Da), resolving power, and the ability to perform MS/MS analysis. The final disadvantage can be overcome by attaching a quadrupole to other analyzers such as additional quadrupoles (triple quadrupole instrument) or a quadrupole linked to a ToF (Q-ToF). An rf-only quadrupole (especially in a hybrid mass spectrometer) will function as an ion focusing device that guides ions to other components of the apparatus. Such functionality can be significantly improved with hexapoles and octapoles, which cannot, however, perform as ion filters.

Time of Flight (ToF) Analyzer

This ion separation methodology is one of the simplest and although it was first described in the middle of the 20th century (58), it was not until the 1990s (59) when it was rediscovered. ToF simply relies on the free flight of the ionized molecules in a tube of 1–2 m in length, before reaching the detector. As seen in Figure 5, if two ions (A_1 and A_2) are formed at the same time with the same charge but the mass of $A_1 < A_2$, A_1 will reach the detector before A_2. The main advantage of a ToF analyzer is that all formed ions will eventually reach the detector (unlike quadrupole or sector instruments). The equation that correlates m/z with total time of flight (t_f) is expressed in the following formula (60):

$$m/z = t_f^2\ 2\ E\ s/(2s + x)$$

where E is the voltage applied, s is the length of the ion acceleration region, and x is the length of the free flight region. Theoretically E, s, and x are fixed; therefore, the above equation can be reduced to

$$m/z = K\ t_f^2$$

where K is the calibrating factor. This equation illustrates the direct relationship between the m/z value and the time of flight.

Though ToF has the advantage of being able to detect a very high mass range, the tube linearity of a conventional ToF analyzer (Figure 5a) can influence its resolving power, because ions entering the ToF carry different kinetic energies (KE) and this subsequently will affect resolution and molecular ion

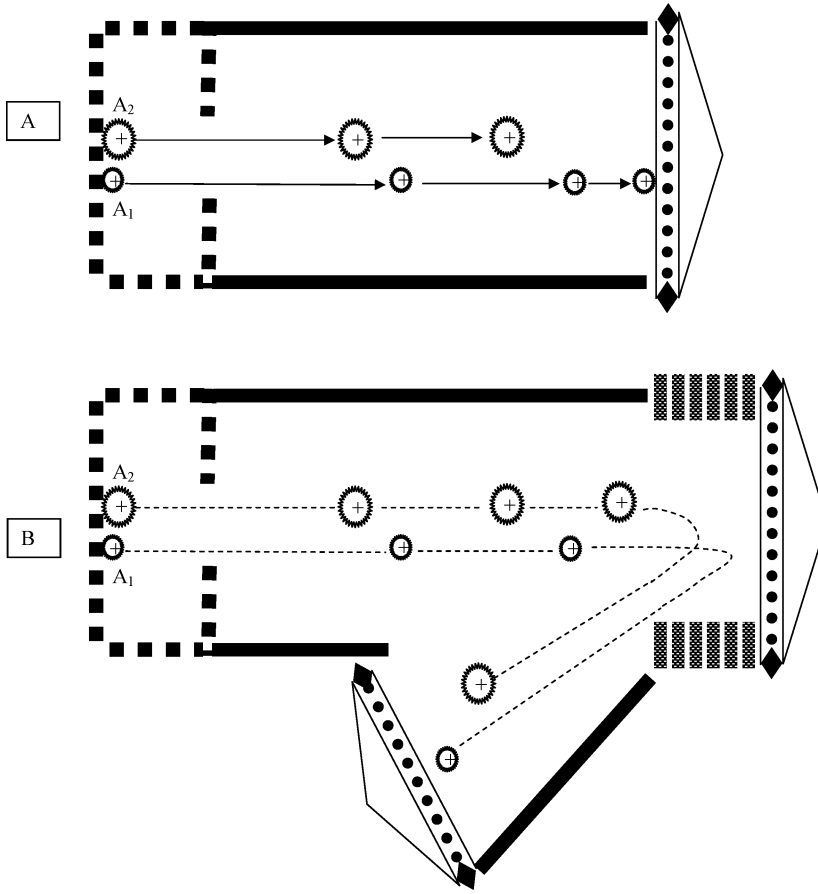

Figure 5. Schematic representation of a conventional ToF (time-of-flight) analyzer (A) and a reflectron-type ToF (B).

measurements. This drawback was overcome by the development of the reflectron (electrostatic ion mirror) (61), which is an ion optic device that changes the path of the ions within the ToF, as shown in Figure 5b. Ions with higher KE will penetrate deeper into the ion mirror and hence ions will be gradually repelled, improving the resolution of the ToF spectrum. Another factor is the tube length; because ions (in reflectron-ToF) are traveling a longer path, better resolution as well as accurate mass measurements can be obtained due to the increase in the flight time (62). Reflectron-type mass analyzers usually have two detectors: the first is beyond the ion mirror, and the second is at the end of the ion path. Due to the fact that a reflectron can result in ion loss, the operator can decide whether to compromise resolution or sensitivity. Due to the nature of ToF, it is widely linked to the MALDI ion source (i.e., MALDI-ToF). Nevertheless, ToF

can also be efficiently attached to an ESI source in hybrid instruments or orthogonal ToF, capable of performing tandem mass spectrometric experiments. These ionization sources in conjunction with mass analyzers such as the time of flight permit the detection of macromolecules that go well beyond the mega Dalton mass range.

Tandem Mass Spectrometry (MS/MS)

Both single-stage ESI-MS and MALDI-MS are very valuable for molecular ion determination and can provide, under certain circumstances, useful structural information. These "circumstances" refer to the induction of fragmentation within the source, such as "post source decay" (63) and "in source fragmentation" (64) in the case of MALDI and ESI, respectively. Tandem mass spectrometry relies on the isolation of a specific m/z (i.e., precursor ion) that can be then subjected to dissociation and subsequent production of fragment or product ions. The user's task is to solve the "puzzle" created with an MS/MS spectrum, revealing valuable information with respect to the molecular structure of the analyte.

To achieve this goal, multiple mass analyzers can be connected in a series, so that ion isolation is performed by the first analyzer followed by ion fragmentation in the collision cell, whereas the final analyzer separates fragment ions based on their m/z values. Figure 6 illustrates the differences between one-stage mass spectrometry and MS/MS instrumentation. In fact, various combinations of mass analyzers can be assembled including sectors, quadrupoles, and ToF (65–67). Such MS/MS instruments are considered tandem-in-space because the analysis is performed by different mass analyzers "in different spaces." Tandem-in-time, however, refers to trapping instruments where all ions are ejected except for one m/z that will be subsequently fragmented "in the same space." These instruments include ITQ and FT-MS, both of which have one analyzer and can perform multiple MS experiments (MS^n), which are powerful tools for structural studies. It was shown, for example, that ion intensity is the only limitation to the extent of an MS^n analysis in an ITQ mass spectrometer (68).

One of the commonly used tandem-in-space mass spectrometers is the triple quadrupole instrument, introduced in late 1970s (69). This instrumentation is currently abbreviated QqQ, where the lower q refers to the collision cell (an rf-only quadrupole). The selected ion for MS/MS analysis is filtered by the first quadrupole and undergoes collision with a stream of inert gas (e.g., helium, nitrogen, argon, xenon) within the collision cell. Such a collision will result in the transfer of some kinetic energy to internal one, causing ions to fragment. This process is referred to as *collisionally activated dissociation* (CAD) or *collision-induced dissociation* (CID) (70, 71). CID can in turn be divided into high and low energy; the latter is associated with QqQ and Q-ToF (introduced

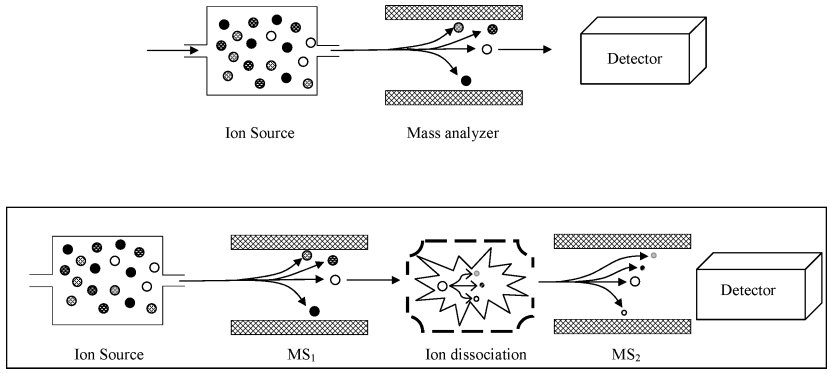

Figure 6. Comparison of one-stage mass spectrometry (top) and tandem mass spectrometry (bottom). In one-stage mass spectrometry, ions formed in the ion source are separated in the mass analyzer before reaching the detector. In tandem mass spectrometry, a specific ion is selected in the first mass analyzer (MS_1) and then subjected to collision in the collision cell, whereas the second analyzer (MS_2) separates the product ions before they reach the detector.

below). High CID is observed with sector instruments that operate at high accelerating potential (in the order of kilo electrvolts; keV) and it has been reported that charge-driven fragmentation is dominant in case of high-energy CID (72). The accelerating potential is, however, less than 100 eV in the case of low-energy CID and it was shown that neutral losses are the main mechanism by which fatty acids, for example, are fragmented (73). CID is not the only process for precursor ion fragmentation, as alternative methods were developed including, for example, surface induced dissociation (74) and electron capture-induced dissociation (75). Discussions about these ion fragmentation methods are beyond the scope of this article.

In a QqQ mass spectrometer, three quadruples are connected; however, an MS/MS, which carries different analyzers, is termed a *hybrid mass spectrometer*. One of the most widely used ones, capable of low-energy CID analysis, is the Q-ToF mass spectrometer.

Hybrid Quadrupole Orthogonal Time-of-Flight Mass Spectrometry (Q-ToF)

The Q-ToF instrument was first described in 1996 (76) as a means of combining the scanning capabilities of a quadrupole and the resolving power of a ToF analyzer. It can provide high-quality, informative, simple, one-stage MS and tandem MS/MS spectra. Readers can refer to an in-depth recent review (77)

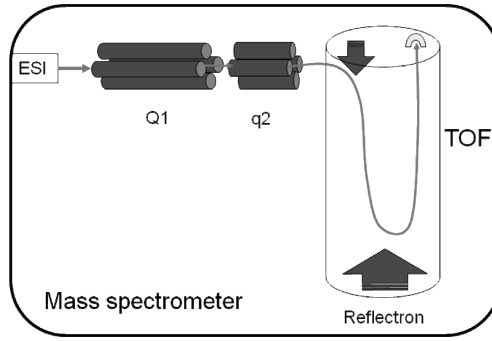

Figure 7. Schematic representation a Q-ToF hybrid instrument with ESI source.

surrounding the development of hybrid instruments over the last 30 years, including Q-ToF instrumentations.

As an example, a diagrammatic representation of a Q-ToF instrument is shown in Figure 7. As seen in this figure, this mass spectrometer is composed of two quadrupoles linked to a ToF analyzer that is geometrically aligned in the orthogonal configuration with respect to the quadrupoles, and, hence, the name Q-ToF orthogonal mass spectrometry. It should be noted that Q-ToF instruments usually include an additional quadrupole before Q_1 as an ion focusing device to provide collisional cooling of the ions so that the quality of the ion beam is improved (78). This quadrupole is usually referred to as q_0 and operates in the rf-only mode similar to q_2, which is the collision cell where low-energy CID fragmentation occurs. Q_1 quadrupole, on the other hand, is the ion filter portion of the instrument, used during MS/MS analysis. All quadrupoles operate in the rf-only mode during simple single-stage MS analysis. The utilization of the Q_1 as the analyzer, instead of the ToF, is only used for tuning the instrument because the ToF is more efficient for this purpose. Due to the crucial role of both the Q_1 and q_2 quadrupoles during MS/MS analysis, these instruments are usually referred to as QqToF mass spectrometers, where the first Q refers to the mass-resolving quadrupole and the second q indicates the collision cell (rf-only quadrupole or hexapole).

One of the major advantages of Q-ToF instrumentation is its ability to be interfaced with either ESI or MALDI with little manipulation of the configuration. The association with MALDI is of great importance because it allows for performing MS/MS experiments. Due to the inherent limitation of a quadrupole in terms of mass range, there are, however, difficulties in orthogonal injection of large singly charged ions to the ToF.

Other advantages of QqToF instruments include ease of operation, high resolution, high mass accuracy, and up to 100-fold increase in sensitivity when compared to triple quadrupole (79). Nevertheless, QqQ instruments are still

favored for quantitative studies as well as for precursor ion scans in which the "parent" ion of a specific fragment can be identified. In such an analysis (with QqQ), the final quadrupole is fixed, whereas the first one is scanning. Such functionality cannot be as efficiently obtained with QqToF instruments as with QqQ mass spectrometers.

We have successfully used QqToF instrument in the structural characterizations of numerous biological and pharmaceutical compounds. We showed that lipid A, isolated from the *A. salmonicida* lipopolysaccharide, had two phosphate groups at position *O*-1 and *O*-4′. In addition, both D-GlcN units of the β-D-(1→6) disaccharide were *O*- and *N*-substituted with various fatty acids, which were identified to be C12:0 and C14 (3-OH). MS/MS analysis illustrated the presence of two C14:0 (3-OH) fatty acids on the reducing end group and two C14:0(3-O(12:0)) on the non-reducing end group of the disaccharide (80). Similarly, the ESI-QqToF-MS/MS has assisted in the complete structural characterization of the core oligosaccharide containing an *O*-4 phosphorylated and *O*-5 glycosylated Kdo reducing end group (81). It was shown for the first time through MS/MS that the Kdo unit is indeed phosphorylated at position *O*-4.

More recently, we reported an MS evidence for the formation of an unprecedented *C*-glycosylation reaction during the ESI-MS and MS/MS analysis of a serious of novel synthetic liposomal neoglycolipids (82). Such a reaction does not occur easily and requires reasonable efforts on a bench-top in a chemistry lab. It occurred, however, in a timeframe of milliseconds within the mass spectrometer. We and Eberlin's research groups are the first to show that such reactions can occur under atmospheric pressure in the ESI source (83, 84).

SUMMARY

Mass spectrometry is evolving into an increasingly powerful and useful tool that can analyze traces of biological samples and identify the structure of unknown proteins and other biological extracts. It complements other traditional biochemical methods and instruments such as NMR. Mass spectrometers are currently available in numerous configurations with various ionization techniques and mass analyzers. Each seems to be optimal for a specific series of compounds or applications. It is therefore not surprising that many journals dedicate their complete contents to the new knowledge surrounding mass spectrometric studies. Mass spectrometry can help answer two continuously asked questions in life science: what and how much. Science around mass spectrometry will continue to attract researchers either to develop new MS applications or to create new hardware or software components.

REFERENCES

1. Henderson, W. and McIndoe, J.S. (2005) *Mass Spectrometry of Inorganic and Organometallic Compounds*. John Wiley & Sons: Chichester.
2. Banoub, J.H., Newton, R.P., Esmans, E., Ewing, D.F., and Mackenzie, G. (2005) Recent developments in mass spectrometry for the characterization of nucleosides, nucleotides, oligonucleotides, and nucleic acids. *Chem. Rev.*, 105: 1869–1915.
3. Korfmacher, W.A., Ed. (2005) *Using Mass Spectrometry for Drug Metabolism Studies*. CRC Press: Boca Raton, FL.
4. Cho, W.C. (2007) Proteomics technologies and challenges. *Genom. Proteom. Bioinformat.*, 5: 77–85.
5. Grayson, M.A., Ed. (2002) *Measuring Mass: From Positive Rays to Proteins*. Chemical Heritage Press: Philadelphia.
6. Hoffmann, E. and Stroobant, V. (2007) *Mass Spectrometry: Principles and Applications*, 3rd ed. John Wiley & Sons: Chicester, England.
7. Thomson, J.J. (1913) *Rays of Postive Electricity and their Applications to Chemical Analysis*. Longmans, Greens and Co.: London.
8. Aston, F.W. (1942) *Mass Spectra and Isotopes*. Longmans, Green and Co.: New York.
9. James, A.T. and Martin, A.J.P. (1952) Gas-liquid partition chromatography: The separation and microestimation of volatile fatty acids from formic acid to dodecanoic acid. *Biochem. J.*, 50: 679–690.
10. Holmes, J.C. and Morrell, F.A. (1957) Ocillographic mass spectrometric monitoring of gas chromatography. *Appl. Spectros.*, 11: 86–87.
11. Kitson, F.G., Larsen, B.S., and McEwen, C.N. (1996) *Gas Chromatography and Mass Spectrometry: A Practical Guide*. Academic Press: San Diego.
12. Herbert, C.G. and Johnstone, R.A.W. (2003) *Mass Spectrometry Basics*. CRC Press: Boca Raton, FL.
13. Munson, M.S.B. and Field, F.H. (1966) Chemical ionization mass spectrometry. I. General introduction. *J. Am. Chem. Soc.*, 88: 2621–2630.
14. Torgerson, D.F., Skowronski, R.P., and Macfarlane, R.D. (1974) New approach to the mass spectroscopy of non-volatile compounds. *Biochem. Biophys. Res. Comm.*, 60: 616–621.
15. Beckey, H.D. (1969) Field desorption mass spectrometry: A technique for the study of thermally unstable substances of low volatility. *Int. J. Mass Spectrom. Ion Phys.*, 2: 500–503.
16. Inghram, M.G. and Gomer, R. (1954) Mass spectrometric analysis of ions from the field microscope. *J. Chem. Phys.*, 22: 1279–1280.
17. Barber, M., Bordoli, R.S., Sedgwick, R.D., and Tyler, A. (1981) Fast atom bombardment of solids as an ion source in mass spectrometry. *Nature*, 293: 270–275.
18. Morris, H.R., Panico, M., Barber, M., Bordoli, R.S., Sedgwick, R.D., and Tyler, A. (1981) Fast atom bombardment: A new mass spectrometric method for peptide sequence analysis. *Biochem. Biophys. Res. Comm.*, 101: 623–631.
19. Shackleton, C.H. and Straub, K.M. (1982) Direct analysis of steroid conjugates: The use of secondary ion mass spectrometry. *Steroids*, 40: 35–51.
20. Tanaka, K., Waki, Y., Ido, Y., Akita, S., Yoshida, Y., and Yoshida, T. (1988) Protein and polymer analyses up to m/z 100,000 by laser ionization time-of-flight mass spectrometry. *Rapid Comm. Mass Spectrom.*, 2: 151–153.

21. Karas, M. and Hillenkamp, F. (1988) Laser desorption ionization of proteins with molecular masses exceeding 10,000 Daltons. *Anal. Chem.*, 60: 2299–2301.
22. Yamashita, M. and Fenn, J.B. (1984) Electrospray ion source. Another variation on the free-jet theme. *J. Phys. Chem.*, 88: 4451–4459.
23. Yamashita, M. and Fenn, J.B. (1984) Negative ion source production with electrospray ion source. *J. Phys. Chem.*, 88: 4671–4675.
24. Hillenkamp, F. and Karas, M. (1990) Mass spectrometry of peptides and proteins by matrix-assisted ultraviolet laser desorption/ionization. *Meth. Enzymol.*, 193: 280–295.
25. Hillenkamp, F., Karas, M., Beavis, R.C., and Chait, B.T. (1991) Matrix-assisted laser desorption/ionization mass spectrometry of biopolymers. *Anal. Chem.*, 63: 1193A–1203A.
26. Dole, M., Mach, L., Hines, R.L., Mobley, R.C., Ferguson, L.D., and Alice, M.B. (1968) Molecular beams of macroions. *J. Chem. Phys.*, 49: 2240–2247.
27. Pramanik, B.N., Ganguly, A.K., and Gross, M.L. (Eds.). (2002) *Applied Electrospray Mass Spectrometry*. Marcel Dekker: New York.
28. Iribarne, J.V. and Thomson, B.A. (1976) One the evaporation of small ions from charged droplets. *J. Chem. Phys.*, 64: 2287–2294.
29. Kebarle, P. (2000) A brief overview of the present status of the mechanisms involved in electrospray mass spectrometry. *J. Mass Spectrom.*, 35: 804–817.
30. Baldwin, M.A. (2005) Mass spectrometers for the analysis of biomolecules. *Meth. Enzymol.*, 402: 3–48.
31. Griffiths, W.J., Jonsson, A.P., Liu, S., Rai, D.K., and Wang, Y. (2001) Electrospray and tandem mass spectrometry in biochemistry. *Biochem. J.*, 355: 545–561.
32. Whitehouse, C.M., Dreyer, R.N., Yamashita, M., and Fenn, J.B. (1985) Electrospray interface for liquid chromatographs and mass spectrometers. *Anal. Chem.*, 57: 675–679.
33. Emmett, M.R. and Caprioli, R.M. (1994) Micro electrospray mass spectrometry: Ultra-high sensitivity analysis of peptides and proteins. *J. Am. Chem. Soc. Mass Spectrom.*, 5: 605–613.
34. Gale, D.C. and Smith, R.D. (1993) Small volume and low flow-rate electrospray ionization mass spectrometry of aqueous samples. *Rapid Comm. Mass Spectrom.*, 7: 1017–1021.
35. Karas, M., Bahr, U., and Dulcks, T. (2000) Nano-electrospray ionization mass spectrometry: Addressing analytical problems beyond routine. *Fresen. J. Anal. Chem.*, 366: 669–676.
36. Banoub, J., Thibault, P., Mansour, A., Cohen, A., Heeley, D.H., and Jackman, D. (2003) Characterisation of the intact rainbow trout vitellogenin protein and analysis of its derived tryptic and cyanogen bromide peptides by matrix-assisted laser desorption/ionisation time-of-flight-mass spectrometry and electrospray ionisation quadrupole/time-of-flight mass spectrometry. *Eur. J. Mass Spectrom. (Chichester, Eng).*, 9: 509–524.
37. Banoub, J., Cohen, A., Mansour, A., and Thibault, P. (2004) Characterization and de novo sequencing of Atlantic salmon vitellogenin protein by electrospray tandem and matrix-assisted laser desorption/ionization mass spectrometry. *Eur J Mass Spectrom.*, 10: 121–134.

38. Gut, I.G. (2004) DNA analysis by MALDI-TOF mass spectrometry. *Hum. Mutat.*, 23: 437–441.
39. Balazy, M. (2004) Eicosanomics: Targeted lipidomics of eicosanoids in biological systems. *Prostag. Other Lipid Mediat.*, 73: 173–180.
40. Harvey, D.J. (2003) Matrix-assisted laser desorption/ionization mass spectrometry of carbohydrates and glycoconjugates. *Int. J. Mass Spectrom.*, 226: 1–35.
41. Laiko, V.V., Baldwin, M.A., and Burlingame, A.L. (2000) Atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry. *Anal. Chem.*, 72: 652–657.
42. Cohen, A.M., Mansour, A.A., and Banoub, J.H. (2006) Absolute quantification of Atlantic salmon and rainbow trout vitellogenin by the "signature peptide" approach using electrospray ionization QqToF tandem mass spectrometry. *J. Mass Spectrom.*, 41: 646–658.
43. Biroccio, A., Urbani, A., Massoud, R., di Ilio, C., Sacchetta, P., Bernardini, S., Cortese, C., and Federici, G. (2005) A quantitative method for the analysis of glycated and glutathionylated hemoglobin by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *Anal. Biochem.*, 336: 279–288.
44. Grant, G.A., Frison, S.L., Yeung, J., Vasanthan, T., and Sporns, P. (2003) Comparison of MALDI-TOF mass spectrometric to enzyme colorimetric quantification of glucose from enzyme-hydrolyzed starch. *J. Agr. Food Chem.*, 51: 6137–6144.
45. Fung, K.Y., Askovic, S., Basile, F., and Duncan, M.W. (2004) A simple and inexpensive approach to interfacing high-performance liquid chromatography and matrix-assisted laser desorption/ionization-time of flight-mass spectrometry. *Proteomics*, 4: 3121–3127.
46. Breaux, G.A., Green-Church, K.B., France, A., and Limbach, P.A. (2000) Surfactant-aided, matrix-assisted laser desorption/ionization mass spectrometry of hydrophobic and hydrophilic peptides. *Anal. Chem.*, 72: 1169–1174.
47. Kallweit, U., Bornsen, K.O., Kresbach, G.M., and Widmer, H.M. (1996) Matrix compatible buffers for analysis of proteins with matrix-assisted laser desorption ionization mass spectrometry. *Rapid Comm. Mass Spectrom.*, 10: 845–849.
48. Karas, M., Gluckmann, M., and Schafer, J. (2000) Ionization in matrix-assisted laser desorption/ionization: Singly charged molecular ions are the lucky survivors. *J. Mass Spectrom.*, 35: 1–12.
49. Hutchens, T.W. and Yip, T.T. (1993) New desorption strategies for mass spectrometric analysis of macromolecules. *Rapid Comm. Mass Spectrom.*, 7: 576–580.
50. Semmes, O.J., Feng, Z., Adam, B.L., Banez, L.L., Bigbee, W.L., Campos, D., Cazares, L.H., Chan, D.W., Grizzle, W.E., Izbicka, E., Kagan, J., Malik, G., McLerran, D., Moul, J.W., Partin, A., Prasanna, P., Rosenzweig, J., Sokoll, L.J., Srivastava, S., Srivastava, S., Thompson, I., Welsh, M.J., White, N., Winget, M., Yasui, Y., Zhang, Z., and Zhu, L. (2005) Evaluation of serum protein profiling by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry for the detection of prostate cancer: I. Assessment of platform reproducibility. *Clin. Chem.*, 51: 102–112.
51. El Aneed, A. and Banoub, J. (2006) Proteomics in the diagnosis of hepatocellular carcinoma: Focus on high risk hepatitis B and C patients. *Anticancer Res.* 2006, 26(5A): 3293–3300.
52. Provan, F., Bjornstad, A., Pampanin, D.M., Lyng, E., Fontanillas, R., Andersen, O.K., Koppe, W., and Bamber, S. (2006) Mass spectrometric profiling—A diagnostic tool in fish? *Mar. Environ. Res.*, 62 (Suppl.): S105–S108.

53. Cooks, R.G., Ouyang, Z., Takats, Z., and Wiseman, J.M. (2006) Detection technologies. Ambient mass spectrometry. *Science*, 311: 1566–1570.
54. Takats, Z., Wiseman, J.M., Gologan, B., and Cooks, R.G. (2004) Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science*, 306: 471–473.
55. Pasa-Tolic, L., Masselon, C., Barry, R.C., Shen, Y., and Smith, R.D. (2004) Proteomic analyses using an accurate mass and time tag strategy. *Biotechniques*, 37: 621–624, 626–633, 636 passim.
56. Downard, K. (2004) *Mass Spectrometry: A Foundation Course*. TJ International Ltd.: Cornwall.
57. March, R.E., Hughes, R.J. (1989) *Quadrupole Storage Mass Spectrometry*. John Wiley & Sons: New York.
58. Stephens, W.E. (1946) A pulsed mass spectrometer with time dispersion. *Phys. Rev.*, 69: 691.
59. Brown, R.S. and Lennon, J.J. (1995) Mass resolution improvement by incorporation of pulsed ion extraction in a matrix-assisted laser desorption/ionization linear time-of-flight mass spectrometer. *Anal. Chem.*, 67: 1998–2003.
60. Merchant, M. and Weinberger, S.R. (2000) Recent advancements in surface-enhanced laser desorption/ionization-time of flight-mass spectrometry. *Electrophoresis*, 21: 1164–1177.
61. Mamyrin, B.A. and Shmikk, D.V. (1979) The linear mass reflectron. *Sov. Phys. JETP*, 49: 762–764.
62. Clauser, K.R., Baker, P., and Burlingame, A.L. (1999) Role of accurate mass measurement (+/−10 ppm) in protein identification strategies employing MS Or MS/MS and database searching. *Anal. Chem.*, 71: 2871–2882.
63. Purcell, A.W. and Gorman, J.J. (2001) The use of post-source decay in matrix-assisted laser desorption/ionisation mass spectrometry to delineate T cell determinants. *J. Immunol. Meth.*, 249: 17–31.
64. Joly, N., El-Aneed, A., Martin, P., Cecchelli, R., and Banoub, J. (2005) Structural determination of the novel fragmentation routes of morphine opiate receptor antagonists using electrospray ionization quadrupole time-of-flight tandem mass spectrometry. *Rapid Comm. Mass Spectrom.*, 19: 3119–3130.
65. Chernushevich, I.V., Loboda, A.V., and Thomson, B.A. (2001) An introduction to quadrupole-time-of-flight mass spectrometry. *J. Mass Spectrom.*, 36: 849–865.
66. Shukla, A.K. and Futrell, J.H. (2000) Tandem mass spectrometry: Dissociation of ions by collisional activation. *J. Mass Spectrom.*, 35: 1069–1090.
67. Vestal, M.L. and Campbell, J.M. (2005) Tandem time-of-flight mass spectrometry. *Meth. Enzymol.*, 402: 79–108.
68. Louris, J.N., Brodbelt-Lusting, J.S., Cooks, R.G., Glish, G.L., van Berkel, G.J., and McLuckey, S.A. (1990) Ion isolation and sequential stages of mass spectrometry in a quadrupole ion trap mass spectrometer. *Int. J. Mass Spectrom. Ion Process.*, 96: 117–137.
69. Yost, R.A. and Enke, C.G. (1978) Selected ion fragmentation with a tandem quadrupole mass spectrometer. *J. Am. Chem. Soc.*, 100: 2274–2275.
70. Jennings, K.R. (1968) Collision-induced decompositions of aromatic molecular ions. *Int J. Mass Spectrom. Ion Phys.*, 1: 227–235.
71. McLafferty, F.W., Bente, P.F., Kornfeld, R., Tsai, S., and Howe, I. (1972) Metastable ion characteristics. XXII. Collisional activation spectra of organic ions. *J. Am. Chem. Soc.*, 95: 2120–2129.

72. Gross, M.L. (1992) Charge-remote fragmentations: Method, mechanism and applications. *Int. J. Mass Spectrom. Ion Process.*, 118–119: 137–165.
73. Kerwin, J.L., Wiens, A.M., and Ericsson, L.H. (1996) Identification of fatty acids by electrospray mass spectrometry and tandem mass spectrometry. *J. Mass Spectrom.*, 31, 184–192.
74. Dongre, A.R., Somogyi, A., and Wysocki, V.H. (1996) Surface-induced dissociation: An effective tool to probe structure, energetics and fragmentation mechanisms of protonated peptides. *J. Mass Spectrom.*, 31, 339–350.
75. Kelleher, N.L., Zubarev, R.A., Bush, K., Furie, B., Furie, B.C., McLafferty, F.W., and Walsh, C.T. (1999) Localization of labile posttranslational modifications by electron capture dissociation: The case of gamma-carboxyglutamic acid. *Anal. Chem.*, 71: 4250–4253.
76. Morris, H.R., Paxton, T., Dell, A., Langhorne, J., Berg, M., Bordoli, R.S., Hoyes, J., and Bateman, R.H. (1996) High sensitivity collisionally-activated decomposition tandem mass spectrometry on a novel quadrupole/orthogonal-acceleration time-of-flight mass spectrometer. *Rapid Comm. Mass Spectrom.*, 10: 889–896.
77. Glish, G.L. and Burinsky, D.J. (2008) Hybrid mass spectrometers for tandem mass spectrometry. *J. Am. Soc. Mass Spectrom.*, 19: 161–172.
78. Krutchinsky, A.N., Chernushevich, I.V., Spicer, V., Ens, W., and Standing, K.G. (1998) A collisional damping interface for an electrospray ionization TOF mass spectrometer. *J. Am. Soc. Mass Spectrom.* 9: 569–579.
79. Ens, W. and Standing, K.G. (2005) Hybrid quadrupole/time-of-flight mass spectrometers for analysis of biomolecules. *Meth. Enzymol.*, 402: 49–78.
80. El-Aneed, A. and Banoub, J. (2005) Elucidation of the molecular structure of lipid A isolated from both a rough mutant and a wild strain of *Aeromonas salmonicida* lipopolysaccharides using electrospray ionization quadrupole time-of-flight tandem mass spectrometry. *Rapid Comm. Mass Spectrom.*, 19: 1683–1695.
81. Banoub, J., El Aneed, A., Cohen, A., and Martin, P. (2004) Characterization of the O-4 phosphorylated and O-5 substituted Kdo reducing end group and sequencing of the core oligosaccharide of *Aeromonas salmonicida* ssp *salmonicida* lipopolysaccharide using tandem mass spectrometry. *Eur. J. Mass Spectrom.*, 10: 715–730.
82. El-Aneed, A., Banoub, J., Koen-Alonso, M., Boullanger, P., and Lafont, D. (2007) Establishment of mass spectrometric fingerprints of novel synthetic cholesteryl neoglycolipids: The presence of a unique C-glycoside species during electrospray ionization and during collision-induced dissociation tandem mass spectrometry. *J. Am. Soc. Mass Spectrom.*, 18: 294–310.
83. Banoub, J., Boullanger, P., Lafont, D., Cohen, A., El Aneed, A., and Rowlands, E. (2005) in situ formation of C-glycosides during electrospray ionization tandem mass spectrometry of a series of synthetic amphiphilic cholesteryl polyethoxy neoglycolipids containing N-acetyl-D-glucosamine. *J. Am. Soc. Mass Spectrom.*, 16: 565–570.
84. Meurer, E.C., Sparrapan, R., Tomazela, D.M., Eberlin, M.N., and Augusti, R. (2005) Cyclization reactions of acylium and thioacylium ions with isocyanates and isothiocyanates: Gas phase synthesis of 3,4-dihydro-2,4-dioxo-2H-1,3,5-oxadiazinium ions. *J. Am. Soc. Mass Spectrom.*, 16: 1602–1607.