

Chapter 4

Experimental Methods and Instrumentation

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4.1 Introduction

This chapter discusses a variety of experimental methods and introduces instrumentation for the study of gas-phase ions and ion–molecule chemistry in general and of ion attachment processes in particular, with an emphasis on mass spectrometry (MS) methods. The section starts with a discussion on common methods to generate gas-phase alkali metal ions, to be applied in various ion attachment experiments. Subsequently, (tandem) MS methods are discussed that enable the investigation of the structure of ions and the results of ion–molecule reactions. Separate sections are presented for beam instruments and ion-trapping instruments. In some cases, these instruments can be used to perform ion–molecule reactions as part of the measurement protocol. Emphasis is put on commercially available MS and MS–MS instruments. In subsequent sections, various other tools are discussed that may be combined with MS and allow the study of gas-phase reactions of ions. These tools comprise (a) flowing afterglow methods (FA-MS), including derived methods like selected ion flow tubes (SIFT-MS) and proton-transfer reaction devices (PTR-MS), (b) drift tubes, ion-mobility spectrometry (IMS) and IMS–MS, and (c) high-pressure MS instruments. The text provides ample references for further reading.

Currently, MS is primarily used in analytical applications. In fact, rather than the study of ion structures, gas-phase ion–molecule reactions, or the structure elucidation of unknown compounds, the routine quantitative analysis of target analytes in complex (biological) matrices using combined gas chromatography (GC–MS) or liquid chromatography (LC–MS) is by far the most important application area of MS. Nevertheless, mass spectrometers have proven to be powerful tools for studying the kinetics, mechanisms, and product distributions of gas-phase bi- and termolecular organic reactions. A wide variety of ion–molecule reactions may be studied [1].

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These studies provide us with fundamental understanding of the organic reactions. Gas-phase chemistry can reveal details of reaction mechanisms that are obscured by solvation and ion pairing, when studied in the condensed phase.

4.2 Production of Gas-phase Alkali Metal ions

4.2.1 Thermoionic Emission

Thermoionic emission involves the heat-induced emission of charge-carrying particles from a surface. The process occurs when the thermal energy given to the carrier overcomes the work function of the metal. The charge-carrying particles may be electrons or ions. The emission of electrons, known as the Edison effect, can be achieved from a heated filament in vacuum, like with the hot (tungsten or rhenium) filament used as primary source of ionization in electron ionization. In the current context, the emission of (alkali) metal ions is of more interest. This can be achieved by heating aluminum silicates doped with (alkali) metal oxides [2–5]. A schematic setup of a thermoionic emitter of K^+ mounted onto a solid probe is shown in Fig. 4.1 [5]. In this way, chemical ionization with alkali metal ions as primary ionization source was performed, e.g., studying gas-phase reactions of Li^+ with fluoroethane and hydrocarbons [6] or of K^+ with a wide variety of analytes, including ketones, ethers, esters, crown ethers, and small peptides [5]. Li^+ -emitters are also applied in ion attachment MS experiments [3].

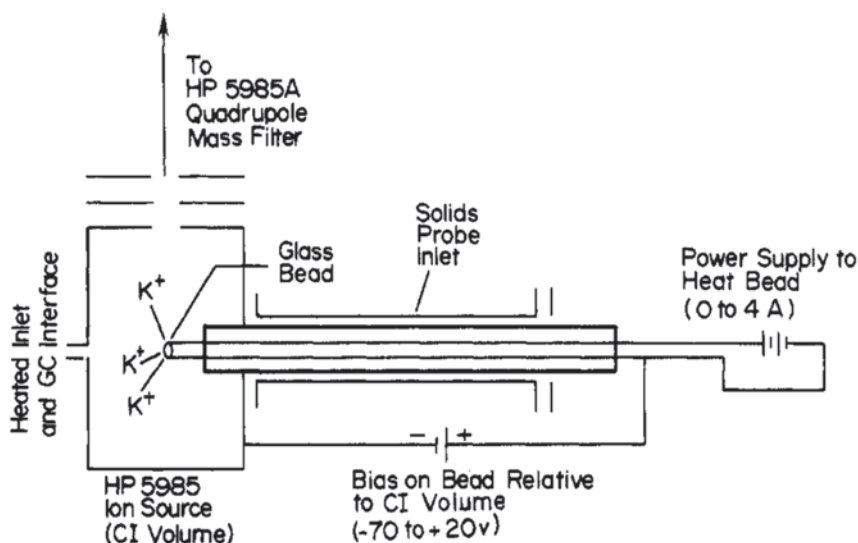


Fig. 4.1 Ion source for thermoionic emission of alkali metal ions. (Reprinted with permission from ref. 5. ©1984, American Chemical Society)

4.2.2 *Laser Ionization*

Laser ablation is the process of removing material from a solid surface by irradiating it with a (pulsed) laser beam. At low laser flux, the material evaporates or sublimates due to heating by the absorbed laser energy. At high laser flux, the material is converted into plasma. If ions are produced in the plasma, the process is usually called laser ionization. In this way, gas-phase metal ions can be produced, which can subsequently be applied in gas-phase ion–molecule studies. Initially, Cu^+ and Ag^+ were generated from their respective metals, and Cr^+ , Fe^+ , and Ni^+ from a stainless steel sample [7]. The method seems to be especially useful to generate gas-phase ions and ion clusters of transition metals [8, 9]. As such, it was, for instance, applied to characterize pollutants on dust particles [10].

4.2.3 *Desorption Ionization: Doping with Alkali Metal Salts*

In desorption ionization methods, like field desorption [11], fast-atom bombardment [12], and matrix-assisted laser desorption (MALDI) [13], adding small amounts of alkali metal salts to the sample on the emission wire (in field desorption) or target (in fast-atom bombardment and MALDI) may result in the observation of adduct ions $[\text{M} + \text{Alkali}]^+$ (see also Sect. 7.2). This is generally termed “doping with alkali metal salts.” In principle, when a platinum or tungsten wire, to be used in thermoionic emission, is doped with alkali metal salts, gas-phase alkali metal ions may be produced as well. It may be questioned whether this is due to a thermoionic or desorption/ionization effect.

A typical example of this type of doping is a fragmentation study of the flavonoid glycoside rutin, adducted with different alkali metal ions (Li^+ , Na^+ , or K^+), by post-source decay MALDI time-of-flight (TOF) MS [14]. Differences in fragment ions and especially the relative abundance of fragment ions were observed (see Sect. 7.5.2). Numerous other examples of this type of studies are available.

4.2.4 *Solvent Additives in Electrospray Ionization*

Adduct formation of analytes to alkali metal ions is also frequently observed in electrospray ionization (ESI) MS [15]. For compounds with high affinities to alkali metal ions, the residual concentrations of 10^{-5} – 10^{-4} M of alkali metal ions, commonly present in solvents used in LC-MS, is sufficient for this type of adduct formation. Alternatively, low concentrations of alkali metal ion salts (up to 1 mM) may be added to the mobile phase; higher concentrations (> 1 mM) result in significant ionization suppression. Because in many applications of ESI-MS compounds are analyzed from biological matrices; these matrices also act as a source of alkali metal ions, especially if no rigorous desalting protocol is adapted.

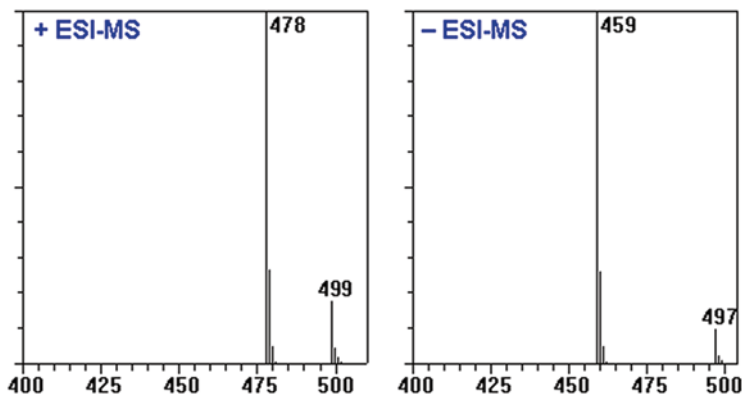


Fig. 4.2 Electrospray mass spectra of a compound with two carboxylic acid functions analyzed as dipotassium salt in a mobile phase containing ammonium acetate. (©2006, hyphen MassSpec)

It appears that adduct formation especially takes place for compounds with a number of oxygen (or sulfur) atoms in such an orientation that chelation or complex formation with the alkali metal ions is possible (see Chap. 7 for a more detailed discussion). This is, for instance, the case with oligosaccharides. However, next to “real” adduct formation, that is formation of $[M+Na]^+$, apparent adduct formation in ESI-MS may be due to liquid-phase H^+/Na^+ -exchange reactions in analytes with acidic functions, resulting in salts which are subsequently transferred to the gas phase as protonated molecules [15]. Thus, the analyte $RCOOH$ is converted into $RCOONa$, protonated, and transferred to the gas-phase for mass analysis as $[(RCOOH-H+Na)+H]^+$ rather than as $[M+Na]^+$ [15, 16]. This behavior is observed with any compound with acidic functions, e.g., peptides and (oligo) nucleotides. The possibility to generate such H^+/Na^+ -exchange reaction products by a gas-phase reaction in the vacuum interface of an ESI-MS system has also been investigated [17]. As expected, methyl ester formation in peptides reduces the adduct formation, because the H^+/Na^+ -exchange is taken away [18].

An example of this behavior is shown in Fig. 4.2, where the positive-ion and negative-ion ESI-MS spectra of a compound are shown with two carboxylic acid functions, analyzed as di-potassium salt in a mobile phase containing ammonium acetate. In positive-ion mode, both the ammoniated molecule $[M+NH_4]^+$ and the potassiated molecule $[M+K]^+$ (or the H^+/K^+ -exchange product $[(M-H+K)+H]^+$) are observed, whereas in negative-ion mode, the H^+/K^+ -exchange product $[(M-H+K)-H]^-$ is observed next to the deprotonated molecule $[M-H]^-$. Thus, apparent alkali metal ion adducts may be observed in negative-ion ESI-MS as well.

4.3 Tandem Mass Spectrometry

4.3.1 Introduction

From instrumental point of view, tandem mass spectrometry (MS–MS) comprises a combination of two mass analyzers in series (in time or space, see below) with a reaction chamber in between. In MS–MS, the m/z values of ions are measured before and after a chemical reaction within the mass spectrometer. In most cases, a change in mass and thus in m/z is involved, although a change in charge, e.g., charge stripping of multiple-charge ions, is also possible. For positive ions, the precursor or parent ion m_p^+ is converted into the product or daughter ion m_d^+ via the loss of a neutral fragment m_n . Whereas the neutral fragment m_n is generally not detected in the mass spectrometer, its mass can be inferred from the difference in m/z of m_p^+ and m_d^+ . In the product-ion analysis mode, which is the most basic MS–MS experiment, the precursor ion m_p^+ is selected in the first stage of mass analysis within the instrument (MS1), while the product ions m_d^+ are mass analyzed and detected in the second stage of mass analysis (MS2). Thus, MS–MS involves the detection of ions that, after their initial formation in the ion source, have undergone a change in m/z and/or charge during the course of their analysis with a mass spectrometer [19].

The observation and explanation in the 1940s of the occurrence of metastable ions in a mass spectrum acquired using a magnetic-sector instrument can be considered as the starting point of the history of MS–MS [20]. In the 1960s, it was discovered that the abundance of the metastable ions can be increased by the introduction of a collision gas in a collision cell. From the mid-1970s onwards, instruments were especially designed for MS–MS experiments. Important landmarks in the development of MS–MS are: (a) the introduction in 1978 of the triple quadrupole (TQ) instrument by Yost and Enke [21]; (b) the demonstration in 1987 of (multiple stages of) MS–MS in an ion trap instrument by Louris et al. [22]; (c) the introduction in the early 1990s of various MS–MS technologies in Fourier transform ion cyclotron resonance (FT-ICR) instrument [23]; (d) the introduction in 1996 of a hybrid quadrupole-time-of-flight instrument (Q-TOF) by Morris et al. [24]; (e) the introduction in 2002 of the hybrid quadrupole-linear ion trap instrument (Q-LIT) by Hager [25]; (f) the introduction in 2002 of tandem TOF–TOF instrument [26], and (g) the introduction in 2005 of the hybrid linear ion trap–Orbitrap instrument by the group of Makarov [27, 28].

Convenient symbolism and terminology for the wide variety of MS–MS and MSⁿ experiments that can be performed in various data acquisition modes have been proposed by Schwartz et al. [29] and are frequently used ever since.

Nowadays, partly initiated by the advent of ESI as a powerful soft ionization technique for highly polar biomolecules and as a convenient method to couple LC and MS, MS–MS is frequently applied. For routine quantitative bioanalysis of target compounds, the selected reaction monitoring (SRM) mode is extensively applied. In the SRM mode, both stages of mass analysis perform the selection of ions with a particular m/z value, i.e., in MS1 a precursor ion, mostly the protonated or

deprotonated molecule of the target analyte, is selected, subjected to dissociation in the collision cell, while in MS2 a preferably structure-specific product ion of the selected precursor is selected and detected. Due to the high selectivity involved in SRM, excellent sensitivity may be achieved in target quantitative analysis. The SRM mode is the method of choice in quantitative bioanalysis using LC-MS [30], e.g., in (pre)clinical studies for drug development within the pharmaceutical industry, and has been implemented in quantitative analytical strategies using GC-MS as well [31].

4.3.2 Ion Dissociation Techniques

Either metastable ions or activated ions may be involved in MS-MS experiments. Metastable ions are ions with sufficient internal energy that survive long enough to be extracted from the ion source before they fragment, but may then fragment in the mass analyzer region prior to detection. The charged fragments of metastable ions that dissociate in the reaction region of the instrument may be detected. Alternatively, ions may be activated after they have left the ion source. Collision activation is the most widely applied method to increase the internal energy of ions. Upon acceleration and collision of an ion with a target gas (He, N₂, or Ar) in a collision cell, part of the ion translational energy is converted into internal energy. If subsequent dissociation of the ion occurs in the collision cell, the process is called collision-induced dissociation (CID). Next to collision activation with a target gas, there is a wide variety of other activation methods [32, 33], including surface-induced dissociation (SID), laser photodissociation (LPD), infrared multiphoton photodissociation (IRMPD), sustained off-resonance irradiation (SORI), black-body infrared radiative dissociation (BIRD), electron-capture dissociation (ECD), and electron-transfer dissociation (ETD) [34]. Most of these alternative techniques are primarily applied to induce fragmentation in FT-ICR-MS instruments, although ETD can also be implemented on ion trap (and other) instruments [34].

The CID, being the most widely applied method to induce fragmentation in MS-MS, is a two-step process, where in the first step, ion translational energy is converted into ion internal energy due to the collision event, while in the second step unimolecular decomposition of the excited ions may yield various product ions by competing reaction pathways. The first step is much faster than the second one. In between the two steps of the process, energy redistribution within the ion may take place. CID can be performed in two different energy regimes [33]. With most instruments, low-energy CID is performed involving multiple collisions with a target gas such as He, N₂, or Ar ($\sim 10^{-3}$ mbar) with a laboratory collision energy generally not exceeding 60 eV. In sector and TOF-TOF instruments, high-energy CID can be performed, which involves single keV collisions with He as a target gas. High-energy collisions open a wider range of fragmentation reactions, thus resulting in more informative and more complex MS-MS spectra. In the low-energy CID regime, one may further discriminate between collision cell CID and ion trap CID. In collision cell CID, that is, in TQ and Q-TOF instruments, after acceleration of the precursor ions with 10–60 V, collisions are performed with N₂ or Ar.

4.3.3 *General Aspects of MS–MS Instrumentation*

The MS–MS instrument comprises a combination of two mass analyzers. The first and second stage of mass analysis may be performed by the same type of mass analyzer, like in a triple quadrupole or an ion trap instrument. In TQ instruments, the three steps of the MS–MS process (precursor ion selection, CID, and mass analysis of product ions) are performed in spatially separated devices (“tandem-in-space”), whereas in an ion trap instrument, the three steps are performed one-after-another in the same device (“tandem-in-time”) [35]. In hybrid instruments, the first and second stage of mass analysis is performed in two different types of mass analyzers, e.g., in a first-stage quadrupole and second-stage TOF in a Q–TOF instrument, or a first-stage quadrupole and second-stage linear ion trap in a Q–LIT instrument.

An MS–MS instrument may be used to study the fragment ions of selected precursor ions and is therefore an indispensable tool in fundamental studies on ion generation, ion–molecule reactions, unimolecular fragmentation reactions, and identity of ions. It also plays an important role in analytical applications of MS, both in qualitative and in quantitative analysis, e.g., in applications involving the online coupling of MS as a detector to GC–MS and LC–MS.

4.3.4 *MS–MS in Sector Instruments*

As indicated before, the basis of MS–MS lies in the observation of metastable ions or, perhaps more accurately, of the fragment ions of metastable ions. In order to detect these ions, linked scan procedures are required. In a magnetic sector instrument, featuring kV-acceleration of ions from the ion source and thus providing significant kinetic energy to the ions, an in-source-generated fragment ion with a particular m/z has a higher kinetic energy than a post-source-generated fragment ion, the so-called metastable ion. Adjustment of the acceleration voltage (V), or the electric (E) and magnetic (B) fields in a double-focusing sector instrument is required to observe the metastable ions or their fragments. In a linked scan, the two fields are automatically adjusted at the same time. In most cases, linked scan procedures are applied in the first field-free region, e.g., E^2/V or B/E to observe the fragment ions and B^2/E to observe the precursor ions [19, 36].

As a result of the introduction of alternative MS–MS instruments, which are more cost-effective and easier to operate, the double-focusing sector instruments are hardly used in MS–MS. The same holds for hybrid MS–MS systems comprising sector instruments combined with quadrupole or ion trap building blocks.

4.3.5 *MS–MS in Tandem Quadrupole Instruments*

Probably, the most widely used MS–MS configuration is the TQ instrument, where mass analysis is performed in the first and third quadrupoles, while the second

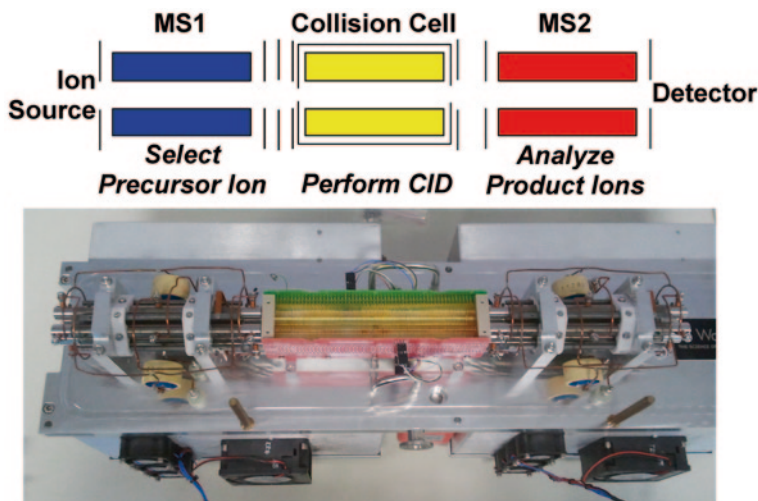


Fig. 4.3 Schematic diagram and photo of tandem quadrupole mass spectrometer. The photo is taken from a Waters tandem-quadrupole instrument, featuring a travelling wave stacked ring RF-only collision cell. (©2013, hyphen MassSpec)

quadrupole is used as collision cell in the radiofrequency (RF)-only mode, i.e., in a $Q-q_{\text{coll}}-Q$ configuration (Fig. 4.3). The TQ instrument was initially developed by Yost and Enke [21] in the late 1970s. Upon its introduction, the TQ instrument yielded significantly better product-ion resolution than the sector instruments, used for MS–MS at that time. The acquisition of a product-ion spectrum was greatly facilitated.

In addition, various structure-specific screening procedure of triple quadrupole instruments were introduced, e.g., the precursor ion and neutral loss analysis mode [37, 38]. In the precursor ion analysis mode, MS1 is operated in scanning or full-spectrum mode, whereas in MS2 a structure-specific product ion is monitored. In the neutral loss analysis mode, both quadrupole mass analyzers are operated in scanning mode, but with a fixed m/z offset corresponding to a structure-specific neutral loss in the fragmentation reaction. These scan modes have been successfully applied for structure-specific screening in order to search for specific compound classes in complex matrices, as demonstrated by the screening for two classes designer drugs in urine [39], or for glutathione and cyanide-trapped reactive drug metabolites [40], to quote two recent examples. Apart from these analytical applications, these analysis modes can be very useful in the study of fragmentation reactions. The precursor ion analysis mode allows to determine which is (or are) the precursor ion(s) of a particular product ion, thus answering the question whether a particular product ion is formed from a particular precursor ion in a one-step dissociation reaction or an intermediate step has been involved. This enables

more detailed studies on fragmentation pathways, as for instance demonstrated for naloxonazine and naloxone, zwitterionic morphine opiate antagonists [41], and for morphine and related opiates [42].

Although usually called a triple quadrupole instrument, because of the initial lineup of two analyzing quadrupoles and a quadrupole collision cell, the term tandem quadrupole (TQ) would nowadays be more appropriate to describe the commercially available instruments. The gas-filled collision cells operated in RF-only mode and enabling refocusing of ions scattered by collisions result in significant transmission losses. In attempts to reduce these losses, alternative collision cells have been implemented by various instrument manufacturers, e.g., RF-only hexapoles or octapoles, a linear acceleration high-pressure collision cell (LINAC) [43], or a stacked ring collision cell (see Fig. 4.3), featuring an axial travelling wave or transient DC voltage to propel the ions and to reduce the transit times [44].

4.3.6 MS–MS in Q–TOF Instruments

The Q–TOF instrument can be considered as a modified TQ instrument, where the third quadrupole has been replaced by an orthogonal acceleration reflectron-TOF mass analyzer. The first commercially available Q–TOF instrument was produced in 1996, especially aiming at peptide sequencing analysis [24], but the instrument has found much wider application, especially in the structure elucidation studies. Q–TOF instruments are now available from various instrument manufacturers. Where fragmentation characteristics in the collision cell are the same for TQ and Q–TOF, a significant advantage of Q–TOF over TQ in structure elucidation is the ability to determine the m/z values of both precursor and product ions with higher mass accuracy (<5 ppm), which is due to the high resolving power of the reflectron-TOF analyzer. Principles and applications of Q–TOF hybrid instruments have been reviewed by Chernushevich et al. [45].

4.3.7 MS–MS in Q–LIT instruments

The hybrid quadrupole-linear ion trap (Q–LIT) instrument, introduced in 2002, also has the general layout of a TQ instrument, but in the Q–LIT, the third quadrupole can be operated (under software control) as either a normal linear quadrupole or a linear ion trap [25]. When used as a linear ion trap, it provides accumulation of ions prior to detection, thus enabling ion trap full-spectrum sensitivity, while still acquiring collision cell CID spectra. Hardware, electronics, and software control of the Q–LIT instrument have been optimized to allow very rapid switching between various MS and MS–MS experiments. The potential of Q–LIT instruments in structure elucidation can be readily demonstrated, e.g., by comparing the information

content between collision cell and ion trap product ion mass spectra [46]. Commercial Q-LIT systems provide a wide variety of typical operating modes [47] (see also: Sect. 4.4.5).

4.3.8 Reactive Gases in Collision Cells

Instead of using inert collision gases like He, N₂, or Ar in CID, one might also consider using reactive gases. The use of reactive collision gases like H₂, CH₄, and NH₃ in RF-only quadrupoles, hexapoles, or octapoles has become a common practice in inductively coupled plasma (ICP) MS in order to reduce contributions of interfering ions in trace level determination of elements by ICP-MS [48, 49]. Different setups have been described by different instrument manufacturers.

Reactive collision gases have also been used in organic MS. Pioneering experiments involved the study of the formation of an adduct ion between protonated esters and ammonia in the collision cell of a TQ instrument [50]. Since then, numerous examples have been reported such as the study of gas-phase reactions of dilactones, psorospermin, and quabalactone diterpenes [51], different phospholipid classes [52], ethyl vinyl ether, the transacetalization with gaseous carboxonium and carbosulfonium ions by collision cell reactions with cyclic acetals and ketals [53], and ketalization of phosphonium ions using 1,4-dioxane [54].

This type of gas-phase ion–molecule reactions have especially been used to discriminate between isomeric species. Collision cell reactions of isomeric tetrachloro-dibenzo-*p*-dioxins (TCDDs) molecular anions, generated by electron capture negative ionization, and O₂ were applied to discriminate isomeric forms by determining the number of chlorine atoms on each ring [55]. Other examples comprise reactive collisions between isomeric C₂H₃O⁺ and C₂H₆O⁺ ions and 1,3-butadiene or benzene [56], and differentiation of isomeric 1,2-cyclopentadiols via reactive collision with NH₃ [57].

Data acquisition in the neutral gain analysis mode, where both quadrupole mass analyzers are operated in scanning mode but with a fixed *m/z* offset depending on the reaction performed, has been applied to efficiently monitor these type of gas-phase reactions in complex mixtures of, e.g., phospholipids [52] and phosphonium ions in relation to chemical warfare agents [54]. The ethyl vinyl ether reaction with phospholipids yields a neutral gain of 26 Da for phosphatidylglycerols and of 57 Da for phosphatidylinositols [52], whereas the reaction of the phosphonium ion with 1,4-dioxane yields a neutral gain of 44 Da [54].

A pentaquadrupole (PQ-MS) instrument, thus featuring Q-q_{coll}-Q-q_{coll}-Q, has been developed and extensively used for the study of gas-phase ion–molecule reactions [58, 59]. The PQ-MS instruments obviously allow sequential product-ion analysis, i.e., MS³ experiments, but other types of experiments are possible as well. The PQ-MS instrument can be used to perform reactive collision of selected ions either in the first or the second collision cell, as illustrated with a wide variety of examples [59].

4.4 Ion-Trapping Devices: Quadrupole Ion Traps and FT-ICR

4.4.1 Introduction

In most of its applications, the mass spectrometer can be considered as a detector, providing a tool to perform mass analysis, that is to separate ions according to their m/z value either in time, as is mostly done, or in space. Some instruments, especially those providing the trapping of ions over a considerable period of time, allow additional experiments as during trapping some parameters may be changed, reactive species may be introduced, and the results of such actions may be monitored. The instruments showing best perspectives in this respect are FT-ICR and ion trap instruments. This section provides an introduction to MS using ion traps and FT-ICR instruments and highlights some examples in fundamental ion chemistry studies.

4.4.2 Ion Trap Mass Analysis

A typical ion trap, also called quadrupole ion trap or three-dimensional ion trap, consists of a cylindrical ring electrode to which a quadrupole RF field is applied, and two end-cap electrodes [60, 61] (Fig. 4.4). The end-cap electrodes contain holes for the introduction of ions from an external ion source and for the ejection of ions out of the trap towards the external electron multiplier detector. The ion trajectories in the trap are stabilized by a He bath gas (~ 1 mbar). With respect to ion trap actions, the individual steps in the mass analysis process are performed

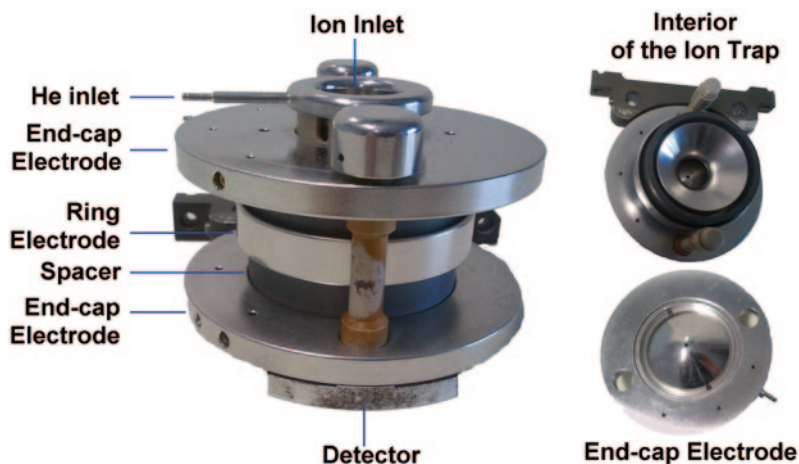


Fig. 4.4 Explanatory photo of a three-dimensional ion trap featuring essential elements of the device. (©2013, hyphen MassSpec)

consecutively in time. Thus, the acquisition of a mass spectrum requires two steps: (a) injection of ions by means of an ion injection pulse of variable duration and storage of the ions in the trap by application of an appropriate low RF voltage to the ring electrode, (b) ramping the RF voltage at the ring electrode (and eventually the application of additional waveforms to the end-cap electrodes) to consecutively eject ions with different m/z -values (resonant ion ejection) from the trap towards the external detector [60].

As the number of ions that can be stored in the trap is limited by space charge effects, software procedures have been developed to control the number of ions in the ion trap by varying the duration of the ion injection pulse from the external ion source with the ion current at the time [61]. Too high numbers of ions in the ion trap will adversely influence mass resolution and accuracy. Ion ejection and subsequent detection can be achieved with unit-mass resolution, or at enhanced resolution by slowing down the scan rate.

More recently, instruments with a linear two-dimensional ion trap (LIT), i.e., a linear quadrupole as ion trap, have become commercial available [24, 62, 63]. As a LIT is less prone to space charging effects, a higher number of ions can be accumulated, and enhanced sensitivity can be achieved. Initially, LITs were applied in hybrid Q-LIT [24] a LIT and hybrid LIT-FT-ICR-MS [64] instruments, but later on stand-alone versions of an LIT were introduced too, thus competing the three-dimensional ion traps. A dual-pressure two-stage LIT has been reported as well: the first high-pressure ion trap serves to capture, select, and fragment ions, whereas the second low-pressure ion trap is used to perform fast scanning of product ions, eventually at enhanced resolution [65].

4.4.3 MS^n in Ion Trap Instruments

Multistage MS-MS (MS^n) in ion traps is based on three features of the ion trap technology: the possibility to m/z -selectively eject ions from the trap, the constant He pressure in the trap which may serve as collision gas, and the possibility to apply an m/z -selective RF waveform to the end-cap electrodes to excite ions of the selected m/z . Thus, after filling the ion trap with ions in the usual way, MS-MS can be achieved by (a) selective ejection of all ions except the precursor ion, (b) excitation of the selected precursor ion while a low RF voltage is applied to the ring electrode to trap the product ions generated, (c) scan out the ions towards the detector to acquire the spectrum. Excitation of the ions means that the selected ions move with wider amplitude, and thus at greater speed through the ion trap. The resulting more energetic collisions with the He atoms of the bath gas lead to a gain in internal energy and subsequent fragmentation of the precursor ion.

However, the process can also be considered in more general terms (Fig. 4.5): one starts with the population of ions, from which the precursor ion is selected, excited, and fragmented, resulting in a new population of (product or daughter) ions. The latter population can either be scanned out to be detected, or can serve in a new

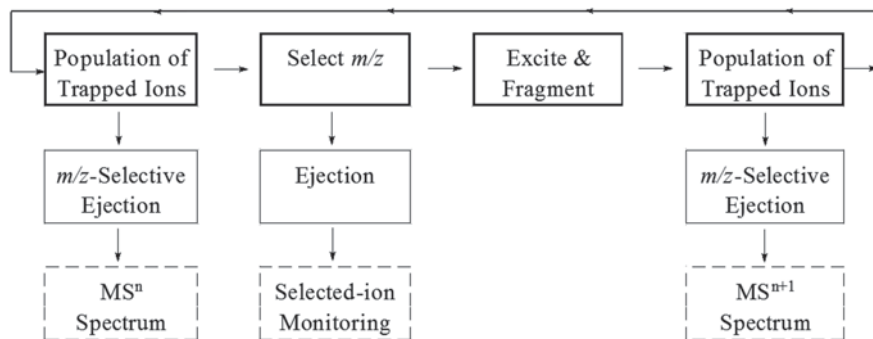


Fig. 4.5 Diagram of the steps involved in multistage MS–MS (MS^n) in an ion trap mass spectrometer. *Top row* represents the processes in the ion trap. By means of the processes in the second row, output may be generated, either the MS^n full spectrum or selected ion (MS^1), selected reaction (MS^2) or consecutive-reaction monitoring ($MS^{>2}$). (©2014, hyphen MassSpec)

series of subsequent steps of the process: selection of a product ion as precursor ion in a new MS–MS experiment, to be excited and fragmented, and leading to a new population of (granddaughter) ions. In this way, multiple stages of MS–MS or MS^n (up to 10 stages in most instruments) can be achieved consecutively (“tandem-in-time” [21]) with the ion trap instrument.

The ion trap MS^n thus enables step-wise fragmentation and the acquisition of fragmentation trees [66]. Such a fragmentation tree is generated by further fragmenting selected fragment ions of a particular stage of MS^n into a next stage, i.e., MS^{n+1} . This provides a wealth of information in structure elucidation and identification of unknowns, as is nicely exemplified for polyphenols [67].

4.4.4 Applications of Ion Trap MS^n

Currently, ion trap MS instruments are primarily used for analytical applications, i.e., in combination with GC or LC in, among other, environmental, food-safety, clinical, pharmaceutical, or biochemical application areas. In many of these applications, the potential of multistage- MS^n plays an important role. The acquisition of fragmentation trees enables detailed study on the fragmentation behavior of target compounds and/or compound classes, e.g., [67].

However, ion trap MS can also be applied in more fundamental ion chemistry studies [68], especially because the reaction time can be varied over several orders of magnitude. Due to the He bath gas in the ion trap, the pressure in the ion trap is several orders of magnitude higher than in an FT-ICR cell, but much lower than in high-pressure MS and FA-MS.

Giving the residence time of ions in the trap, one may anticipate that ion–molecule reactions would readily take place in the ion trap. In fact, self-protonation of several compounds, present at higher concentrations in the ion trap, was observed

in electron ionization (EI) spectra, thus resulting in $[M+H]^+$ next to M^+ [69]. From this observation, it was anticipated that chemical ionization (CI), either via proton-transfer or charge-exchange reactions, should be possible. Initially, ion trap CI reactions with methane and isobutane as reagent gas were studied [70], but later on the headspace vapor of solvents like water, methanol, acetonitrile, acetone, or furan were used in CI on ion trap MS instruments [71–74]. Given the ease at which such experiments can be done and the interaction time can be varied, gas-phase ion–molecule reactions with unusual reagent gases can be readily performed in an ion trap. Similarly, this type of procedures can be used to determine the proton affinity or gas-phase basicity of compounds via the bracketing method using ion trap MS [75]. Alternatively, the kinetic method can be applied. By mass-selection and subsequent dissociation of proton-bound dimers ($A-H^+-B$), the proton affinity (PA) can be determined, provided the PA of A is known and several proton-bound dimers with similar functionalities are available [76].

It allows reaction products to be observed from reactions under either kinetic or thermodynamic control. Numerous reports are available, where ion trap MS is applied in ion chemistry studies [68], e.g., involving reactions between 1,4-benzodiazepines and dimethyl ether ions [77], dissociation of [Alanine + Alkali cation]⁺ ions to study the role of the metal cation [78], or regioselective ion–molecule reactions to enable MS differentiation of protonated isomeric aromatic diamines [79]. The three-dimensional ion trap mass spectrometer has even been described as a complete chemical laboratory for fundamental gas-phase studies of metal-mediated chemistry [80].

Ion trap instruments also provide the possibility to study ion–ion reactions, e.g., reactions of multiple-charge peptide or protein ions with ions of opposite polarity. Mostly, proton-transfer reactions are performed, but electron transfer, fluoride transfer reactions, and even attachment reactions may occur as well [81]. Ion traps, either three-dimensional or linear ones, with multiple ion sources are applied in such experiments [82].

As indicated before, the ion trap CID process differs from collision cell CID in a number of ways, including collisions with a smaller target (He instead of Ar), ion excitation by an RF waveform pulse rather than by acceleration of ions in an electric field, and the interaction time, which is milliseconds in ion trap CID rather than microseconds in collision CID. As a result, different fragmentation pathways may be accessible in ion trap CID compared to collision cell CID. Ion trap CID is generally considered to be softer, thus both requiring and enabling multistage MSⁿ experiments to generate a wealth of structural information. The lower energy involved in ion trap CID also is at the basis of the generation of fragmentation trees and stepwise fragmentation strategies [66]. Another feature is that $[M+Na]^+$ -ions may be fragmented in ion trap MSⁿ, whereas they are generally not in TQ and Q–TOF instruments. This is extensively applied in the structure characterization of oligosaccharides [83]. Interestingly, $[M+Li]^+$ -ions, e.g., of (phospho)lipids, may be fragmented by both TQ and ion trap instruments [84, 85], suggesting that it is the short ion residence time that prevents $[M+Na]^+$ to be fragmented in TQ instruments. Other interesting features of the fragmentation of lithiated (phospho)lipids is

the loss of lithium salts of fatty acids, next to the loss of fatty acids [84, 85], and the ability to apply charge remote fragmentation of lithiated lithium salts of unsaturated fatty acids to determine double bond positions [86].

4.4.5 *Hybrid Systems Involving Ion Traps*

A number of hybrid tandem mass spectrometer systems, in which ion traps are involved, are commercially available. Ion traps can be applied either in the first stage (MS1) or in the second stage (MS2) of mass analysis.

In the Q–LIT hybrid [25] instruments, an ion trap is implemented as the second stage of mass analysis, either for accumulation of ions to achieve improved sensitivity after collision cell CID [25, 46], and/or to perform MSⁿ [25, 46, 47]. The Q–LIT instrument can either be operated as a conventional TQ instrument or as the hybrid instrument. In TQ mode, the instrument is capable of all acquisition modes of a TQ, including SRM. In the hybrid mode, full-spectrum data can be acquired in the enhanced product ion (EPI) mode with up to 60-fold enhanced sensitivity compared to TQ instruments. Next to the enhanced multiple-charge scan and the time-delayed fragmentation scan, the system allows the acquisition of MS³ spectra, with the second dissociation step to be performed in the LIT [46, 47].

If the ion trap is implemented as the first stage of mass analysis (MS1), it generally serves several functions. Next to acting as a “filter” with respect to the number of ions that are transferred to the second stage, based on the duration of the ion accumulation in the ion trap, MSⁿ experiments may be performed prior to transferring a package of ions to the second stage. Thus, in such hybrid systems, no collision cell has to be present between the first and second stage of mass analysis.

This is true for ion trap hybrids with FT-ICR-MS (see Sect. 4.4.7) and Orbitrap (see Sect. 4.4.9) instruments, but also for the ion trap–time-of-flight hybrid system. The latter system has been pioneered by the group of Lubman [87, 88]. It has become commercial available for both MALDI and LC–MS applications [89]. All these hybrid MS system are frequently applied in combination with LC and electrospray ionization in, for instance, drug metabolite identification studies and in various proteomics-related studies.

4.4.6 *Mass Analysis in Fourier Transform Ion Cyclotron Resonance Instruments*

A Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR-MS) can be considered as an ion trap system, where the ions are trapped in the magnetic field rather than in a quadrupole electric field. The ICR cell is a cubic or cylindrical cell positioned in a strong magnetic field B (up to 15 T). The cell consists of two opposite trapping plates, two opposite excitation plates, and two opposite receiver plates (Fig. 4.6). Extreme high vacuum should be achieved in the cell, e.g., 10^{-9} mbar.

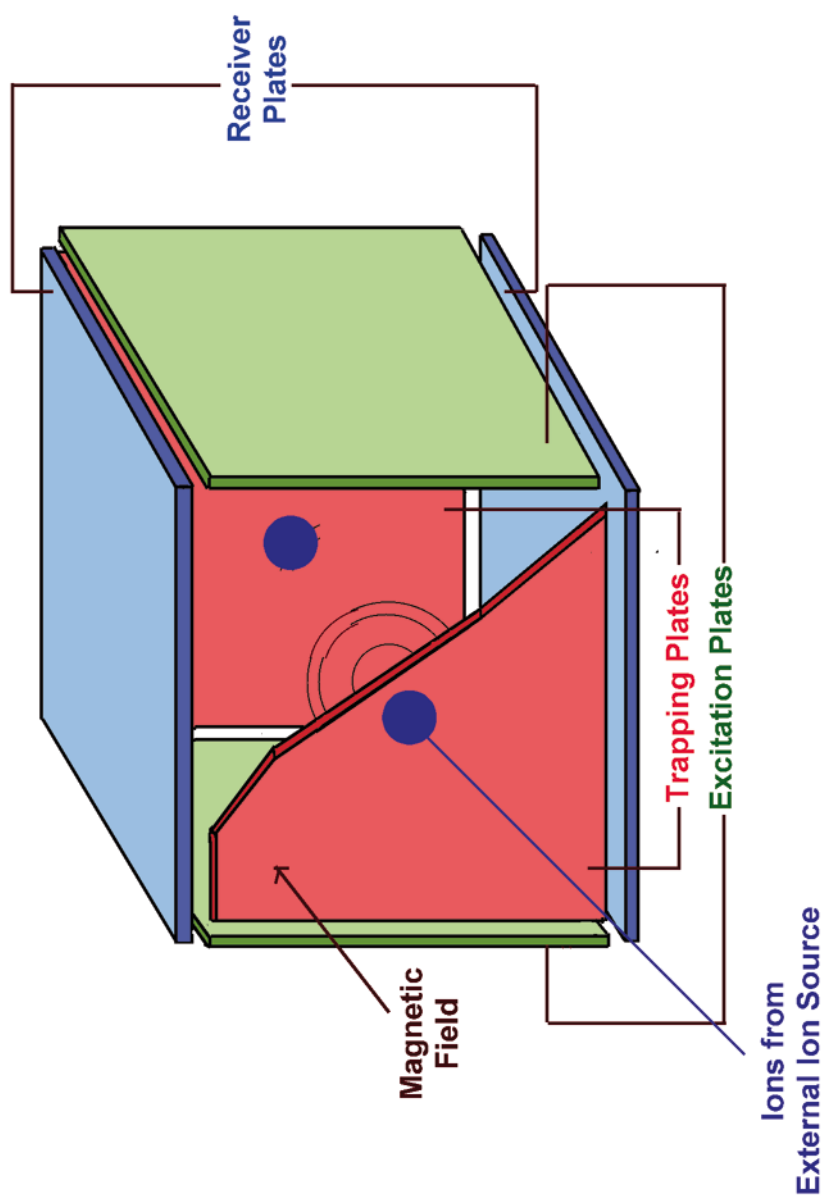


Fig. 4.6 Schematic diagram of a cubic FT-ICR cell. (©2014, hyphen MassSpec)

An ion of mass m , with velocity v , and z elementary charges describes a circle of radius r , perpendicular to and around the magnetic field lines. The resulting cyclotron frequency ω_c can be written as:

$$\omega_c = 2\pi f = v / r = Bez / m$$

where f is the frequency in Hz. The cyclotron frequency is thus inversely proportional to the m/z value. When ions, trapped in their cyclotron motion in the cell, are excited by means of an RF pulse at the excitation plates, the radius of the cyclotron motion increases and ions with the same m/z values start moving in phase. This coherent movement of the ions generates an image current at the receiver plates. As the coherency of the ion movement is disturbed in time, the image current signal decays in time as well. The time domain signal from the receiver plates contains all frequency information of the moving ions present in the cell. By applying Fourier transformation, the time domain signal can be transformed into a frequency domain signal, which can subsequently be transformed in a regular mass spectrum by application of the equation above [23].

Characteristic features of an FT-ICR-MS instrument are an extremely high (mass-dependent) resolution, i.e., in excess of 10^5 (FWHM), and a dynamic range of five orders of magnitude. For many years, FT-ICR-MS has primarily been used in fundamental studies of gas-phase ion–molecule reactions only. Due to its high-resolution and MS–MS capabilities, the application of FT-ICR-MS in combination with electrospray ionization for the characterization of large biomolecules has been investigated more recently [90]. At present, FT-ICR-MS plays an important role in top-down strategies to characterize proteins [91, 92]. For this purpose, more user friendly instruments have been introduced by the instrument manufacturers, featuring hybrid systems with either front end quadrupole [93] or ion trap systems [64, 94].

4.4.7 MS–MS in FT-ICR-MS

As targeted ions can be selectively trapped in the ICR cell, while unwanted ions can be eliminated by the application of RF pulses, the MSⁿ procedures in an FT-ICR-MS instrument greatly resemble those in an ion trap instrument. However, successful operation of an FT-ICR-MS instrument requires extreme low pressures in the cell. Thus, the vacuum constraints hamper the possibilities of performing CID in the FT-ICR cell [23]. This problem can be elegantly solved by the use of hybrid systems where fragmentation is performed prior to transfer of ions to the ICR cell [64, 93, 94]. Alternatively, alternative ion activation methods can be applied to induce fragmentation in the FT-ICR, such as infrared multiphoton photodissociation (IRMPD) and sustained off-resonance irradiation (SORI) [32, 33]. More recently, electron-capture (ECD) and electron-transfer dissociation (ETD) have been introduced as powerful ion dissociation tools, especially for peptides and proteins [34].

4.4.8 *Application of FT-ICR-MS in Fundamental Studies*

Numerous examples are available in the scientific literature, where the application of FT-ICR-MS in fundamental studies concerning ion–molecule reactions is reported. FT-ICR-MS instruments are highly versatile tools in the study of ion–molecule reactions, as they enable control of reaction time and energy, selection of reactant and product ions, as well as structure characterization of reactants and products using MS^n procedures [95]. Gas-phase ion–molecule reactions of organic anions have been discussed by Nibbering [96]. Gas-phase reaction molecules of transition metal ions and biomolecules have been discussed by Freiser [9].

Combined with electrospray ionization or MALDI, FT-ICR-MS is a very attractive tool for gas-phase studies of biomolecules such as peptides and proteins, oligonucleotides, and oligosaccharides. The possibility to trap ions for prolonged periods of time, even up to thousands of seconds, can be applied in the study of gas-phase ion–molecule reactions. Application of proton-transfer reactions in ICR cells in the study of biomolecules has been reviewed [97]. Detailed structural as well as conformational studies on biomolecules rely on H/D-exchange experiments, for which FT-ICR is an excellent tool, e.g., [98, 99].

4.4.9 *Orbitrap Mass Spectrometry*

Although both FT-ICR-MS and Orbitrap-MS are based on the acquisition of mass spectra by the Fourier transformation of image currents of trapped ions, the FT-ICR can be considered as a high-vacuum gas-phase reaction cell, whereas the Orbitrap is just applied to perform high-resolution measurement of populations of ions. However, the ion optical tools required to deliver the package of analyte ions in the Orbitrap provides ample possibilities for advanced ion chemistry experiments and or ion dissociation steps. The initial instrumental setup of the Orbitrap consisted of a LIT–Orbitrap hybrid configuration, featuring a LIT to control the number of ions transferred to the Orbitrap and to perform MS^n , when necessary, a so-called C-trap which essentially is a curved high-pressure quadrupole to direct the ion package into the Orbitrap, and the Orbitrap itself [28]. As the ion trap system in this commercial LIT–Orbitrap instrument is equipped with separate off-axis detectors, simultaneous acquisition of high-resolution precursor ion and unit-mass resolution product-ion spectra can be achieved. Subsequently, it was demonstrated that CID could be achieved in the C-trap, which turned to be more like collision cell CID than like ion trap CID [100]. Separate higher energy collision RF-only octapoles (higher energy CID, HCD) were mounted on LIT–Orbitrap systems to make optimum use of this feature. Such a system can be considered as a gas-phase chemistry laboratory by its own, featuring different ways to perform fragmentation, i.e., ion trap CID, HCD, and eventually ETD, as well as different ways to measure the m/z values of the resulting ion (unit-mass resolution with the ion trap, up to ultra-high resolution at the Orbitrap). The HCD-cells also lead to stand-alone Orbitrap (Exactive™) and Q–Orbitrap hybrid systems (Q-Exactive™) [101].

4.4.10 Comparison of MS–MS Strategies

It is obviously difficult to compare the different instruments and ion dissociation techniques. Some comparison between high-energy CID and low-energy CID are given in Sect. 7.5. However, in this respect, a review paper of Wührer et al. [102] on glycopeptide characterization by MS–MS is of interest. Wührer et al. [102] compared a range of fragmentation techniques with respect to the information content upon fragmentation of a tryptic glycopeptide (Ser²⁹⁵–Arg³¹³ from horseradish peroxidase). For this glycopeptide, CID in ion trap instruments primarily provided information on the glycan sequence, whereas in collision cell CID, e.g., in a Q–TOF instrument, cleavage of glycosidic bonds are induced at low-collision energy and peptide backbone cleavages at higher collision energies. Electron-transfer dissociation (ETD) in an ion trap instrument or electron-capture dissociation (ECD) in FT-ICR-MS leaves the glycan unaffected and provides peptide backbone cleavages. Infrared multiphoton dissociation (IRMPD) in FR-ICR-MS again provides information on the glycan structure. In high-energy CID in a TOF–TOF instrument after MALDI, both peptide sequence ions and fragmentation of glycosidic bonds is obtained [102].

4.5 Flowing Afterglow Mass Spectrometry

4.5.1 Introduction

The flowing afterglow (FA) is a flow reactor tube. Ions are produced by an ion source at the upstream end of the tube. These ions are carried by a buffer gas (He or Ar) and thermalized to room temperature down the flow tube. On their way down, they react with neutral molecules added downstream in the tube. The (ionic) reaction products can be monitored in a number of ways, including optical spectroscopy and MS. In the latter case, the resulting swarm of ions is sampled through an orifice into a high-vacuum chamber where they are mass analyzed and detected.

The FA-MS technique was originally developed in the early 1960s by the group of Ferguson [103] at the Environmental Science Services Administration (ESSA) Aeronomy Laboratory (now the National Oceanic and Atmospheric Administration, NOAA) in Boulder, CO. Their FA device, coupled to a quadrupole mass spectrometer, was initially used to study reactions of He⁺-ions with atmospheric components, such as O₂, N₂, CO, NO, and NO₂. It provided new insights on ion–molecule dissociative charge-transfer reactions [103].

Over the years, the FA technique has undergone continuous refinement and development and found a wide variety of applications, e.g., in fundamentals of ion–molecule reactions and in atmospheric and interstellar chemistry [104]. The FA technique enables the generation of high-density, steady state populations of ions and reactive neutral species with well-defined thermal energy distributions. The reaction conditions can be carefully controlled among others due to the temporal

and spatial separation of the source and reaction regions. The inherent simplicity and flexibility of the FA technique allows for straightforward adaptation to other experiments.

An important adaptation of the FA technique comprises the implementation of ion separation methods, which allows for more advanced flow drift tubes and selected ion flow tubes (SIFT) [105, 106] (see below). More recently, flow drift tubes (see Sect. 4.6) and flowing atmospheric pressure afterglow (FAPA) devices [107] have been developed.

4.5.2 Instrumentation

A typical FA-MS instrument consists of a 1-m long, 7–10-cm inner diameter (ID) pyrex, quartz, or stainless steel tube. A typical setup is shown in Fig. 4.7. By means of a high-speed, high-capacity mechanical pump, a pressure between 0.2 and 2.0 mbar is maintained, while a continuous flow of buffer gas (He or Ar, 100–200 STP ml/s) is introduced. At the upstream end, ions are generated, which are thermalized by collisions with the buffer gas. Initially, ions were generated by a weak microwave discharge in the He or Ar carrier gas, resulting in $\text{He}^{+\bullet}$ or $\text{Ar}^{+\bullet}$ ions. As the linear velocity of the buffer gas is in the range of 50–100 m/s, the ion residence time in the flow reactor is a few ms. The primary ions may then react in ion–molecule reactions with neutrals, introduced via an inlet. As a result, the afterglow may be observed, resulting from visible photoemission from excited ionic and neutral

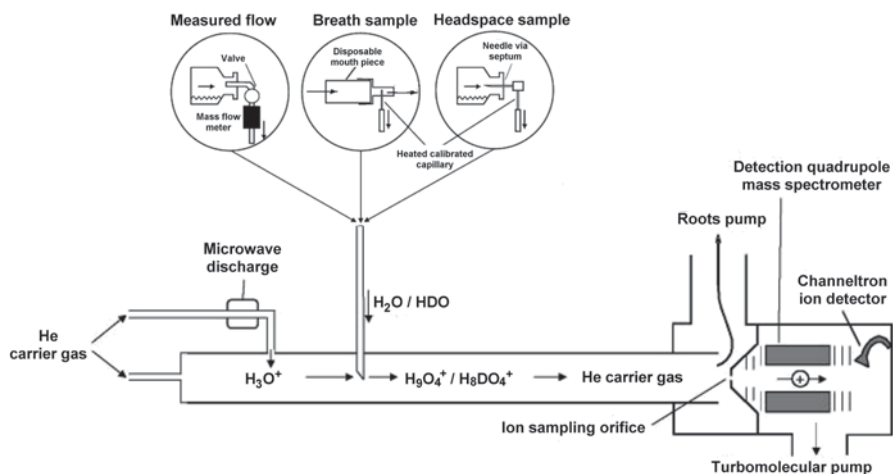


Fig. 4.7 Schematic diagram of a flowing afterglow mass spectrometry setup. The system is setup to perform reactions with H_3O^+ . Other primary ions can be generated by other types of ion sources. (Adapted from ref. [121] and Smith, D.; Španěl, P.: Selected ion flow tube mass spectrometry (SIFT-MS) for online trace gas analysis. *Mass Spectrom Rev.* 24, 2005, 661–700] with permission of Wiley. ©2001 and 2005, Wiley, Ltd.)

species. The reaction mixture is sampled by an ion sampling orifice (0.2–2 mm ID) into a differentially pumped housing of the mass analyzer. While the pressure is reduced to high vacuum, the ions are focused and mass analyzed by a quadrupole mass analyzer and detected by an ion multiplier. Next to establishing the identity of the reaction products based on the m/z of the ions detected, the setup allows determining reaction rate coefficients by monitoring the decay of reactant ion intensity as a function of the flow of neutral reactants at a constant reaction time.

From the basic setup, just described, one may conclude that an FA instrument is a modular instrument, consisting of an ion source, a flow reactor tube, and a (mass spectrometric) detector as the building blocks. Each of the building blocks may be modified or adapted to the specific needs of the experiment to be performed.

Different types of ion sources may be introduced, e.g., instead of the microwave discharge ion source, ions may be generated by electron ionization, using electron emission from a heated filament. Ion generation in a high-pressure ion source allows the generation of cluster ions by termolecular association of ions with one or more neutrals, which may then be studied in FA-MS. Thermoionic emission filaments doped with alkali metal salts allows the generation of alkali metal ions [108] or even Ag^+ or Cu^+ ions [109]. Even, ion generation by electrospray ionization in combination with FA-MS has been reported [110]. In SIFT [105, 106], the ion source is combined with a quadrupole mass filter in order to select a particular ion from the various ions generated in the source to be introduced into the flow reactor. This allows an even wider range of potential ion sources to be used.

The inlet for the neutrals may have a fixed or a variable position at the flow reactor. The sample inlet may be a controlled flow of a trace gas, a breath sample, or a headspace sample, introduced via a heated sampling line (Fig. 4.7). Variation of temperature, by external heating of the flow tube, and ion kinetic energy may be applied as well.

Instead of the quadrupole mass analyzer, other types of detectors may be used, e.g., other types of mass analyzers like TOF-MS, tandem mass spectrometers (TQ instruments), but also devices for optical and photoelectron spectroscopy or neutral product analysis, ion photodissociation, or photoemission [104].

4.5.3 Application of FA-MS

The FA-MS has been applied in a wide variety of areas, including atmospheric and interstellar chemistry [111, 112], the study of a wide variety of ion–molecule reactions in order to determine parameters like rate constants and temperature dependence [113], the study of ion chemistry of, for instance, triazoles [114], the study of electron attachment to a wide variety of compounds including halogenated alkenes and alkanes [115, 116], transition metal carbonyls [117], and sulfuroxyhalides [118], the study of gas-phase proton-transfer and hydride-transfer reactions [119]. Analytical applications of FA-MS involve, among others, online breath analysis [120–122] and screening for pesticides in food [123].

The online breath analysis by FA-MS is based on measuring the H/D ratio before and after introduction of a known amounts of D₂O or HDO into the system. Initially, a swarm of primary H₃O⁺ ions is generated in the FA-MS ion source. These primary ions are allowed to react with the water in the exhaled breath analyzed, that is, with the H₂O, HDO, and D₂O molecules in the water vapor (Fig. 4.7). As a result, hydrated ions, H₃O⁺·(H₂O)₃ or H₉O₄⁺ with *m/z* 73 are formed, together with their isotopic variants H₈DO₄⁺ with *m/z* 74 and eventually H₇D₂O₄⁺ with *m/z* 75 (correction is required for the known amounts of H₂¹⁷O and H₂¹⁸O in natural water supplies). By comparing ion intensities for *m/z* 73, *m/z* 74 and eventually *m/z* 75 before and after introduction of D₂O or HDO, a noninvasive determination of total body water can be performed [121, 122]. More recently, monitoring of H/D abundance in breath has found clinical applications, e.g., in determining the extent of abnormal accumulation of fluid in the extravascular space in the alveoli of patients with pulmonary edema [124, 125].

Reactions between Li⁺, Na⁺, and K⁺-ion clusters with O₃, N₂O₅, and SO₂ with NO[•] and CO were studied. In these reactions, the alkali metal ions did not play a chemical role, i.e., in forming bonds for instance. Reaction products of the reactions with NO[•] were NO₂, O₂, and the alkali metal ion. In the reaction with CO, the products were CO₂, O₂, and the alkali metal ions. Interestingly, the measured reaction rate constants are much larger than the rate constants for the analogous gas-phase reaction in the absence of the alkali metal ion [108].

4.5.4 Selected Ion Flow Tube Mass Spectrometry

As indicated before, SIFT-MS can be considered as an adaptation of FA-MS [105, 106]. The major adaptation concerns the selection of the precursor ion by means of a quadrupole mass spectrometer prior to its injection in the fast-flowing He carrier gas of the FA-MS. In most cases, H₃O⁺, NO⁺, or O₂^{+•} are generated in a microwave discharge and used as selected precursor ion. The precursor ion is used to react to and ionize trace gases in air or breath samples, introduced downstream in the flow tube. The characteristic product ions identify the trace gas constituents; their count rates allow quantification. Computer-searchable libraries have been compiled to facilitate the identification of the products from reactions of the selected precursor ions with various classes of compounds, including hydrocarbons, alcohols, aldehydes, and ketones, which may be present in breath samples. For identification of reaction products, the downstream mass spectrometer is operated in full-spectrum mode. More accurate quantitation of target compounds can be achieved by operating the downstream mass spectrometer in selected ion monitoring (SIM) mode [105, 106]. Developments in SIFT-MS instrumentation have been reviewed in detail [106]. Portable SIFT-MS instruments have been developed for diagnostic breath analysis in a clinical setting. Other applications concern headspace analysis of volatiles in the skin or in urine, and the emission of toxic compounds and explosives. The SIFT-MS technique and its applications are discussed in more detail in Sect. 8.3. SIFT-MS systems are commercially available.

4.5.5 Proton-Transfer Reaction Mass Spectrometry

Proton-transfer reaction mass spectrometry (PTR-MS) can be considered as a derivative of the SIFT technique [126, 127]. Two changes are made, relative to SIFT. In PTR-MS, the front-end mass analyzer to select the reactant ion has been replaced by a hollow-cathode discharge source, capable of a highly efficient generation of primary H_3O^+ ions, taking away the need for the mass filter. The long FA or SIFT flow tube has been replaced by a short drift tube, where the air from the sample is applied as carrier gas. As a result, PTR-MS has become a very easy-to-use and sensitive tool for the detection of volatile organic compounds (VOCs) in air. As such, it is frequently applied for environmental studies related to industrial and anthropogenic emission of VOCs, in plant studies, in relation to flavors in food, food quality, as well as in (clinical) breath analysis [127]. A recent adaptation of PTR-MS is the so-called selective reagent ionization approach (SRI-MS), where other primary ions such as O_2^+ , NO^+ , Kr^+ , or Xe^+ can be chosen as reagent ions [128]. PTR-MS systems are commercially available.

4.6 Drift Tubes and Ion Mobility

4.6.1 Introduction

Whereas FA-MS is applied to study reactive collisions between ions and neutrals, static drift tubes are used for the study of the nonreactive ion-neutral interactions to determine diffusion and mobility characteristics [104, 129, 130]. A flow drift tube consists of (a) an ion-generation region, (b) a charge-separation region, (c) an ion shutter, (d) the actual drift-reaction region, and (e) an analyzer, which in the present discussion is a mass spectrometer. Ions can, in principle, be generated by any means. A radioactive ^{63}Ni -foil is the most common ionization source in drift-tube experiments, although techniques like photoionization, thermionic emission, and corona discharges have been used as well. The charge-separation region is applied to assure only either positive or negative ions to enter the drift region via the ion shutter. The ion shutter, consisting of two fine-mesh grids connected to a modulating power supply, enables pulse-wise introduction of ions into the drift tube, thus allowing for drift time measurements. In the drift reaction region, a uniform axial electric field gradient is maintained with a series of guard rings, separated by electrically insulating spacers and connected with appropriate precision resistors. Once in the drift tube, the ions are subjected to this homogeneous electric field (typically 1–1000 V/cm). This electric field drives the ions through the drift tube, where they interact with the (countercurrent) buffer gas (He, N_2 , Ar). In absence of the drift field, the device functions as a flowing-afterglow device. However, with the drift field on, the ion velocity is the sum of flow velocity and drift velocity, the latter being determined by the mobility of the ion. The drift tube experiments may be considered as gas-phase electrophoresis.

Drift-tube experiments have been applied to study the energy dependences of a wide range of reactions and to correlate these to temperature dependences. Reactions like proton transfer and charge exchange, which proceed at or near the limiting collision rate, generally show little variation with ion kinetic energy. Slow reactions that show significant temperature dependence, often show substantial kinetic energy dependence. However, data must always be interpreted with care [104].

4.6.2 *Ion Mobility*

In its simplest form, the drift-tube ion-mobility system measures how fast a given ion moves in a uniform electric field through a given atmosphere. Thus, an ion-mobility system separates ions by shape and charge. The flow drift technique can be applied to determine quantities like ion mobility and diffusion coefficient, as these are functions of the nonreactive attractive and repulsive ion-neutral interactions [129, 130]. Ion mobilities have been measured for a wide range of ions in several buffer gases (He, N₂, Ar). With Ar as buffer gas, the mobility can be predicted with reasonable accuracy, whereas the measured mobility in He shows poor agreement with theoretical predictions [131].

Ion mobility is a measure of how fast an ion moves through the buffer gas under the influence of an electric field. As larger ions undergo more collisions with the buffer gas, they will have longer drift times than smaller ions. Higher charge states of an ion experience a greater effective drift force, and thus show higher mobility than the lower charge states. In practice, expressing ion mobility as reduced mobility is more useful, as it allows comparison between different experimental conditions. From the reduced mobility, the experimental collision cross-section can be determined. There is a good correlation between experimentally determined collision cross-sections and theoretically predicted ones [132, 133].

Based on these concepts, ion-mobility spectrometry initially called plasma chromatography or gaseous electrophoresis [134], or gas-phase ion chromatography [135], have been developed in the 1970s [129, 130]. Both atmospheric pressure and low-pressure ion-mobility spectrometry (IMS) systems have been used to study gas-phase chemistry, including differentiation between isomeric species [129, 130]. As chemical species can be separated based on their ion mobility, the drift time can be used to generate a response characteristic for the chemical composition of the measured sample. Given the speed, at which the separation and detection occurs (ms range), its ease of use, relatively high sensitivity, and the highly compact design, commercial ion-mobility spectrometers are used as a routine tool for the field detection of explosives [136], drugs of abuse, and chemical weapons [137], for instance at airports. Handheld IMS-based systems have been developed for this purpose. IMS systems have also been used as detectors for chromatography [130], including GC, LC, and supercritical fluid chromatography (SFC).

4.6.3 Ion-Mobility Spectrometry–Mass Spectrometry

The online combination of ion-mobility spectrometry devices with mass spectrometry obviously results in a very attractive tool to combine analysis of conformation and shape, as performed in IMS, with the analysis of m/z and structural features, as performed in MS. Ion-mobility spectrometry–mass spectrometry (IMS–MS) has been pioneered by the groups of Bowers [138] and Clemmer [139, 140]. In most cases, ion-mobility devices have been interfaced to quadrupole or TOF instruments. IMS–MS provides a rapid gas-phase separation step prior to MS analysis, enabling the identification of ions with different drift time, thus with different collisional cross-sections, as well as the measurement of collisional cross-sections and correlations to size, shape, and conformation. It may also help understanding ionization characteristics and fragmentation pathways, as a better understanding of gas-phase ion structures is obtained [141]. Instrumental developments in IMS–MS have been reviewed by the group of Hill [142]. The technology is intensively used to study protein conformations in the gas phase, which, for instance, provides interesting insights in the development and possible causes of several neurodegenerative and neuropathic diseases like Parkinson's and Alzheimer's disease [143]. However, IMS–MS is also very useful in the study of small molecules [141] (Fig. 4.8).

Ion-mobility may be implemented in IMS–MS systems in the form of (a) drift tubes, as already discussed [138, 139, 144], (b) differential ion-mobility or high-field asymmetric waveform ion mobility spectrometry (FAIMS) devices [145–147], and (c) traveling-wave ion guide devices [44, 148]. Tandem IMS–IMS–MS systems

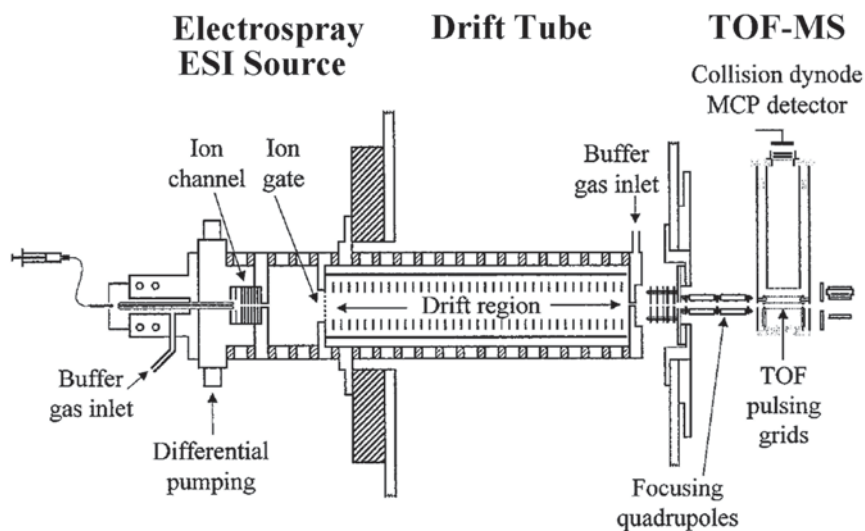


Fig. 4.8 Schematic diagram of a setup for ion-mobility spectrometry–mass spectrometry. (Reprinted with permission from ref. [140]. Copyright 1999, American Chemical Society)

have also been described [149]. This instrument performs an initial ion-mobility separation of individual components in a mixture of ions, collisional activation of selected ions inside the drift tube, transfer of product ions and subsequent ion-mobility separation to a second drift tube, and mass analysis and detection of the ions by TOF-MS, eventually after another CID step [149]. Whereas drift-tube devices have been the initial devices to measure and apply ion mobility in combination with MS, drift-tube IMS-MS system has only recently become commercially available. Nevertheless, successful application of and fundamental studies using IMS-MS have been developed in various laboratories [141–144]. In its various forms, IMS-MS plays an important role in many forefront application areas of biological MS, including structural proteomics [150, 151] characterization of protein assemblies [152], and chiral and structural analysis of biomolecules [153].

4.6.4 High-Field Asymmetric Waveform Ion-Mobility Spectrometry (FAIMS)

In FAIMS, the gas-phase mobility separation of ions in an electric field is achieved at atmospheric pressure [145–147]. In its simplest design, the FAIMS device comprises two parallel rectangular electrodes at a uniform distance (Fig. 4.9). One of the electrodes is grounded, while at the other an asymmetrical waveform is applied. The asymmetric waveform is characterized by a significant difference in voltage in the positive and negative polarities of the waveform. FAIMS uti-

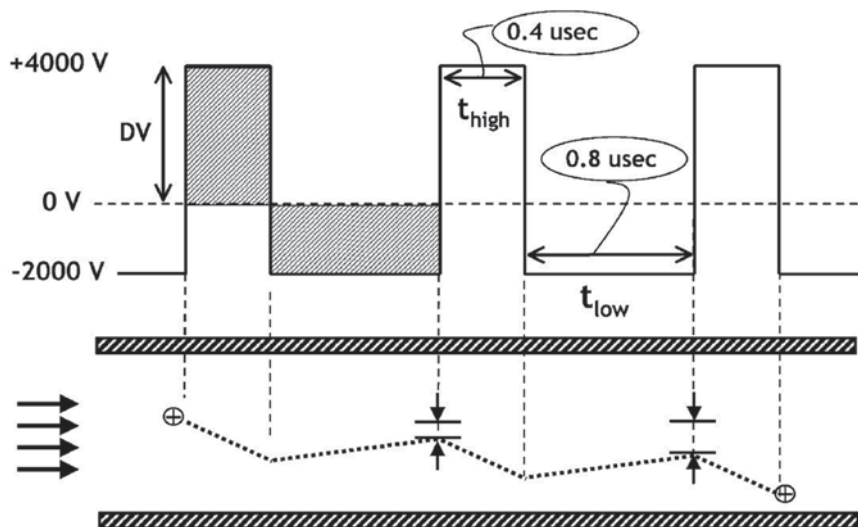


Fig. 4.9 Operation of an FAIMS device. (Reprinted with permission from [145]. Copyright 2004, Elsevier Science Publishers)

lizes much higher electrical fields (typically 10,000 V/cm) than conventional IMS (typically 200 V/cm). Ions drift through the gas between the electrodes and are separated depending on their mobility. At low field, the ion mobility is independent of the electric field: the drift velocity is proportional to the field strength, whereas at high field, the ion mobility becomes dependent on the applied electric field. This electric-field dependence of the ion mobility is the basis of FAIMS. As a result of the applied asymmetric waveform to one of the electrodes, the ions would show a net displacement towards the grounded plate. This net displacement can be corrected or compensated for by a DC voltage (compensation voltage), and assures that the ions remain between the plates. Scanning of the compensation voltage allows ions with different mobilities to be monitored (Fig. 4.9). In this way, for instance, the mobility separation of phthalic acid isomers has been demonstrated [145]. Next to ion mobility separation of ions, focusing of the beam is achieved, thus improving the sensitivity in an FAIMS–MS system. In current FAIMS devices, various electrode configurations, including various geometries of cylindrical electrode devices, are applied [145–147]. Currently, FAIMS devices are commercially available and primarily applied in combination with electrospray ionization and MS to improve sensitivity and to reduce matrix effects in quantitative analysis [147, 154].

4.6.5 *IMS–MS Using Traveling Wave Ion Guide Devices*

Traveling-wave stacked ring ion guides were initially developed to replace RF-only hexapole ion guides, present in commercial MS systems equipped with atmospheric pressure ion sources (electrospray or atmospheric pressure chemical ionization), to achieve high-transmission ion transport in the vacuum interface. Similarly, they were used to replace RF-only hexapole collision cells in tandem quadrupole systems, both to improve ion transmission and to reduce crosstalk by reducing the ion-residence time in the collision cell [44]. The very nature of the device, which greatly resembles a drift tube, suggests its application for ion-mobility experiments. To this end, a Q–TOF instrument featuring travelling wave ion guides in both the vacuum-interface region and the collision cell region was constructed. Initially, ion-mobility spectra were acquired by storing ions in the source ion guide and gating them periodically to the collision cell ion guide. The mobility-separated ions were subsequently analyzed using the TOF-MS system [44]. For ion-mobility measurement, the collision cell was operated at 0.2 mbar pressure of Ar.

The initial setup was developed into a hybrid quadrupole–ion-mobility–TOF instrument [148]. The collision cell region of this instrument features three traveling-wave stacked ring ion guides, of which the middle one is used as ion-mobility drift tube and the other two may be used as collision cell, when applicable. The 185 mm long IMS part is operated at pressures up to 1 mbar with up to 200 ml/min Ar gas, whereas the collision cells are 100 mm long and operated at 10^{-2} mbar with up to 10 ml/min gas [148]. The system can be used for a wide variety of applications,

involving ion-mobility studies, such as protein conformation studies and differentiation of heterogeneities in glycoproteins [155]. By correlating theoretically predicted collision cross-sections to measured drift times of a parent drug and its fragments, Shimizu et al. [156] generated a calibration plot, which could subsequently be used to differentiate between chromatographically separated isomeric glucuronic acid conjugates, generated in biotransformation of the parent drug. For isomeric hydroxylated metabolites, the differences in drift time are too small, compared to the resolution of the IMS separation [157, 158]. Therefore, Shimizu and Chiba [158] introduced selective derivatization of aromatic hydroxyl groups of drug metabolites to increase the differences in collision cross-sections, and thus in drift times. This allowed differentiation between isomeric forms in the same way as demonstrated for the glucuronic acid conjugates [158].

4.7 High-Pressure Mass Spectrometry

The term “high-pressure mass spectrometry” (HP-MS) has been used in various contexts over the years. In many studies, the term refers to performing MS experiments with a high-pressure or atmospheric pressure ion source in combination with a high-vacuum mass analyzer (in contrast to the high-vacuum ion source), whereas in some studies the actual mass analysis is also performed at higher pressures as well, e.g., at ~ 1 mbar.

4.7.1 *HP-MS: Mass Analysis at Higher Pressure*

Mass analysis at higher pressures than the conventional high-vacuum conditions is especially of interest in the construction of portable mass spectrometers [159, 160]. The most widely used approach in portable mass spectrometers seems to be the use of (miniaturized) ion-trap instruments, as these are generally operated at relatively higher pressure (~ 1 mbar) due to the He bath gas present [159, 161, 162]. These types of instruments are primarily used for environmental monitoring and other field studies (explosives, chemical weapons, etc.). As such, they are frequently equipped with atmospheric pressure ion sources. Nevertheless, portable mass spectrometers based on double-focusing sector, quadrupole, and TOF technology have been described as well [159, 163].

4.7.2 *HP-MS: High-Pressure Ion Sources*

Historically, the introduction of chemical ionization (CI) by Munson and Field [164, 165] may be considered as the starting point of MS using high-pressure ion sources. They studied gas-phase ion–molecule reactions between reagent gas ions, generated

in a ~ 1 -mbar ion source, with analyte molecules introduced into the source. The general purpose of subsequent HP-MS experiments is to perform fundamental studies of gas-phase reactivity in ion–molecule reactions, preferably under equilibrium thermochemical conditions. Several HP-MS instruments have been built and applied by various groups.

The group of Kebarle [166, 167] initially applied an HP-MS instrument, consisting of a pulsed electron beam, a high-pressure ion source, and a sector or quadrupole mass analyzer. The high-pressure ion source comprises a temperature-controlled (-190 – 650 °C) reaction chamber in which a suitable reaction mixture at pressures of 0.5 – 10 mbar is ionized by short electron pulses. The ions created react and reach equilibrium while diffusing towards the walls of the ion source vessel. The ion source is sampled by means of a $3\text{ mm} \times 10\text{ }\mu\text{m}$ slit towards a high-vacuum chamber containing a magnetic sector or quadrupole mass analyzer. A similar system was developed to study gas-phase ion equilibria of alkali metal ions like K^+ with various molecules. The alkali metal ions were generated by thermoionic emission from platinum filaments doped with appropriate alkali metal salts [168]. Later on, similar studies were performed to get a better understanding of electrospray ionization [169]. Also, alkali metal ions generated by electrospray ionization at atmospheric pressure were pulsed into a ~ 10 -mbar reaction chamber to study reactions with amines and small peptides, introduced in the reaction chamber [170].

A very similar experimental setup was applied by the group of Castleman [171, 172] in their gas-phase ion-equilibrium studies involving gas-phase hydration of metal cations like Pb^+ and Bi^+ . A thermocouple in the reaction chamber enabled continuous monitoring of the temperature. A gating grid between ion source and reaction chamber allowed more accurate control of the energy of the ions entering the reaction cell.

Instrumentation for pulsed ionization HP-MS has been developed by the group of McMahon [173] and applied in thermochemical studies of relatively nonvolatile biomolecules. The instrument consists of a laboratory-built high-pressure ion source, focusing and alignment optics, and a double-focusing reversed geometry (BE) sector instrument. The ion source is kept in a separate vacuum chamber. The setup can be used with either continuous ionization to acquire mass spectra or in pulsed mode (5 – 40 Hz), where the mass spectrometer is operated in the selected ion monitoring mode. The temperature-controlled high-pressure ion source is operated at 1 – 10 -mbar pressure of a gas mixture prepared in an external reservoir. Ionization is achieved by means of a 2 -kV electron gun. The high pressure is sampled through a 200 - μm ID ion-exit aperture. When operated in the pulsed ion mode, the time evolution of the ion populations can be monitored to determine reaction rates of the ion–molecule reactions. Thermochemical properties and structures of, for instance, protonated dimers and trimers of glycine are studied in this way [173]. Later on, other gas-phase reactions of, among others, other amino acids are studied [174]. Instrumentation and experimental results with HP-MS have been reviewed and compared with FT-ICR-MS results [95].

4.8 Conclusion and Perspectives

This chapter deals with instrumentation and experimental methods to study gas-phase ion–molecule reactions, especially in relation to ion attachment with alkali metal ions. The obvious tools for this are mass spectrometers and tandem mass spectrometers. These are discussed in Sects. 4.3 and 4.4. Alternatively, gas-phase reactions may be studied in a variety of reaction chambers, either in static mode or in flow systems. Examples of the static reaction chambers are high-pressure MS devices (see Sect. 4.7). Examples of flow devices are flowing afterglow and drift-tube systems (see Sects. 4.5 and 4.6, respectively). However, next to these tools, there are a number of other tools that may be helpful.

In a crossed beam instrument [175], a beam of mass analyzed low-energy ions is intersected with a supersonic molecular beam of neutrals of a known chemical species. Products of the resulting ion–molecule reaction are energy and mass analyzed by a second mass spectrometer, which can be partly, typically for an octant, moved around the collision point. In this way, information is gathered on the chemical composition and energy of the fragments of the ion–molecule reaction involved.

In neutralization-reionization mass spectrometry (NRMS) [176], the mass spectrometer is used to study neutrals rather than ions. The neutrals are generated from mass-selected ions by intermolecular charge exchange or intramolecular dissociation reactions. Reionization of the neutrals is subsequently achieved by collision with gas-phase target atoms (although using photons or electrons has been described as well). NRMS can provide valuable information on the structures of the neutrals, especially if they are prepared by intramolecular dissociation reactions, as well as of their ionic precursors. To exemplify the wealth of information that can be obtained in NRMS, NR-spectra can be obtained for all four precursor ion–product ion combinations, i.e., positive-ion precursor and product ions, positive-ion precursor ion and negative-ion product ion, negative-ion precursor and product ions, or negative-ion precursor ion and positive-ion product ion.

Next to using a mass spectrometer to monitor and detect the product ions generated in ion–molecule reactions, these ions can also be studied using (laser) spectroscopy. One of the challenges in such studies is the low concentration of ions present in the source or reaction chamber. Informative reviews on the spectroscopy of molecular ions have been published [177–179].

References

1. Gronert S. Mass spectrometric studies of organic ion/molecule reactions. *Chem Rev.* 2001;101:329–60.
2. Blewett J, Jones EJ. Filament sources of positive ions. *Phys Rev.* 1936;50:464–8.
3. Selvin PC, Fujii T. Lithium ion attachment mass spectrometry: instrumentation and features. *Rev Sci Instrum.* 2001;72:2248–52.
4. Campbell EEB, Levine RD. Delayed ionization and fragmentation en route to thermoionic emission: statistics and dynamics. *Annu Rev Phys Chem.* 2000;51:65–98.

5. Bombick D, Pinkston JD, Allison J. Potassium ion chemical ionization and other uses of an alkali thermoionic emitter in mass spectrometry. *Anal Chem.* 1984;56:396–402.
6. Hodges RV, Beauchamp JL. Application of alkali ions in chemical ionization mass spectrometry. *Anal Chem.* 1976;48:825–9.
7. Cody RB, Burnier RC, Reents WD Jr, Carlin TJ, McCreary DA, Lengel RK, Freiser BS. Laser ionization source for ion cyclotron resonance spectroscopy. Application to atomic metal ion chemistry. *Int J Mass Spectrom Ion Phys.* 1980;33:37–43.
8. Aubriet F, Muller JF. Laser ablation mass spectrometry of inorganic transition metal compounds. Additional knowledge for the understanding of ion formation. *J Am Soc Mass Spectrom.* 2008;19:488–501.
9. Freiser BS. Gas-phase metal ion chemistry, *J Mass Spectrom.* 1996;31:703–15.
10. Carré V, Aubriet F, Scheepers PT, Krier G, Muller JF. Potential of laser ablation and laser desorption mass spectrometry to characterize organic and inorganic environmental pollutants on dust particles. *Rapid Commun Mass Spectrom.* 2005;19:871–80.
11. Lattimer RP, Schulten HR. Field ionization and field desorption mass spectrometry: past, present, and future. *Anal Chem.* 1989;61:1201A–15A.
12. Fenselau C, Cotter RJ. Chemical aspects of fast atom bombardment. *Chem Rev.* 1987;87:501–12.
13. Zenobi R, Knochenmuss R. Ion formation in MALDI mass spectrometry. *Mass Spectrom Rev.* 1998;17:337–66.
14. Kéki S, Deák G, Zsuga M. Fragmentation study of rutin, a naturally occurring flavone glycoside cationized with different alkali metal ions, using post-source decay matrix-assisted laser desorption/ionization mass spectrometry. *J Mass Spectrom.* 2001;36:1312–6.
15. Niessen WMA. Information from atmospheric-pressure ionization mass spectra. In: Niessen WMA, editor. *Encyclopedia of mass spectrometry*, Vol. 8. Oxford: Elsevier; 2006. p. 202–32.
16. Jang S, Song MJ, Kim H, Choi SS. Formation of metal complex ions from amino acid in the presence of Li^+ , Na^+ and K^+ by electrospray ionization: metal replacement of hydrogen in the ligands. *J Mass Spectrom.* 2011;46:496–501.
17. Rodriquez CF, Guo X, Shoeib T, Hopkinson AC, Siu KW. Formation of $[\text{M}-n\text{H} + m\text{Na}]^{(m-n)+}$ and $[\text{M}-n\text{H} + m\text{K}]^{(m-n)+}$ ions in electrospray mass spectrometry of peptides and proteins. *J Am Soc Mass Spectrom.* 2000;11:967–75.
18. Poon C, Kaplan H, Mayer PM. Methylating peptides to prevent adduct ion formation also directs cleavage in collision-induced dissociation mass spectrometry. *Eur J Mass Spectrom.* 2004;10:39–46.
19. Busch KL, Glish GL, McLuckey SA. *Mass spectrometry/mass spectrometry: Techniques and applications of tandem mass spectrometry.* New York: VCH; 1988.
20. Hipple JA, Fox RE, Condon EU. Metastable ions formed by electron impact in hydrocarbon gases. *Phys Rev.* 1946;69:347–356.
21. Yost RA, Enke CG. Selected ion fragmentation with a tandem quadrupole mass spectrometer. *J Am Chem Soc.* 1978;100:2274–5.
22. Lourijs JN, Cooks RG, Syka JEP, Kelley PE, Stafford GC Jr, Todd JFJ. Instrumentation, applications, and energy deposition in quadrupole ion-trap tandem mass spectrometry. *Anal Chem.* 1987;59:1677–85.
23. Marshall A, Hendrickson CL, Jackson GS. Fourier transform ion cyclotron resonance mass spectrometry: a primer. *Mass Spectrom Rev.* 1998;17:1–35.
24. Morris HR, Paxton T, Dell A, Langhorne J, Berg M, Bordoli RS, Hoyes J, Bateman RH. High-sensitivity collisionally-activated decomposition tandem mass spectrometry on a novel quadrupole-orthogonal acceleration time-of-flight mass spectrometer. *Rapid Commun Mass Spectrom.* 1996;10:889–96.
25. Hager JW. A new linear ion trap mass spectrometer, *Rapid Commun Mass Spectrom.* 2002;16:512–26.
26. Bienvenu WV, Déon C, Pasquarello C, Campbell JM, Sanchez JC, Vestal ML, Hochstrasser DF. Matrix-assisted laser desorption/ionization-tandem mass spectrometry with high resolution and sensitivity for identification and characterization of proteins. *Proteomics.* 2002;2:868–76.

27. Hu Q, Noll RJ, Li H, Makarov A, Hardman M, Cooks GR. The Orbitrap: a new mass spectrometer. *J Mass Spectrom.* 2005;40:430–43.
28. Makarov A, Denisov E, Kholomeev A, Balschun W, Lange O, Strupat K, Horning S. Performance evaluation of a hybrid linear ion trap/orbitrap mass spectrometer. *Anal Chem.* 2006;78:2113–20.
29. Schwartz JC, Wade AP, Enke CG, Cooks RG. Systematic delineation of scan modes in multidimensional mass spectrometry. *Anal Chem.* 1990;62:1809–18.
30. van Dongen WD, Niessen WMA. LC-MS systems for quantitative bioanalysis. *Bioanalysis.* 2012;4:2391–9.
31. Cherta L, Portolés T, Beltran J, Pitarch E, Mol JG, Hernández F. Application of gas chromatography-(triple quadrupole) mass spectrometry with atmospheric pressure chemical ionization for the determination of multiclass pesticides in fruits and vegetables. *J Chromatogr A.* 2013;1314:224–40.
32. Sleno L, Volmer DA. Ion activation methods for tandem mass spectrometry. *J Mass Spectrom.* 2004;39:1091–12.
33. Laskin J, Futrell JH. Activation of large ions in FT-ICR mass spectrometry. *Mass Spectrom Rev.* 2005;24:135–67.
34. Zhurov KO, Fornelli L, Wodrich MD, Laskay ŪA, Tsybin YO. Principles of electron capture and transfer dissociation mass spectrometry applied to peptide and protein structure analysis. *Chem Soc Rev.* 2013;42:5014–30.
35. Johnson JV, Yost RA, Kelley PE, Bradford DC. Tandem-in-space and tandem-in-time mass spectrometry: triple quadrupoles and quadrupole ion traps. *Anal Chem.* 1990;62:2162–72.
36. De Hoffmann E, Stroobant V. *Mass spectrometry. Principles and applications.* 3. ed. Chichester: Wiley; 2007.
37. Johnson JV, Yost RA. Tandem mass spectrometry for trace analysis *Anal Chem.* 1985;57:758A–68A.
38. Hunt DF, Shabanowitz J, Harvey TM, Coates ML. Analysis of organics in the environment by functional group using a triple quadrupole mass spectrometer. *J Chromatogr.* 1983;271:93–105.
39. Montesano C, Sergi M, Moro M, Napoletano S, Romolo FS, Del Carlo M, Compagnone D, Curini R. Screening of methylenedioxymphetamine- and piperazine-derived designer drugs in urine by LC-MS/MS using neutral loss and precursor ion scan. *J Mass Spectrom.* 2013;48:49–59.
40. Jian W, Liu HF, Zhao W, Jones E, Zhu M. Simultaneous screening of glutathione and cyanide adducts using precursor ion and neutral loss scans-dependent product ion spectral acquisition and data mining tools. *J Am Soc Mass Spectrom.* 2012;23:964–76.
41. Joly N, Vaillant C, Cohen AM, Martin P, El Essassi M, Massoui M, Banoub J. Structural determination of the novel fragmentation routes of zwitterionic morphine opiate antagonists naloxonazine and naloxone hydrochlorides using electrospray ionization tandem mass spectrometry. *Rapid Commun Mass Spectrom.* 2007;21:1062–74.
42. Bijlsma L, Sancho JV, Hernández F, Niessen WMA. Fragmentation pathways of drugs of abuse and their metabolites based on QTOF MS/MS and MSE accurate-mass spectra. *J Mass Spectrom.* 2011;46:865–75.
43. Mansoori BA, Dyer EW, Lock CM, Bateman K, Boyd RK, Thomson BA. Analytical performance of a high-pressure radiofrequency-only quadrupole collision cell with a axial field applied by using conical rods. *J Am Soc Mass Spectrom.* 1998;9:775–88.
44. Giles K, Pringle SD, Worthington KR, Little D, Wildgoose JL, Bateman RH. Applications of a travelling wave-based radio-frequency-only stacked ring ion guide. *Rapid Commun Mass Spectrom.* 2004;18:2401–14.
45. Chernushevich IV, Loboda AV, Thomson BA. An introduction to quadrupole–time-of-flight mass spectrometry. *J Mass Spectrom.* 2001;36:849–65.
46. Xia YQ, Miller JD, Bakhtiar R, Franklin RB, Liu DQ. Use of a quadrupole linear ion trap mass spectrometer in metabolite identification and bioanalysis. *Rapid Commun Mass Spectrom.* 2003;17:1137–45.

47. Hopfgartner G, Varesio E, Tschäpät V, Grivet C, Bourgogne E, Leuthold LA. Triple quadrupole linear ion trap mass spectrometer for the analysis of small molecules and macromolecules. *J Mass Spectrom.* 2004;39:845–55.
48. Tanner SD, Baranov VI, Bandua DR. Reaction cells and collision cells for ICP-MS: a tutorial review. *Spectrochim Acta B.* 2002;57:1361–452.
49. Yip Y-C, Sham W-C. Applications of collision/reaction cell technology in isotope dilution mass spectrometry. *Trends Anal Chem.* 2007;26:727–43.
50. Schmitt JP, Dawson PH, Beaulieu N. Chemical synthesis inside the collision cell of a MS/MS system: I—Formation of adduct ions between protonated esters and ammonia. *Org Mass Spectrom.* 1985;20:269–75.
51. Pachuta RR, Kenttämäa HI, Cooks RG, Zennie TM, Ping C, Chang C-J, Cassady JM. Analysis of natural products by tandem mass spectrometry employing reactive collisions with ethyl vinyl ether. *Org Mass Spectrom.* 1988;23:10–15.
52. Cole MJ, Enke CG. Fast atom bombardment tandem mass spectrometry employing ion–molecule reactions for the differentiation of phospholipid classes. *J Am Soc Mass Spectrom.* 1991;2:470–5.
53. Moraes LAB, Mendes MA, Sparrapan R, Eberlin MN. Transacetalization with gaseous carboxonium and carbosulfonium ions. *J Am Soc Mass Spectrom.* 2001;12:14–22.
54. Chen H, Zheng X, Cooks RG. Ketalization of phosphonium ions by 1,4-dioxane: selective detection of the chemical warfare agent simulant DMMP in mixtures using ion/molecule reactions. *J Am Soc Mass Spectrom.* 2003;14:182–8.
55. Kostianen R, Auriola S. Isomer-specific determination of tetrachlorodibenzo-*p*-dioxins by tandem mass spectrometry using low-energy reactive collisions between oxygen and negative molecular ions. *Rapid Commun Mass Spectrom.* 1988;2:135–7.
56. Jalonen J. Application of reactive collisions for differentiation of isomeric organic ions in the gas phase. *J Chem Soc Chem Commun.* 1985:872–4.
57. Meyerhoffer WJ, Bursley MM. Differentiation of the isomeric 1,2-cyclopentanediols by ion–molecule reactions in a triple quadrupole mass spectrometer. *Org Mass Spectrom.* 1989;24:169–75.
58. Schwartz JC, Schey KL, Cooks RG. A penta-quadrupole instrument for reaction intermediate scans and other MS–MS–MS experiments. *Int J Mass Spectrom Ion Proc.* 1990;101:1–20.
59. Eberlin MN. Triple-stage pentaquadrupole (QqQqQ) mass spectrometry and ion–molecule reactions. *Mass Spectrom Rev.* 1997;16:113–44.
60. Jonscher KR, Yates JR 3rd. The quadrupole ion trap mass spectrometer—a small solution to a big challenge. *Anal Biochem.* 1997;244:1–15.
61. March RE. An introduction to quadrupole ion trap mass spectrometry. *J Mass Spectrom.* 1997;32:351–69.
62. Schwartz JC, Senko MW, Syka JEP. A two-dimensional quadrupole ion trap mass spectrometer. *J Am Soc Mass Spectrom.* 2002;13:659–69.
63. Douglas DJ, Frank AJ, Mao D. Linear ion traps in mass spectrometry. *Mass Spectrom Rev.* 2005;24:1–29.
64. Wu SL, Jardine I, Hancock WS, Karger BL. A new and sensitive on-line liquid chromatography/mass spectrometric approach for top-down protein analysis: the comprehensive analysis of human growth hormone in an *E. coli* lysate using a hybrid linear ion trap/Fourier transform ion cyclotron resonance mass spectrometer. *Rapid Commun Mass Spectrom.* 2004;18:2201–7.
65. Olsen JV, Schwartz JC, Griep-Raming J, Nielsen ML, Damoc E, Denisov E, Lange O, Remes P, Taylor D, Splendore M, Wouters ER, Senko M, Makarov A, Mann M, Horning S. A dual pressure linear ion trap orbitrap instrument with very high sequencing speed. *Mol Cell Proteomics.* 2009;8:2759–69.
66. Kind T, Fiehn O. Advances in structure elucidation of small molecules using mass spectrometry. *Bioanal Rev.* 2010;2:23–60.

67. van der Hooft JJ, Vervoort J, Bino RJ, Beekwilder J, de Vos RC. Polyphenol identification based on systematic and robust high-resolution accurate mass spectrometry fragmentation. *Anal Chem.* 2011;83:409–16.
68. Gronert S. Quadrupole ion trap studies of fundamental organic reactions. *Mass Spectrom Rev.* 2005;24:100–120.
69. McLuckey SA, Glish GL, Asano KG, Van Berkel GJ. Self chemical ionization in an ion trap mass spectrometer. *Anal Chem.* 1988;60:2312–4.
70. Brodbelt JS, Louris JN, Cooks RG. Chemical ionization in an ion trap mass spectrometer. *Anal Chem.* 1987;59:1278.
71. Pons A, Lavigne V, Darriet P, Dubourdieu D. Determination of 3-methyl-2,4-nonanedione in red wines using methanol chemical ionization ion trap mass spectrometry. *J Chromatogr A.* 2011;1218:7023–30.
72. Libong D, Bouchonnet S, Ricordel I. A selective and sensitive method for quantitation of lysergic acid diethylamide (LSD) in whole blood by gas chromatography-ion trap tandem mass spectrometry. *J Anal Toxicol.* 2003;27:24–9.
73. Tzing SH, Ghule A, Chang JY, Ling YC. Selective adduct formation by furan chemical ionization reagent in gas chromatography ion trap mass spectrometry. *J Mass Spectrom.* 2003;38:401–8.
74. Bouchonnet S, Kinani S, Sablier M. Does the reagent gas influence collisional activation when performing in situ chemical ionization with an ion trap mass spectrometer? *Eur J Mass Spectrom.* 2007;13:223–6.
75. Ryzhov V, Sunderlin SS, Keller LMM, Gaillard ER. Measuring gas-phase basicities of amino acids using an ion trap mass spectrometer. A physical chemistry laboratory experiment. *J Chem Educ.* 2005;82:1071–3.
76. Nourse BD, Cooks RG. Proton affinity determination using the kinetic method in an ion trap mass spectrometer. *Int J Mass Spectrom Ion Proc.* 1991;106:249–72.
77. McCarley TD, Brodbelt J. Structurally diagnostic ion–molecule reactions and collisionally activated dissociation of 1,4-benzodiazepines in a quadrupole ion trap mass spectrometer. *Anal Chem.* 1993;65:2380–8.
78. Abirami S, Wong CC, Tsang CW, Ma NL. Dissociation of alkaliated alanine in the gas phase: the role of the metal cation. *Chemistry.* 2005;11:5289–301.
79. Fu M, Duan P, Li S, Habicht SC, Pinkston DS, Vinueza NR, Kenttämäa HI. Regioselective ion–molecule reactions for the mass spectrometric differentiation of protonated isomeric aromatic diamines. *Analyst.* 2008;133:452–4.
80. O’Hair RA. The 3D quadrupole ion trap mass spectrometer as a complete chemical laboratory for fundamental gas-phase studies of metal mediated chemistry. *Chem Commun (Camb).* 2006;14:1469–81.
81. Pitterl SJ, McLuckey SA. Recent developments in the ion-ion chemistry of high-mass multiply charged ions. *Mass Spectrom Rev.* 2005;24:931–58.
82. Xia Y, McLuckey SA. Evolution of instrumentation for the study of gas-phase ion-ion chemistry via mass spectrometry. *J Am Soc Mass Spectrom.* 2008;19:173–89.
83. Zaia J. Mass spectrometry of oligosaccharides. *Mass Spectrom Rev.* 2004;23:161–227.
84. Hsu FF, Turk J. Electrospray ionization with low-energy collisionally activated dissociation tandem mass spectrometry of glycerophospholipids: mechanisms of fragmentation and structural characterization. *J Chromatogr B.* 2009;877:2673–95.
85. Hsu FF, Turk J. Structural characterization of triacylglycerols as lithiated adducts by electrospray ionization mass spectrometry using low-energy collisionally activated dissociation on a triple stage quadrupole instrument. *J Am Soc Mass Spectrom.* 1999;10:587–99.
86. Cheng C, Gross ML. Applications and mechanisms of charge-remote fragmentation. *Mass Spectrom Rev.* 2000;19:398–420.
87. Michael SM, Chien BM, Lubman DM. An ion trap storage/time-of-flight mass spectrometer. *Rev Sci Instrum.* 1992;63:4277–84.

88. Michael SM, Chien BM, Lubman DM. Detection of electrospray ionization using a quadrupole ion trap storage/reflectron time-of-flight mass spectrometer. *Anal Chem.* 1993;65:2614–20.
89. Liu ZY. An introduction to hybrid ion trap/time-of-flight mass spectrometry coupled with liquid chromatography applied to drug metabolism studies. *J Mass Spectrom.* 2012;47:1627–42.
90. Römpf A, Taban IM, Mihalca R, Duursma MC, Mize TH, McDonnell LA, Heeren RM. Examples of Fourier transform ion cyclotron resonance mass spectrometry developments: from ion physics to remote access biochemical mass spectrometry. *Eur J Mass Spectrom.* 2005;11:443–56.
91. Kelleher NL, Lin HY, Valaskovic GA, Aserud DJ, Fridriksson EK, McLafferty FW. Top-down versus bottom-up protein characterization by tandem high-resolution mass spectrometry. *J Am Chem Soc.* 1999;121:806–12.
92. Bogdanov B, Smith RD. Proteomics by FTICR mass spectrometry: top down and bottom up. *Mass Spectrom Rev.* 2005;24:168–200.
93. Patrie SM, Charlebois JP, Whipple D, Kelleher NL, Hendrickson CL, Quinn JP, Marshall AG, Mukhopadhyay B. Construction of a hybrid quadrupole–Fourier transform ion cyclotron resonance mass spectrometer for versatile MS–MS above 10 kDa. *J Am Soc Mass Spectrom.* 2004;15:1099–108.
94. Syka JE, Marto JA, Bai DL, Horning S, Senko MW, Schwartz JC, Ueberheide B, Garcia B, Busby S, Muratore T, Shabanowitz J, Hunt DF. Novel linear quadrupole ion trap/FT mass spectrometer: performance characterization and use in the comparative analysis of histone H3 post-translational modifications. *J Proteome Res.* 2004;3:621–6.
95. Wu R, McMahon TB. Structures, energetics, and dynamics of gas phase ions studied by FTICR and HPMS. *Mass Spectrom Rev.* 2009;28:546–85.
96. Nibbering NMM. Gas-phase reactions of organic anions. *Adv Phys Org Chem.* 1988;24:1–55.
97. Raczynska ED, Gal JF, Maria PC, Zientara K, Szlag M. Application of FT-ICR-MS for the study of proton-transfer reactions involving biomolecules. *Anal Bioanal Chem.* 2007;389:1365–80.
98. Somogyi A. Probing peptide fragment ion structures by combining sustained off-resonance collision-induced dissociation and gas-phase H/D exchange (SORI-HDX) in Fourier transform ion-cyclotron resonance (FT-ICR) instruments. *J Am Soc Mass Spectrom.* 2008;19:1771–5.
99. Bou-Assaf GM, Chamoun JE, Emmett MR, Fajer PG, Marshall AG. Complexation and calcium-induced conformational changes in the cardiac troponin complex monitored by hydrogen/deuterium exchange and FT-ICR mass spectrometry. *Int J Mass Spectrom.* 2011;302:116–24.
100. Olsen JV, Macek B, Lange O, Makarov A, Horning S, Mann M. Higher-energy C-trap dissociation for peptide modification analysis. *Nat Methods.* 2007;4:709–12.
101. Michalski A, Damoc E, Hauschild JP, Lange O, Wiegand A, Makarov A, Nagaraj N, Cox J, Mann M, Horning S. Mass spectrometry-based proteomics using Q Exactive, a high-performance benchtop quadrupole orbitrap mass spectrometer. *Mol Cell Proteomics.* 2011;10:1–12 M111.011015.
102. Senko MW, Remes PM, Canterbury JD, Mathur R, Song Q, Eliuk SM, Mullen C, Earley L, Hardman M, Blethrow JD, Bui H, Specht A, Lange O, Denisov E, Makarov A, Horning S, Zabrouskov V. Novel parallelized quadrupole/linear ion trap/orbitrap tribrid mass spectrometer improving proteome coverage and peptide identification rates. *Anal Chem.* 2013;85:11710–4.
103. Fehsenfeld FC, Schmeltekopf AL, Goldan PD, Schiff HI, Ferguson EE. Thermal energy ion-neutral reaction rates. I. Some reactions of helium ions. *J Chem Phys.* 1966;44:4087–94.
104. Graul ST, Squires RR. Advances in flow reactor techniques for the study of gas-phase ion chemistry. *Mass Spectrom Rev.* 1988;7:263–358.

105. Smith D, Španěl P, Holland TA, al Singari W, Elder JB. Selected ion flow tube mass spectrometry of urine headspace. *Rapid Commun Mass Spectrom.* 1999;13:724–9.
106. Španěl P, Smith D. Progress in SIFT-MS: breath analysis and other applications. *Mass Spectrom Rev.* 2011;30:236–67.
107. Andrade FJ, Wetzel WC, Chan GCY, Webb MR, Gamez G, Ray SJ, Hieftje GM. A new, versatile, direct-current helium atmospheric-pressure glow discharge. *J Anal At Spectrom.* 2006;21:1175–84.
108. Rowe BR, Viggiano AA, Fehsenfeld FC, Fahey DW, Ferguson EE. Reactions between neutrals clustered on ions. *J Chem Phys.* 1982;76:742–3.
109. Castleman AW Jr, Weil KG, Sigsworth SW, Leuchtner RE, Keesee RG. Considerations of the rates and lifetimes of intermediate complexes for the association of various ligands to metal ions: Ag^+ and Cu^+ . *J Chem Phys.* 1987;86:3829–35.
110. Poutsma JC, Seburg RA, Chyall LJ, Sunderlin LS, Hill BT, Hu J, Squires RR. Combining electrospray ionization and the flowing afterglow method. *Rapid Commun Mass Spectrom.* 1997;11:489–93.
111. Ferguson EE. A Personal history of the early development of the flowing afterglow technique for ion–molecule reaction studies. *J Am Soc Mass Spectrom.* 1992;3:479–86.
112. Ferguson EE. Mass spectrometry in ionospheric research. *Mass Spectrom Rev.* 2007;26:142–9.
113. Fournier JA, Shuman NS, Melko JJ, Ard SG, Viggiano AA. A novel technique for measurement of thermal rate constants and temperature dependences of dissociative recombination: CO_2^+ , CF_3^+ , N_2O^+ , C_7H_8^+ , C_7H_7^+ , C_6H_6^+ , C_6H_5^+ , C_5H_6^+ , C_4H_4^+ , and C_3H_3^+ . *J Chem Phys.* 2013;138:154201.
114. Ichino T, Andrews DH, Rathbone GJ, Misaizu F, Calvi RM, Wren SW, Kato S, Bierbaum VM, Lineberger WC. Ion chemistry of 1H-1,2,3-triazole. *J Phys Chem B.* 2008;112:545–57.
115. Shuman NS, Friedman JF, Miller TM, Viggiano AA. Electron attachment to 14 halogenated alkenes and alkanes, 300–600 K. *J Chem Phys.* 2012;137:164306.
116. Shuman NS, Miller TM, Viggiano AA, Troe J. Electron attachment to CF_3 and CF_3Br at temperatures up to 890 K: experimental test of the kinetic modeling approach. *J Chem Phys.* 2013;138:204316.
117. Shuman NS, Miller TM, Friedman JF, Viggiano AA. Electron attachment to $\text{Fe}(\text{CO})_n$ ($n=0-5$). *J Phys Chem A.* 2013;117:1102–9.
118. Miller TM, Friedman JF, Caples CM, Shuman NS, Van Doren JM, Bardaro MF Jr, Nguyen P, Zweiben C, Campbell MJ, Viggiano AA. Electron attachment to sulfur oxyhalides: SOF_2 , SOCl_2 , SO_2F_2 , SO_2Cl_2 , and SO_2FCl attachment rate coefficients, 300–900 K. *J Chem Phys.* 2010;132:214302.
119. Garver JM, Yang Z, Kato S, Wren SW, Vogelhuber KM, Lineberger WC, Bierbaum VM. Gas phase reactions of 1,3,5-triazine: proton transfer, hydride transfer, and anionic σ -adduct formation. *J Am Soc Mass Spectrom.* 2011;22:1260–72.
120. Smith D, Španěl P. On-line determination of the deuterium abundance in breath water vapour by flowing afterglow mass spectrometry with applications to measurements of total body water. *Rapid Commun Mass Spectrom.* 2001;15:25–32.
121. Španěl P, Smith D. Accuracy and precision of flowing afterglow mass spectrometry for the determination of the deuterium abundance in the headspace of aqueous liquids and exhaled breath water. *Rapid Commun Mass Spectrom.* 2001;15:867–72.
122. Davies S, Španěl P, Smith D. Rapid measurement of deuterium content of breath following oral ingestion to determine body water. *Physiol Meas.* 2001;22:651–9.
123. Jecklin MC, Gamez G, Touboul D, Zenobi R. Atmospheric pressure glow discharge desorption mass spectrometry for rapid screening of pesticides in food. *Rapid Commun Mass Spectrom.* 2008;22:2791–8.
124. Tan BK, Smith D, Španěl P, Davies SJ. Dispersal kinetics of deuterated water in the lungs and airways following mouth inhalation: real-time breath analysis by flowing afterglow mass spectrometry (FA-MS). *J Breath Res.* 2010;4:017109.

125. Tan BK, Davies SJ, Španěl P, Smith D. Injection of deuterated water into the pulmonary/alveolar circulation; measurement of HDO in exhaled breath and implications to breath analysis. *J Breath Res.* 2012;6:036005.
126. Hansel A, Jordan A, Holzinger R, Prazeller P, Vogel W, Lindinger W. Proton transfer reaction mass spectrometry: on-line trace gas analysis at ppb level. *Int J Mass Spectrom Ion Proc.* 1995;149/150:609–19.
127. Blake RS, Monks PS, Ellis AM. Proton-transfer reaction mass spectrometry. *Chem Rev.* 2009;109:861–96.
128. Jordan A, Haidacher S, Hanel G, Hartungen E, Herbig J, Maerk L, Schottkowsky R, Seehauser H, Sulzer P, Maerk TD. An online ultra-high sensitivity proton-transfer-reaction mass-spectrometer combined with switchable reagent ion capability (PTR + SRI-MS). *Int J Mass Spectrom.* 2009;286:32–8.
129. Creaser CS, Griffiths JR, Bramwell CJ, Noreen S, Hill CA, Thomas CLP. Ion mobility spectrometry: a review. Part 1. Structural analysis by mobility measurement. *Analyst.* 2004;129:984–994.
130. Hill HH Jr, Siems WF, St Louis RH, McMinn DG. Ion mobility spectrometry. *Anal Chem.* 1990;62:1201A–9A.
131. Lindinger W, Albritton DL. Mobilities of various mass-identified positive ions in helium and argon. *J Chem Phys.* 1975;62:3517–22.
132. Shvartsburg AA, Jarrold MF. An exact hard-spheres scattering model for the mobilities of polyatomic ions. *Chem Phys Lett.* 1996;261:86–91.
133. Wyttenbach T, von Helden G, Batka JJ Jr, Carlat D, Bowers MT. Effect of the long-range potential on ion mobility measurements. *J Am Soc Mass Spectrom.* 1997;8:275–82.
134. Revercomb HE, Mason EA. Theory of plasma chromatography/gaseous electrophoresis—A review. *Anal Chem.* 1975;47:970–83.
135. Bowers MT, Kemper PR, von Helden G, van Koppen PA. Gas-phase ion chromatography: transition metal state selection and carbon cluster formation. *Science.* 1993;260(5113):1446–51.
136. Oxley JC, Smith JL, Kirschenbaum LJ, Marimnganti S, Vadlamannati S. Detection of explosives in hair using ion mobility spectrometry. *J Forensic Sci.* 2008;53:690–3.
137. Zimmermann S, Barth S, Baether WK, Ringer J. Miniaturized low-cost ion mobility spectrometer for fast detection of chemical warfare agents. *Anal Chem.* 2008;80:6671–6.
138. Wyttenbach T, von Helden G, Bowers MT. Gas-phase conformation of biological molecules: Bradykinin. *J Am Chem Soc.* 1996;118:8355–64.
139. Clemmer DE, Jarrold MF. Ion mobility measurements and their applications to clusters and biomolecules. *J Mass Spectrom.* 1997;32:577–92.
140. Srebalus CA, Li J, Marshall WS, Clemmer DE. Gas-phase separations of electrosprayed peptide libraries. *Anal Chem.* 1999;71:3918–27.
141. Laphorn C, Pullen F, Chowdhry BZ. Ion mobility spectrometry-mass spectrometry (IMS-MS) of small molecules: separating and assigning structures to ions. *Mass Spectrom Rev.* 2013;32:43–71.
142. Kanu AB, Dwivedi P, Tam M, Matz L, Hill HH Jr. Ion mobility-mass spectrometry. *J Mass Spectrom.* 2008;43:1–22.
143. Williams DM, Pukala TL. Novel insights into protein misfolding diseases revealed by ion mobility-mass spectrometry. *Mass Spectrom Rev.* 2013;32:169–87.
144. Wyttenbach T, Pierson NA, Clemmer DE, Bowers MT. Ion mobility analysis of molecular dynamics. *Annu Rev Phys Chem.* 2014;65:175–96.
145. Guevremont R. High-field asymmetric waveform ion mobility spectrometry: a new tool for mass spectrometry. *J Chromatogr A.* 2004;1058:3–19.
146. Kolakowski BM, Mester Z. Review of applications of high-field asymmetric waveform ion mobility spectrometry (FAIMS) and differential mobility spectrometry (DMS). *Analyst.* 2007;132:842–64.

147. Tsai CW, Yost RA, Garrett TJ. High-field asymmetric waveform ion mobility spectrometry with solvent vapor addition: a potential greener bioanalytical technique. *Bioanalysis*. 2012;4:1363–75.
148. Pringle SD, Giles K, Wildgoose JL, Williams JP, Slade SE, Thalassinos K, Bateman RH, Bowers MT, Scrivens JH. An investigation of the mobility separation of some peptide and protein ions using a new hybrid quadrupole/travelling wave IMS/oa-ToF instrument. *Int J Mass Spectrom*. 2007;261:1–12.
149. Koeniger SL, Merenbloom SI, Valentine SJ, Jarrold MF, Udseth HR, Smith RD, Clemmer DE. An IMS–IMS analogue of MS–MS. *Anal Chem*. 2006;78:4161–74.
150. Zhong Y, Hyung SJ, Ruotolo BT. Ion mobility-mass spectrometry for structural proteomics. *Expert Rev Proteomics*. 2012;9:47–58.
151. Jurneckzo E, Barran PE. How useful is ion mobility mass spectrometry for structural biology? The relationship between protein crystal structures and their collision cross sections in the gas phase. *Analyst*. 2011;136:20–8.
152. Uetrecht C, Rose RJ, van Duijn E, Lorenzen K, Heck AJ. Ion mobility mass spectrometry of proteins and protein assemblies. *Chem Soc Rev*. 2010;39:1633–55.
153. Enders JR, McLean JA. Chiral and structural analysis of biomolecules using mass spectrometry and ion mobility-mass spectrometry. *Chirality*. 2009;21:E253–264.
154. Xia YQ, Wu ST, Jemal M. LC-FAIMS-MS/MS for quantification of a peptide in plasma and evaluation of FAIMS global selectivity from plasma components. *Anal Chem*. 2008;80:7137–43.
155. Olivova P, Chen W, Chakraborty AB, Gebler JC. Determination of N-glycosylation sites and site heterogeneity in a monoclonal antibody by electrospray quadrupole ion-mobility time-of-flight mass spectrometry. *Rapid Commun Mass Spectrom*. 2008;22:29–40.
156. Shimizu A, Ohe T, Chiba M. A novel method for the determination of the site of glucuronidation by ion mobility spectrometry-mass spectrometry. *Drug Metab Dispos*. 2012;40:1456–9.
157. Dear GJ, Munoz-Muriedas J, Beaumont C, Roberts A, Kirk J, Williams JP, Campuzano I. Sites of metabolic substitution: investigating metabolite structures utilising ion mobility and molecular modelling. *Rapid Commun Mass Spectrom*. 2010;24:3157–62.
158. Shimizu A, Chiba M. Ion mobility spectrometry-mass spectrometry analysis for the site of aromatic hydroxylation. *Drug Metab Dispos*. 2013;41:1295–9.
159. Badman ER, Cooks RG. Miniature mass analyzers. *J Mass Spectrom*. 2000;35:659–71.
160. Peng Y, Austin DE. New approaches to miniaturizing ion trap mass analyzers. *Trends Anal Chem*. 2011;30:1560–7.
161. Whitten WB, Reilly PT, Ramsey JM. High-pressure ion trap mass spectrometry. *Rapid Commun Mass Spectrom*. 2004;18:1749–52.
162. Misharin A, Novoselov K, Laiko V, Doroshenko VM. Development and characterization of a field-deployable ion-trap mass spectrometer with an atmospheric pressure interface. *Anal Chem*. 2012;84:10105–12.
163. Sparkman OD, Bierbaum VM. Focus on field-portable and miniature mass spectrometers. Presentations from the 12th Sanibel conference on mass spectrometry. *J Am Soc Mass Spectrom*. 2001;12:617–8.
164. Munson MSB, Franklin JL, Field FH. High pressure mass spectrometric study of alkanes. *J Phys Chem*. 1964;68:3098–107.
165. Munson MSB, Field FH. Chemical ionization mass spectrometry. I. General introduction. *J Am Chem Soc*. 1966;88:2621–30.
166. Kebarle P. Ion thermochemistry and solvation from gas-phase ion equilibria. *Ann Rev Phys Chem*. 1977;28:445–76.
167. Cunningham AJ, Payzant JD, Kebarle P. A kinetic study of the proton hydrate $H^+(H_2O)_n$ equilibria in the gas phase. *J Am Chem Soc*. 1972;94:7627–32.
168. Searles SK, Kebarle P. Hydration of the potassium ion in the gas phase: enthalpies and entropies of hydration reactions: $K^+(H_2O)_{n-1} + H_2O = K^+(H_2O)_n$ for $n = 1$ to $n = 6$. *Can J Chem*. 1969;47:2619–27.

169. Kebarle P, Verkerk UH. Electrospray: from ions in solution to ions in the gas phase, what we know now. *Mass Spectrom Rev.* 2009;28:898–917.
170. Klassen JS, Blades AT, Kebarle P. Determinations of ion–molecule equilibria involving ions produced by electrospray. Hydration of protonated amines, diamines, and some small peptides. *J Phys Chem.* 1995;99:15509–17.
171. Tang IN, Castleman AW Jr. Mass spectrometric study of the gas-phase hydration of the monovalent lead ion. *J Chem Phys.* 1972;57:3638–44.
172. Tang IN, Castleman AW Jr. Mass spectrometric study of gas-phase clustering reactions: hydration of the monovalent bismuth ion. *J Chem Phys.* 1974;60:3981–6.
173. Raspopov SA, McMahon TB. A high-pressure mass spectrometric and density functional theory investigation of the thermochemical properties and structure of protonated dimers and trimers of glycine. *J Mass Spectrom.* 2005;40:1536–45.
174. Wu R, McMahon TB. Stabilization of zwitterionic structures of amino acids (Gly, Ala, Val, Leu, Ile, Ser and Pro) by ammonium ions in the gas phase. *J Am Chem Soc.* 2008;130:3065–78.
175. Vestal ML, Blakley CR, Ryan PW, Futrell JH. New crossed-beam apparatus for the study of ion–molecule collision processes. *Rev Sci Instrum.* 1976;47:15–26.
176. Wesdemiotis C, McLafferty FW. Neutralization–reionization mass spectrometry. *Chem Rev.* 1987;87:485–500.
177. Saykally RJ, Woods RC. High resolution spectroscopy of molecular ions. *Ann Rev Phys Chem.* 1981;32:403–31.
178. Polfer NC, Oomens J. Vibrational spectroscopy of bare and solvated ionic complexes of biological relevance. *Mass Spectrom Rev.* 2009;28:468–94.
179. Polfer NC, Oomens J. Reaction products in mass spectrometry elucidated with infrared spectroscopy. *Phys Chem Chem Phys.* 2007;9:3804–17.