Mass Spectrometry

Principles and Applications

Third Edition

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Contents

Preface to Third Edition

Following the first studies of J.J. Thomson (1912), mass spectrometry has undergone countless improvements. Since 1958, gas chromatography coupled with mass spectrometry has revolutionized the analysis of volatile compounds. Another revolution occurred in the 1980s when the technique became available for the study of non-volatile compounds such as peptides, oligosaccharides, phospholipids, bile salts, etc. From the discoveries of electrospray and matrix-assisted laser desorption in the late 1980s, compounds with molecular masses exceeding several hundred thousands of daltons, such as synthetic polymers, proteins, glycans and polynucleotides, have been analysed by mass spectrometry.

From the time of the second edition published in 2001 until now, much progress has been achieved. Several techniques have been improved, others have almost disappeared. New atmospheric pressure desorption ionization sources have been discovered and made available commercially. One completely new instrument, the orbitrap, based on a new mass analyser, has been developed and is now also available commercially. Improved accuracy in low-mass determination, even at low resolution, improvements in sensitivity, better detection limits and more efficient tandem mass spectrometry even on high-molecular-mass compounds are some of the main achievements. We have done our best to include them is this new edition.

As the techniques continue to advance, the use of mass spectrometry continues to grow. Many new applications have been developed. The most impressive ones arise in system biology analysis.

Starting from the very foundations of mass spectrometry, this book presents all the important techniques developed up to today. It describes many analytical methods based on these techniques and emphasizes their usefulness by numerous examples. The reader will also find the necessary information for the interpretation of data. A series of graduated exercises allows the reader to check his or her understanding of the subject. Numerous references are given for those who wish to go deeper into some subjects. Important Internet addresses are also provided. We hope that this new edition will prove useful to students, teachers and researchers.

We would like to thank Professor Jean-Louis Habib Jiwan and Alexander Spote for their friendly hospitality and competent help.

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Many colleagues and friends have read the manuscript and their comments have been very helpful. Some of them carried out a thorough reading. They deserve special mention:

namely, Magda Claeys, Bruno Domon, Jean-Claude Tabet, and the late François Van Hoof. We also wish to acknowledge the remarkable work done by the scientific editors at John Wiley & Sons.

Many useful comments have been published on the first two editions, or sent to the editor or the authors. Those from Steen Ingemann were particularly detailed and constructive.

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Edmond de Hoffmann and Vincent Stroobant
Louvain-la-Neuve, March 2007

Introduction

Mass spectrometry's characteristics have raised it to an outstanding position among analytical methods: unequalled sensitivity, detection limits, speed and diversity of its applications. In analytical chemistry, the most recent applications are mostly oriented towards biochemical problems, such as proteome, metabolome, high throughput in drug discovery and metabolism, and so on. Other analytical applications are routinely applied in pollution control, food control, forensic science, natural products or process monitoring. Other applications include atomic physics, reaction physics, reaction kinetics, geochronology, inorganic chemical analysis, ion–molecule reactions, determination of thermodynamic parameters (ΔG°_{f}, K_a, etc.), and many others.

Mass spectrometry has progressed extremely rapidly during the last decade, between 1995 and 2005. This progress has led to the advent of entirely new instruments. New atmospheric pressure sources were developed [1–4], existing analysers were perfected and new hybrid instruments were realized by new combinations of analysers. An analyser based on a new concept was described: namely, the orbitrap [5] presented in Chapter 2. This has led to the development of new applications. To give some examples, the first spectra of an intact virus [6] and of very large non-covalent complexes were obtained. New high-throughput mass spectrometry was developed to meet the needs of the proteomic [7, 8], metabolomic [9] and other 'omics'.

Principles

The first step in the mass spectrometric analysis of compounds is the production of gas-phase ions of the compound, for example by electron ionization:

$$M + e^- \longrightarrow M^{\bullet +} + 2e^-$$

This molecular ion normally undergoes fragmentations. Because it is a radical cation with an odd number of electrons, it can fragment to give either a radical and an ion with an even number of electrons, or a molecule and a new radical cation. We stress the important difference between these two types of ions and the need to write them correctly:

$$M^{\bullet +} \rightarrow EE^{+} \text{ (EVEN ION)} + R^{\bullet} \text{ (RADICAL)}$$

$$M^{\bullet +} \rightarrow OE^{\bullet +} \text{ (ODD ION)} + N \text{ (MOLECULE)}$$

These two types of ions have different chemical properties. Each primary product ion derived from the molecular ion can, in turn, undergo fragmentation, and so on. All these ions are separated in the mass spectrometer according to their mass-to-charge ratio, and

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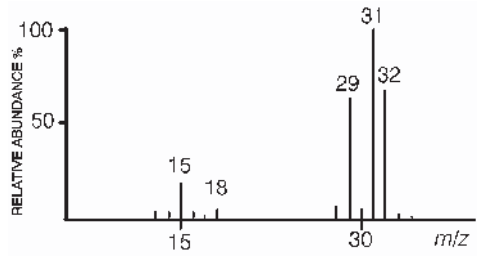

m/z	Relative abundance (%)	*m/z*	Relative abundance (%)
12	0.33	28	6.3
13	0.72	29	64
14	2.4	30	3.8
15	13	31	100
16	0.21	32	66
17	1.0	33	0.73
18	0.9	34	~ 0.1

Figure 1
Mass spectrum of methanol by electron ionization, presented as a graph and as a table.

are detected in proportion to their abundance. A mass spectrum of the molecule is thus produced. It provides this result as a plot of ion abundance versus mass-to-charge ratio. As illustrated in Figure 1, mass spectra can be presented as a bar graph or as a table. In either presentation, the most intense peak is called the base peak and is arbitrarily assigned the relative abundance of 100 %. The abundances of all the other peaks are given their proportionate values, as percentages of the base peak. Many existing publications label the *y* axis of the mass spectrum as number of ions, ion counts or relative intensity. But the term relative abundance is better used to refer to the number of ions in the mass spectra.

Most of the positive ions have a charge corresponding to the loss of only one electron. For large molecules, multiply charged ions also can be obtained. Ions are separated and detected according to the mass-to-charge ratio. The total charge of the ions will be represented by q, the electron charge by e and the number of charges of the ions by z:

$$q = ze \quad \text{and} \quad e = 1.6 \times 10^{-19}\,\text{C}$$

The x axis of the mass spectrum that represents the mass-to-charge ratio is commonly labelled m/z. When m is given as the relative mass and z as the charge number, both of which are unitless, m/z is used to denote a dimensionless quantity.

Generally in mass spectrometry, the charge is indicated in multiples of the elementary charge or charge of one electron in absolute value ($1\,e = 1.602\,177 \times 10^{-19}$ C) and the mass is indicated in atomic mass units ($1\,\text{u} = 1.660\,540 \times 10^{-27}$ kg). As already mentioned, the physical property that is measured in mass spectrometry is the mass-to-charge ratio. When the mass is expressed in atomic mass units (u) and the charge in elementary charge units

(e) then the mass-to-charge ratio has u/e as dimensions. For simplicity, a new unit, the Thomson, with symbol Th, has been proposed [10]. The fundamental definition for this unit is

$$1\,\mathrm{Th} = 1\,\mathrm{u}/e = 1.036\,426 \times 10^{-8}\,\mathrm{kg\,C^{-1}}$$

Ions provide information concerning the nature and the structure of their precursor molecule. In the spectrum of a pure compound, the molecular ion, if present, appears at the highest value of m/z (followed by ions containing heavier isotopes) and gives the molecular mass of the compound. The term molecular ion refers in chemistry to an ion corresponding to a complete molecule regarding occupied valences. This molecular ion appears at m/z 32 in the spectrum of methanol, where the peak at m/z 33 is due to the presence of the ^{13}C isotope, with an intensity that is 1.1 % of that of the m/z 32 peak. In the same spectrum, the peak at m/z 15 indicates the presence of a methyl group. The difference between 32 and 15, that is 17, is characteristic of the loss of a neutral mass of 17 Da by the molecular ion and is typical of a hydroxyl group. In the same spectrum, the peak at m/z 16 could formally correspond to ions $CH_4^{\bullet+}$, O^+ or even CH_3OH^{2+}, because they all have m/z values equal to 16 at low resolution. However, O^+ is unlikely to occur, and a doubly charged ion for such a small molecule is not stable enough to be observed.

The atomic mass units u or Da have the same fundamental definition:

$$1\,\mathrm{u} = 1\,\mathrm{Da} = 1.660\,540 \times 10^{-27}\,\mathrm{kg} \pm 0.59\,\mathrm{ppm}$$

However, they are traditionally used in different contexts: when dealing with mean isotopic masses, as generally used in stoichiometric calculations, Da will be preferred; in mass spectrometry, masses referring to the main isotope of each element are used and expressed in u.

There are different ways to define and thus to calculate the mass of an atom, molecule or ion. For stoichiometric calculations chemists use the average mass calculated using the atomic weight, which is the weighted average of the atomic masses of the different isotopes of each element in the molecule. In mass spectrometry, the nominal mass or the monoisotopic mass is generally used. The nominal mass is calculated using the mass of the predominant isotope of each element rounded to the nearest integer value that corresponds to the mass number, also called nucleon number. But the exact masses of isotopes are not exact whole numbers. They differ weakly from the summed mass values of their constituent particles that are protons, neutrons and electrons. These differences, which are called the mass defects, are equivalent to the binding energy that holds these particles together. Consequently, every isotope has a unique and characteristic mass defect. The monoisotopic mass, which takes into account these mass defects, is calculated by using the exact mass of the most abundant isotope for each constituent element.

The difference between the average mass, the nominal mass and the monoisotopic mass can amount to several Da, depending on the number of atoms and their isotopic composition. The type of mass determined by mass spectrometry depends largely on the resolution and accuracy of the analyser. Let us consider CH_3Cl as an example. Actually, chlorine atoms are mixtures of two isotopes, whose exact masses are respectively 34.968 852 u

and 36.965 903 u. Their relative abundances are 75.77 % and 24.23 %. The atomic weight of chlorine atoms is the balanced average: $(34.968\,852 \times 0.7577 + 36.965\,903 \times 0.2423) = 35.453$ Da. The average mass of CH_3Cl is $12.011 + (3 \times 1.00\,794) + 35.453 = 50.4878$ Da, whereas its monoisotopic mass is $12.000\,000 + (3 \times 1.007\,825) + 34.968\,852 = 49.992\,327$ u. When the mass of CH_3Cl is measured with a mass spectrometer, two isotopic peaks will appear at their respective masses and relative abundances. Thus, two mass-to-charge ratios will be observed with a mass spectrometer. The first peak will be at m/z $(34.968\,852 + 12.000\,000 + 3 \times 1.007\,825) = 49.992\,327$ Th, rounded to m/z 50. The mass-to-charge value of the second peak will be $(36.965\,90 + 12.000\,000 + 3 \times 1.007\,825) = 51.989\,365$ Th, rounded to m/z 52. The abundance at this latter m/z value is $(24.23/75.77) = 0.3198$, or 31.98 % of that observed at m/z 50. Carbon and hydrogen also are composed of isotopes, but at much lower abundances. They are neglected for this example.

For molecules of very high molecular weights, the differences between the different masses can become notable. Let us consider two examples.

The first example is human insulin, a protein having the molecular formula $C_{257}H_{383}N_{65}O_{77}S_6$. The nominal mass of insulin is 5801 u using the integer mass of the most abundant isotope of each element, such as 12 u for carbon, 1u for hydrogen, 14 u for nitrogen, 16 u for oxygen and 32 u for sulfur. Its monoisotopic mass of 5803.6375 u is calculated using the exact masses of the predominant isotope of each element such as C = 12.0000 u, H = 1.0079 u, N = 14.0031 u, O = 15.9949 u and S = 31.9721 u. These values can be found in the tables of isotopes in Appendices 4A and 4B. Finally, an average mass of 5807.6559 Da is calculated using the atomic weight for each element, such as C = 12.011 Da, H = 1.0078 Da, N = 14.0067 Da, O = 15.9994 Da and S = 32.066 Da.

The second example is illustrated in Figure 2. The masses of two alkanes having the molecular formulae $C_{20}H_{42}$ and $C_{100}H_{202}$ are calculated. For the smaller alkane, its nominal mass is $(20 \times 12) + (42 \times 1) = 282$ u, its monoisotopic mass is $(20 \times 12) + (42 \times 1.007\,825) = 282.3287$ u rounded to 282.33 and its average mass is $(20 \times 12.011) + (42 \times 1.007\,94) = 282.5535$ Da. The differences between these different types of masses are small but are more important for the heavier alkane. Indeed, its nominal mass is $(100 \times 12) + (202 \times 1) = 1402$ u, its monoisotopic mass is $(100 \times 12) + (202 \times 1.007\,825) = 1403.5807$ u rounded to 1403.58 and its average mass is $(100 \times 12.011) + (202 \times 1.007\,94) = 1404.7039$ Da.

In conclusion, the monoisotopic mass is used when it is possible experimentally to distinguish the isotopes, whereas the average mass is used when the isotopes are not distinguishable. The use of nominal mass is not recommended and should only be used for low-mass compounds containing only the elements C, H, N, O and S to avoid to making mistakes.

Diagram of a Mass Spectrometer

A mass spectrometer always contains the following elements, as illustrated in Figure 3: a sample inlet to introduce the compound that is analysed, for example a gas chromatograph or a direct insertion probe; an ionization source to produce ions from the sample; one or several mass analysers to separate the various ions; a detector to 'count' the ions emerging

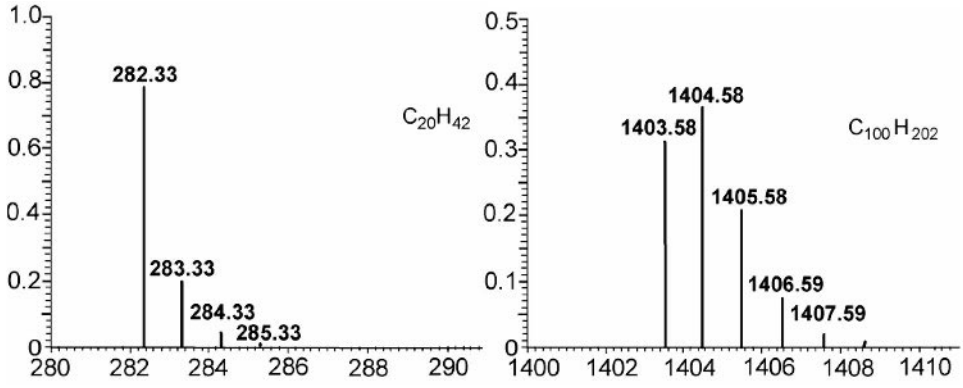

Figure 2
Mass spectra of isotopic patterns of two alkanes having the molecular formulae $C_{20}H_{42}$ and $C_{100}H_{202}$, respectively. The monoisotopic mass is the lighter mass of the isotopic pattern whereas the average mass, used by chemists in stoichiometric calculations, is the balanced mean value of all the observed masses.

from the last analyser; and finally a data processing system that produces the mass spectrum in a suitable form. However, some mass spectrometers combine the sample inlet and the ionization source and others combine the mass analyser and the detector.

A mass spectrometer should always perform the following processes:

1. Produce ions from the sample in the ionization source.
2. Separate these ions according to their mass-to-charge ratio in the mass analyser.
3. Eventually, fragment the selected ions and analyze the fragments in a second analyser.
4. Detect the ions emerging from the last analyser and measure their abundance with the detector that converts the ions into electrical signals.
5. Process the signals from the detector that are transmitted to the computer and control the instrument through feedback.

History

A large number of mass spectrometers have been developed according to this fundamental scheme since Thomson's experiments in 1897. Listed here are some highlights of this development [11, 12]:

1886: E. GOLDSTEIN discovers anode rays (positive gas-phase ions) in gas discharge [13].

1897: J.J. THOMSON discovers the electron and determines its mass-to-charge ratio. *Nobel Prize in 1906*.

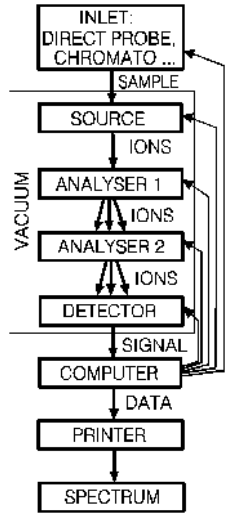

Figure 3
Basic diagram for a mass spectrometer with two analysers and feedback control carried out by a data system.

1898: W. WIEN analyses anode rays by magnetic deflection and then establishes that these rays carried a positive charge [14]. *Nobel Prize in 1911*.

1901: W. KAUFMANN analyses cathodic rays using parallel electric and magnetic fields [15].

1909: R.A. MILLIKAN and H. FLETCHER determine the elementary unit of charge.

1912: J.J. THOMSON constructs the first mass spectrometer (then called a parabola spectrograph) [16]. He obtains mass spectra of O_2, N_2, CO, CO_2 and $COCl_2$. He observes negative and multiply charged ions. He discovers metastable ions. In 1913, he discovers isotopes 20 and 22 of neon.

1918: A.J. DEMPSTER develops the electron ionization source and the first spectrometer with a sector-shaped magnet (180°) with direction focusing [17].

1919: F.W. ASTON develops the first mass spectrometer with velocity focusing [18]. *Nobel Prize in 1922*. He measures mass defects in 1923 [19].

1932: K.T. BAINBRIDGE proves the mass–energy equivalence postulated by Einstein [20].

1934: R. CONRAD applies mass spectrometry to organic chemistry [21].

1934: W.R. SMYTHE, L.H. RUMBAUGH and S.S. WEST succeed in the first preparative isotope separation [22].

1940: A.O. NIER isolates uranium-235 [23].

1942: The Consolidated Engineering Corporation builds the first commercial instrument dedicated to organic analysis for the Atlantic Refinery Company.

1945: First recognition of the metastable peaks by J.A. HIPPLE and E.U. CONDON [24].

1948: A.E. CAMERON and D.F. EGGERS publish design and mass spectra for a linear time-of-flight (LTOF) mass spectrometer [25]. W. STEPHENS proposed the concept of this analyser in 1946 [26].

1949: H. SOMMER, H.A. THOMAS and J.A. HIPPLE describe the first application in mass spectrometry of ion cyclotron resonance (ICR) [27].

1952: Theories of quasi-equilibrium (QET) [28] and RRKM [29] explain the monomolecular fragmentation of ions. R.A. MARCUS receives the *Nobel Prize in 1992*.

1952: E.G. JOHNSON and A.O. NIER develop double-focusing instruments [30].

1953: W. PAUL and H.S. STEINWEDEL describe the quadrupole analyser and the ion trap or quistor in a patent [31]. W. PAUL, H.P. REINHARD and U. Von ZAHN, of Bonn University, describe the quadrupole spectrometer in *Zeitschrift für Physik* in 1958. PAUL and DEHMELT receive the *Nobel Prize in 1989* [32].

1955: W.L. WILEY and I.H. McLAREN of Bendix Corporation make key advances in LTOF design [33].

1956: J. BEYNON shows the analytical usefulness of high-resolution and exact mass determinations of the elementary composition of ions [34].

1956: First spectrometers coupled with a gas chromatograph by F.W. McLAFFERTY [35] and R.S. GOHLKE [36].

1957: Kratos introduces the first commercial mass spectrometer with double focusing.

1958: Bendix introduces the first commercial LTOF instrument.

1966: M.S.B. MUNSON and F.H. FIELD discover chemical ionization (CI) [37].

1967: F.W. McLAFFERTY [38] and K.R. JENNINGS [39] introduce the collision-induced dissociation (CID) procedure.

1968: Finnigan introduces the first commercial quadrupole mass spectrometer.

1968: First mass spectrometers coupled with data processing units.

1969: H.D. BECKEY demonstrates field desorption (FD) mass spectrometry of organic molecules [40].

1972: V.I. KARATEV, B.A. MAMYRIM and D.V. SMIKK introduce the reflectron that corrects the kinetic energy distribution of the ions in a TOF mass spectrometer [41].

1973: R.G. COOKS, J.H. BEYNON, R.M. CAPRIOLI and G.R. LESTER publish the book *Metastable Ions*, a landmark in tandem mass spectrometry [42].

1974: E.C. HORNING, D.I. CARROLL, I. DZIDIC, K.D. HAEGELE, M.D. HORNING and R.N. STILLWELL discover atmospheric pressure chemical ionization (APCI) [43].

1974: First spectrometers coupled with a high-performance liquid chromatograph by P.J. ARPINO, M.A. BALDWIN and F.W. McLAFFERTY [44].

1974: M.D. COMISAROV and A.G. MARSHALL develop Fourier transformed ICR (FTICR) mass spectrometry [45].

1975: First commercial gas chromatography/mass spectrometry (GC/MS) instruments with capillary columns.

1976: R.D. MACFARLANE and D.F. TORGESSON introduce the plasma desorption (PD) source [46].

1977: R.G. COOKS and T.L. KRUGER propose the kinetic method for thermochemical determination based on measurement of the rates of competitive fragmentations of cluster ions [47].

1978: R.A. YOST and C.G. ENKE build the first triple quadrupole mass spectrometer, one of the most popular types of tandem instrument [48].

1978: Introduction of lamellar and high-field magnets.

1980: R.S. HOUK, V.A. FASSEL, G.D. FLESCH, A.L. GRAY and E. TAYLOR demonstrate the potential of inductively coupled plasma (ICP) mass spectrometry [49].

1981: M. BARBER, R.S. BORDOLI, R.D. SEDGWICK and A.H. TYLER describe the fast atom bombardment (FAB) source [50].

1982: First complete spectrum of insulin (5750 Da) by FAB [51] and PD [52].

1982: Finnigan and Sciex introduce the first commercial triple quadrupole mass spectrometers.

1983: C.R. BLAKNEY and M.L. VESTAL describe the thermospray (TSP) [53].

1983: G.C. STAFFORD, P.E. KELLY, J.E. SYKA, W.E. REYNOLDS and J.F.J. TODD describe the development of a gas chromatography detector based on an ion trap and commercialized by Finnigan under the name Ion Trap [54].

1987: M. GUILHAUS [55] and A.F. DODONOV [56] describe the orthogonal acceleration time-of-flight (oa-TOF) mass spectrometer. The concept of this technique was initially proposed in 1964 by G.J. O'Halloran of Bendix Corporation [57].

1987: T. TANAKA [58] and M. KARAS, D. BACHMANN, U. BAHR and F. HILLENKAMP [59] discover matrix-assisted laser desorption/ionization (MALDI). TANAKA receives the *Nobel Prize in 2002*.

1987: R.D. SMITH describes the coupling of capillary electrophoresis (CE) with mass spectrometry [60].

1988: J. FENN develops the electrospray (ESI) [61]. First spectra of proteins above 20 000 Da. He demonstrated the electrospray's potential as a mass spectrometric technique for small molecules in 1984 [62]. The concept of this source was proposed in 1968 by M. DOLE [63]. FENN receives the *Nobel Prize in 2002*.

1991: V. KATTA and B.T. CHAIT [64] and B. GAMEN, Y.T. LI and J.D. HENION [65] demonstrate that specific non-covalent complexes could be detected by mass spectrometry.

1991: B. SPENGLER, D. KIRSCH and R. KAUFMANN obtain structural information with reflectron TOF mass spectrometry (MALDI post-source decay) [66].

1993: R.K. JULIAN and R.G. COOKS develop broadband excitation of ions using the stored-waveform inverse Fourier transform (SWIFT) [67].

1994: M. WILM and M. MANN describe the nanoelectrospray source (then called microelectrospray source) [68].

1999: A.A. MAKAROV describes a new type of mass analyser: the orbitrap. The orbitrap is a high-performance ion trap using an electrostatic quadro-logarithmic field [5,69].

The progress of experimental methods and the refinements in instruments led to spectacular improvements in resolution, sensitivity, mass range and accuracy. Resolution ($m/\delta m$) developed as follows:

	$m/\delta m$	
1913	13	Thomson [16]
1918	100	Dempster [17]
1919	130	Aston [18]
1937	2000	Aston [70]
1998	8 000 000	Marshall and co-workers [71]

A continuous improvement has allowed analysis to reach detection limits at the pico-, femto- and attomole levels [72, 73]. Furthermore, the direct coupling of chromatographic techniques with mass spectrometry has improved these limits to the atto- and zeptomole levels [74,75]. A sensitivity record obtained by mass spectrometry has been demonstrated by using modified desorption/ionization on silicon DIOS method to measure concentration of a peptide in solution. This technique has achieved a lower detection limit of 800 yoctomoles, which corresponds to about 480 molecules [76].

Regarding the mass range, DNA ions of 10^8 Da were weighed by mass spectrometry [77]. In the same way, non-covalent complexes with molecular weights up to 2.2 MDa were measured by mass spectrometry [78]. Intact viral particles of tobacco mosaic virus with a theoretical molecular weight of 40.5 MDa were analysed with an electrospray ionization charge detection time-of-flight mass spectrometer [6].

The mass accuracy indicates the deviation of the instrument's response between the theoretical mass and the measured mass. It is usually expressed in parts per million (ppm) or in 10^{-3} u for a given mass. The limit of accuracy in mass spectrometry is about 1 ppm. The measurement of the atomic masses has reached an accuracy of better than 10^{-9} u [79].

In another field, Litherland *et al.* [80] succeeded in determining a $^{14}C/^{12}C$ ratio of $1{:}10^{15}$ and hence in dating a 40 000-year-old sample with a 1 % error. A quantity of ^{14}C corresponding to only 10^6 atoms was able to be detected in less than 1 mg of carbon [81].

Ion Free Path

All mass spectrometers must function under high vacuum (low pressure). This is necessary to allow ions to reach the detector without undergoing collisions with other gaseous molecules. Indeed, collisions would produce a deviation of the trajectory and the ion would lose its charge against the walls of the instrument. On the other hand, ion–molecule collisions could produce unwanted reactions and hence increase the complexity of the spectrum. Nevertheless, we will see later that useful techniques use controlled collisions in specific regions of a spectrometer.

According to the kinetic theory of gases, the mean free path L (in m) is given by

$$L = \frac{kT}{\sqrt{2}p\sigma} \tag{1}$$

where k is the Boltzmann constant, T is the temperature (in K), p is the pressure (in Pa) and σ is the collision cross-section (in m^2); $\sigma = \pi d^2$ where d is the sum of the radii of the stationary molecule and the colliding ion (in m). In fact, one can approximate the mean free path of an ion under normal conditions in a mass spectrometer ($k = 1.38 \times 10^{-21}$ J K^{-1}, $T \approx 300$ K, $\sigma \approx 45 \times 10^{-20}$ m^2) using either of the following equations where L is in centimetres and pressure p is, respectively, in pascals or milliTorrs:

$$L = \frac{0.66}{p} \tag{2}$$

$$L = \frac{4.95}{p} \tag{3}$$

Table 1 is a conversion table for pressure units. In a mass spectrometer, the mean free path should be at least 1 m and hence the maximum pressure should be 66 nbar. In instruments using a high-voltage source, the pressure must be further reduced to prevent the occurrence of discharges. In contrast, some trap-based instruments operate at higher pressure.

However, introducing a sample into a mass spectrometer requires the transfer of the sample at atmospheric pressure into a region of high vacuum without compromising the latter. In the same way, producing efficient ion–molecule collisions requires the mean free path to be reduced to around 0.1 mm, implying at least a 60 Pa pressure in a region of the

Table 1 Pressure units. The official SI unit is the pascal.

1 pascal (Pa) = 1 newton (N) per m^2
1 bar = 10^6 dyn cm^{-2} = 10^5 Pa
1 millibar (mbar) = 10^{-3} bar = 10^2 Pa
1 microbar (μbar) = 10^{-6} bar = 10^{-1} Pa
1 nanobar (nbar) = 10^{-9} bar = 10^{-4} Pa
1 atmosphere (atm) = 1.013 bar = 101 308 Pa
1 Torr = 1 mmHg = 1.333 mbar = 133.3 Pa
1 psi = 1 pound per square inch = 0.07 atm

spectrometer. These large differences in pressure are controlled with the help of an efficient pumping system using mechanical pumps in conjunction with turbomolecular, diffusion or cryogenic pumps. The mechanical pumps allow a vacuum of about 10^{-3} Torr to be obtained. Once this vacuum is achieved, the operation of the other pumping systems allows a vacuum as high as 10^{-10} Torr to be reached.

The sample must be introduced into the ionization source so that vacuum inside the instrument remains unchanged. Samples are often introduced without compromising the vacuum using direct infusion or direct insertion methods. For direct infusion, a capillary is employed to introduce the sample as a gas or a solution. For direct insertion, the sample is placed on a probe, a plate or a target that is then inserted into the source through a vacuum interlock. For the sources that work at atmospheric pressure and are known as atmospheric pressure ionization (API) sources, introduction of the sample is easy because the complicated procedure for sample introduction into the high vacuum of the mass spectrometer is removed.

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1
Ion Sources

In the ion sources, the analysed samples are ionized prior to analysis in the mass spectrometer. A variety of ionization techniques are used for mass spectrometry. The most important considerations are the internal energy transferred during the ionization process and the physico-chemical properties of the analyte that can be ionized. Some ionization techniques are very energetic and cause extensive fragmentation. Other techniques are softer and only produce ions of the molecular species. Electron ionization, chemical ionization and field ionization are only suitable for gas-phase ionization and thus their use is limited to compounds sufficiently volatile and thermally stable. However, a large number of compounds are thermally labile or do not have sufficient vapour pressure. Molecules of these compounds must be directly extracted from the condensed to the gas phase.

These direct ion sources exist under two types: liquid-phase ion sources and solid-state ion sources. In liquid-phase ion sources the analyte is in solution. This solution is introduced, by nebulization, as droplets into the source where ions are produced at atmospheric pressure and focused into the mass spectrometer through some vacuum pumping stages. Electrospray, atmospheric pressure chemical ionization and atmospheric pressure photoionization sources correspond to this type. In solid-state ion sources, the analyte is in an involatile deposit. It is obtained by various preparation methods which frequently involve the introduction of a matrix that can be either a solid or a viscous fluid. This deposit is then irradiated by energetic particles or photons that desorb ions near the surface of the deposit. These ions can be extracted by an electric field and focused towards the analyser. Matrix-assisted laser desorption, secondary ion mass spectrometry, plasma desorption and field desorption sources all use this strategy to produce ions. Fast atom bombardment uses an involatile liquid matrix.

The ion sources produce ions mainly by ionizing a neutral molecule in the gas phase through electron ejection, electron capture, protonation, deprotonation, adduct formation or by the transfer of a charged species from a condensed phase to the gas phase. Ion production often implies gas-phase ion–molecule reactions. A brief description of such reactions is given at the end of the chapter.

1.1 Electron Ionization

The electron ionization (EI) source, formerly called electron impact, was devised by Dempster and improved by Bleakney [1] and Nier [2]. It is widely used in organic mass spectrometry. This ionization technique works well for many gas-phase molecules but induces extensive fragmentation so that the molecular ions are not always observed.

As shown in Figure 1.1, this source consists of a heated filament giving off electrons. The latter are accelerated towards an anode and collide with the gaseous molecules of

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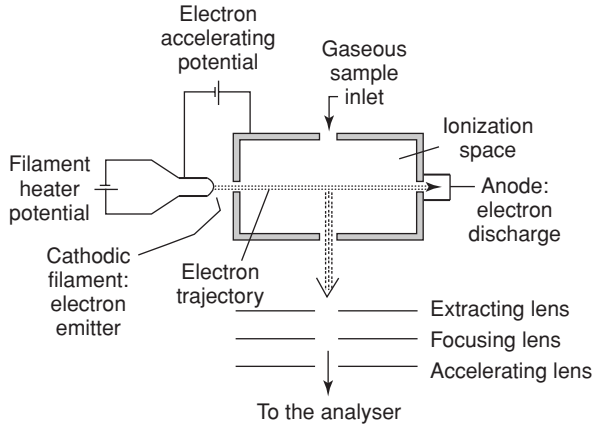

Figure 1.1
Diagram of an electron ionization source.

the analysed sample injected into the source. Gases and samples with high vapour pressure are introduced directly into the source. Liquids and solids are usually heated to increase the vapour pressure for analysis.

Each electron is associated to a wave whose wavelength λ is given by

$$\lambda = \frac{h}{mv}$$

where m is its mass, v its velocity and h Planck's constant. This wavelength is 2.7 Å for a kinetic energy of 20 eV and 1.4 Å for 70 eV. When this wavelength is close to the bond lengths, the wave is disturbed and becomes complex. If one of the frequencies has an energy hv corresponding to a transition in the molecule, an energy transfer that leads to various electronic excitations can occur [3]. When there is enough energy, an electron can be expelled. The electrons do not 'impact' molecules. For this reason, it is recommended that the term electron impact must be avoided.

Figure 1.2 displays a typical curve of the number of ions produced by a given electron current, at constant pressure of the sample, when the acceleration potential of the electrons (or their kinetic energy) is varied [4]. At low potentials the energy is lower than the molecule ionization energy. At high potentials, the wavelength becomes very small and molecules become 'transparent' to these electrons. In the case of organic molecules, a wide maximum appears around 70 eV. At this level, small changes in the electron energy do not significantly affect the pattern of the spectrum.

On average, one ion is produced for every 1000 molecules entering the source under the usual spectrometer conditions, at 70 eV. Furthermore, between 10 and 20 eV is transferred to the molecules during the ionization process. Since approximately 10 eV is enough to ionize most organic molecules, the excess energy leads to extensive fragmentation. This fragmentation can be useful because it provides structural information for the elucidation of unknown analytes.

At a given acceleration potential and at constant temperature, the number of ions I produced per unit time in a volume V is linked to the pressure p and to the electron current