CHAPTER 1

BASICS OF MASS SPECTROMETRY

Mass spectrometry (MS) is an analytical technique that measures the molecular masses of individual compounds and atoms precisely by converting them into charged ions. Quite often, the structure of a molecule can also be deduced. Mass spectrometry is also uniquely qualified to provide quantitative information of an analyte at levels of structure specificity and sensitivity that are beyond imagination (e.g., in the zeptomole range). In addition, mass spectrometry allows one to study reaction dynamics and chemistry of ions, to provide data on physical properties such as ionization energy, appearance energy, enthalpy of a reaction, proton and ion affinities, and so on, and to verify molecular orbital calculations-based theoretical predictions. Thus, mass spectrometry probably is the most versatile and comprehensive analytical technique currently at the disposal of chemists and biochemists. Several areas of physics, chemistry, medicinal chemistry, pharmaceutical science, geology, cosmochemistry, nuclear science, material science, archeology, petroleum industry, forensic science, and environmental science have benefited from this highly precise and sensitive instrumental technique.

1.1. BRIEF HISTORY OF MASS SPECTROMETRY

As early as 1898, Wien demonstrated that canal rays could be deflected by passing them through superimposed parallel electric and magnetic fields. Sir Joseph J. Thomson (1856–1940) is credited with the birth of mass spectrometry through his work on the analyses of negatively charged cathode ray particles [1] and of positive rays with a parabola mass spectrograph [2]. His prophesy was that this

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new technique would play a profound role in the field of chemical analysis. In the next two decades, however, the developments of mass spectrometry continued in the hands of renowned physicists like Aston, Dempster, Bainbridge, and Nier [3]. During this time, mass spectrometry played a pivotal role in the discovery of new isotopes and in determining their relative abundances and accurate masses. In the 1940s, mass spectrometry played a major role in the Manhattan Project, a wartime program to separate on a preparative scale the fissionable ^{235}U isotope and as a leak detector in a UF_6 gaseous diffusion plant.

In the 1940s, chemists ultimately recognized the potential of mass spectrometry as an analytical tool and applied it to monitor a petroleum refinery stream. The first commercial mass spectrometer became available in 1943 through the Consolidated Engineering Corporation. The principles of time-of-flight (TOF) and ion cyclotron resonance (ICR) mass spectrometry were introduced in 1946 and 1948, respectively [4,5]. Applications to organic chemistry began in the 1950s and exploded in the 1960s and 1970s. Double-focusing high-resolution mass spectrometers, which became available in the early 1950s, paved the way for accurate mass measurements of a variety of compounds. The concept of quadrupole mass analyzer and ion traps as mass detectors was described by Wolfgang Paul et al. in 1953 [6,7]. The development of gas chromatography (GC)/MS in the 1960s marked the beginning of the analysis of seemingly complex mixtures by mass spectrometry [8,9]. The 1960s also witnessed the development of tandem mass spectrometry (MS/MS) [10]; the emergence of this technique is a high point in the field of structure analysis and unambiguous quantification by mass spectrometry. Chemical ionization, a "soft" mode of ionization, was also introduced during this period [11].

By the 1960s, mass spectrometry had become a standard analytical tool in the analysis of organic compounds. Its applications to biological fields were, however, miniscule, owing primarily to the lack of suitable ionization techniques for fragile and nonvolatile compounds of biological origin. Over the last two decades, that situation has changed. Several unique developments in gentler modes of ionization have allowed the production of ions from compounds of large molecular mass and compounds of biological relevance. These methods include fast atom bombardment (FAB) in 1981 [12], electrospray ionization (ESI) (in 1984–1988) [13], and matrix-assisted laser desorption/ionization (MALDI) in 1988 [14,15]. The last two methods have extended the upper mass range beyond 100 kilodaltons (kDa) and had an enormous impact on the use of mass spectrometry in biology and the life sciences. Concurrent with these developments, several innovations in mass analyzer technology, such as the introduction of high-field and superfast magnets and improvements in the TOF and Fourier transform (FT) ion cyclotron resonance–mass spectrometry (ICR–MS) analysis concepts, have also improved the sensitivity and upper mass range amenable to mass spectrometry. The current decade has seen the introduction of two new types of ion traps, the quadrupole linear ion trap (LIT) and the orbitrap, for mass spectrometric analysis [16,17]. A variety of hybrid tandem mass spectrometry systems are available for enhanced performance in tandem mass spectrometry (see Chapter 4). The coupling of high-performance liquid chromatography (HPLC) with mass

spectrometry, first demonstrated in the 1970s [18,19] and later optimized with an ESI interface [20], is another high point that has provided chemists and biochemists with one of their most useful instruments. Improvements in detection devices and the introduction of fast data systems have also paralleled these developments. Currently, mass spectrometry has found a niche in the biomedical field and life sciences and is at the forefront of proteomics techniques.

1.2. DESIRABLE FEATURES OF MASS SPECTROMETRY

The wide popularity of mass spectrometry is the result of its unique capabilities:

- It provides unsurpassed molecular specificity because of its unique ability to measure accurate molecular mass and to provide information on structurally diagnostic fragment ions of an analyte.
- It provides ultrahigh detection sensitivity. In theory, mass spectrometry has the ability to detect a single molecule; the detection of molecules in attomole and zeptomole amounts has been demonstrated.
- It has unparalleled versatility to determine the structures of most classes of compounds.
- It is applicable to all elements.
- It is applicable to all types of samples: volatile or nonvolatile; polar or nonpolar; and solid, liquid, or gaseous materials.
- In combination with high-resolution separation devices, it is uniquely qualified to analyze "real-world" complex samples.

1.3. BASIC PRINCIPLES OF MASS SPECTROMETRY

Mass spectrometry measurements deal with ions because unlike neutral species, it is easy to manipulate the motion and direction of ions experimentally and detect them. Three basic steps are involved in mass spectrometry analysis (Figure 1.1):

1. The first step is ionization that converts analyte molecules or atoms into gas-phase ionic species. This step requires the removal or addition of an

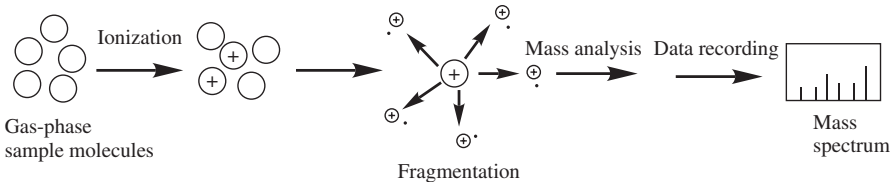

Figure 1.1. Basic concept of mass spectrometry analysis. (Reproduced from C. Dass, *Principles and Practice of Biological Mass Spectrometry*, Wiley-Interscience, 2001.)

electron or proton(s). The excess energy transferred during an ionization event may break the molecule into characteristic fragments.

2. The next step is the separation and mass analysis of the molecular ions and their charged fragments on the basis of their m/z (mass-to-charge) ratios.
3. Finally, the ion current due to these mass-separated ions is measured, amplified, and displayed in the form of a mass spectrum.

The first two steps are carried out under high vacuum, which allows ions to move freely in space without colliding or interacting with other species. Collisions may lead to fragmentation of the molecular ions and may also produce a different species through ion–molecule reactions. These processes will reduce sensitivity, increase ambiguity in the measurement, and decrease resolution. In addition, the atmospheric background will introduce interference.

A simplistic view of the essential components of a mass spectrometer is given in Figure 1.2. These components are:

- *An inlet system:* transfers a sample into the ion source. An essential requirement is to maintain the integrity of the sample molecules during their transfer from atmospheric pressure to the ion-source vacuum.
- *An ion source:* converts the neutral sample molecules into gas-phase ions. Several ionization techniques have been developed for this purpose (see Chapter 2).
- *A mass analyzer:* separates and mass-analyzes the ionic species. Magnetic and/or electric fields are used in mass analyzers to control the motion of ions. A magnetic sector, quadrupole, time-of-flight, quadrupole ion trap, quadrupole linear ion trap, orbitrap, and Fourier transform ion cyclotron resonance instrument are the most common forms of mass analyzers currently in use (discussed in Chapter 3).

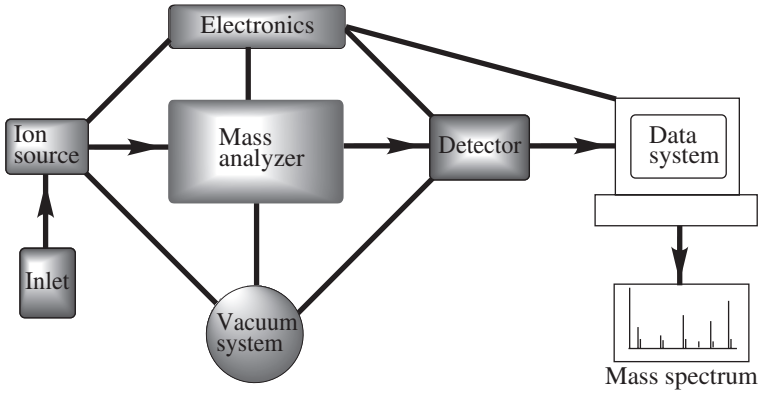

Figure 1.2. Basic components of a mass spectrometer. (Reproduced from C. Dass, *Principles and Practice of Biological Mass Spectrometry*, Wiley-Interscience, 2001.)

- *A detector:* measures and amplifies the ion current of mass-resolved ions.
- *A data system:* records, processes, stores, and displays data in a form that a human eye can easily recognize (computer screen or printer output).
- *A vacuum system:* maintains a very low pressure in the mass spectrometer. The ion source region is usually maintained at a pressure of 10^{-4} to 10^{-8} torr; somewhat lower pressure is required in the mass analyzer region (around 10^{-8} torr). Most instruments use a differential pumping system to maintain an optimal vacuum.
- *Electronics:* controls the operation of various units.

1.4. ANATOMY OF A MASS SPECTRUM

The simplest and most common means of ion formation in mass spectrometry is to bombard the gas-phase sample molecules with a beam of electrons. During this process, an electron is removed from the highest-occupied molecular orbital (HOMO) of the sample molecule to form a positively charged molecular ion:

$$M + e^- \rightarrow M^{+\bullet} + 2e^- \tag{1.1}$$

Fragment ions may also form. The data output is in the form of a mass spectrum.

It is essential to become familiar with a mass spectrum. A common form, the computer-generated bar-graph plot, is shown in Figure 1.3. It is a plot of m/z values (on the x-axis) of all ions (i.e., the molecular ion and its fragment ions, plus background ions, if any) that reach the detector versus their abundances (on the y-axis). The spectrum in Figure 1.3 is a positive ion electron ionization–generated mass spectrum of acetophenone. A mass spectrum is usually characterized by a molecular ion region (i.e, the molecular ion signal plus

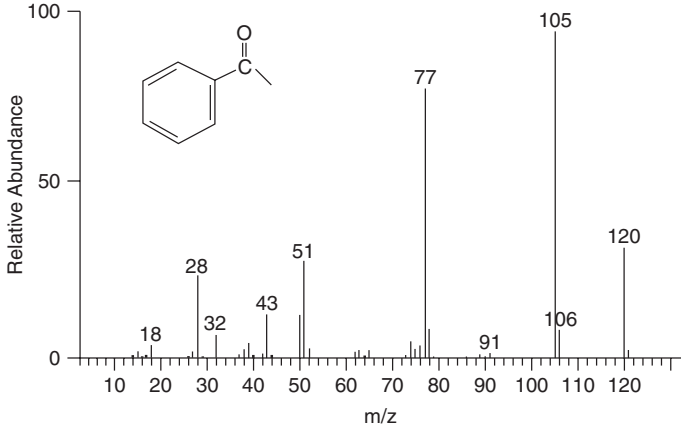

Figure 1.3. The 70-eV EI mass spectrum of acetophenone (MW = 120 u).

associated heavy isotope satellite ions) and a fragment ion region (the sample molecule-related fragments). It is general practice to designate the most abundant ion in the spectrum as the base peak (here, m/z 105), which is arbitrarily assigned a relative height of 100. The abundances of all other ions in the spectrum are reported as percentage abundances relative to this base peak. Before the advent of computers, a photographic chart paper recorder was used to provide a mass spectrum. An advantage of this type of recording was that information derived from metastable ion fragmentation could also be retrieved. The ions from a metastable fragmentation appear as diffuse peaks at nonintegral masses. The computer-generated spectrum can also be presented in list format, a tabulation of all ion m/z values versus their abundances.

A mass spectrum is a useful source of structure-specific information, the most important datum being the molecular mass of the analyte. The molecular mass of an analyte can readily be inferred from the molecular ion because this ion represents the intact molecule minus an electron [reaction. (1.1)]. The molecular ion usually is the largest peak among the high-mass cluster of peaks in the spectrum (e.g., the m/z 120 in Figure 1.3). From the m/z values of the fragment ions, the structure of the analyte can be deduced. The structure determination of organic compounds by mass spectrometry is discussed in more detail in Chapter 6. In some ionization techniques (see Chapter 2), the molecular ion is obtained as a protonated or deprotonated molecule (i.e., $[M + H]^+$ or $[M - H]^-$). The molecular mass from those spectra is obtained by subtracting the mass of a proton from the m/z value of the $[M + H]^+$ ion. For example, Figure 1.4 is the ESI

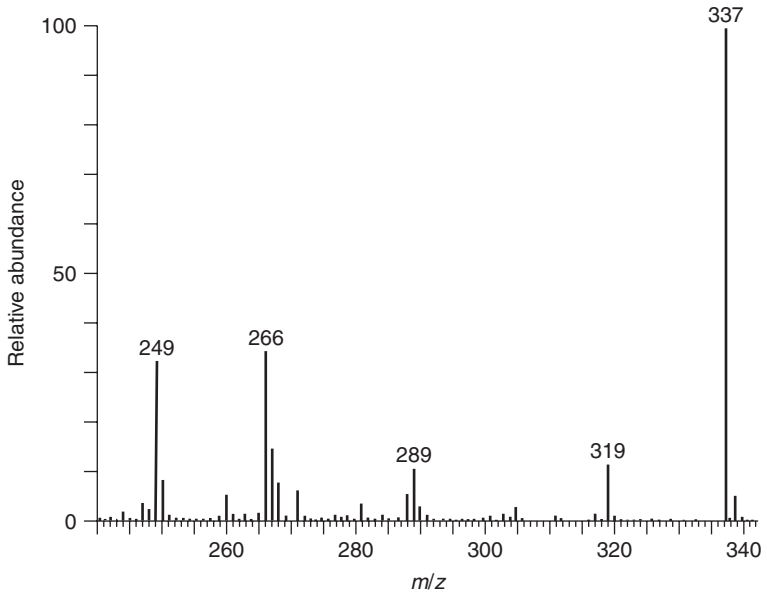

Figure 1.4. ESI mass spectrum of acetebutol (MW = 336 u).

mass spectrum of a β-blocker, acetebutol (molecular mass = 336.205 u). From the m/z observed for the $[M + H]^+$ ion (i.e., 337), the molecular mass of acetebutol can readily be determined. The molecular mass can also be obtained from a negative-ion spectrum. The structural information is, however, very sparse in negative-ion spectra because negative ions are more stable. The structural information is also sparse in a mass spectrum that is acquired by ionizing the molecule with a gentler mode of ionization such as FAB, ESI, or MALDI. The structural information from FAB-, ESI-, and MALDI-generated ions is obtained by subjecting the molecular ions to collision-induced dissociation (CID) and acquiring the MS/MS spectrum (see Chapter 4 for more details).

1.5. ATOMIC AND MOLECULAR MASSES

The SI base unit of mass is the kilogram (kg). The mass of microscopic species, such as atoms and molecules, is very small, and to express this small quantity in kilograms is cumbersome. For example, to denote the mass of a single carbon atom as 1.99266×10^{-26} kg is very inconvenient. Therefore, the mass of atoms and molecules is expressed in terms of the unified atomic mass unit (u). By international agreement, the mass of one atom of the ^{12}C isotope is assigned the exact value 12. One unified atomic mass unit is defined as equal to $\frac{1}{12}$ the mass of a single atom of the ^{12}C isotope. Alternatively, the dalton (Da) is also used in place of u, especially when expressing the mass of large biomolecules. The mass of other atoms is expressed relative to the mass of the ^{12}C isotope. Thus,

$$1 \text{ u} = 1 \text{ Da} = 1.6605402 \times 10^{-27} \text{ kg}$$

Several different molecular mass terms are in use:

- *Nominal ion mass:* the mass of the ion for a given empirical formula, calculated by adding the integer mass of the most abundant isotope of each element (e.g., $^1H = 1$ and $^{12}C = 12$).
- *Monoisotopic ion mass:* the mass of the ion for a given empirical formula, calculated from the exact mass of the most abundant isotope of each element (e.g., $^1H = 1.007825$ and $^{12}C = 12.000000$). The exact mass of the elements and their isotopes are provided in Appendix C.
- *Most abundant ion mass:* the mass that corresponds to the most abundant peak in the isotopic cluster of the ion of a given empirical formula.
- *Average mass:* the mass of an ion for a given empirical formula calculated with the atomic weight of each element (e.g., $C = 12.01115$ and $H = 1.00797$): that is, the average of the isotopic masses of each element, weighted for isotopic abundance. The average mass represents the centroid of the distribution of the isotopic peaks of the molecular ion and is used by chemists in stoichiometric calculations.

► **Example 1.1** Calculate the nominal and exact mass of acetophenone.

Solution The nominal mass of acetophenone (C_8H_8O) = $(8 \times 12) + (8 \times 1) + (1 \times 16) = 120$ u.

The exact mass of acetophenone = $(8 \times 12.000000) + (8 \times 1.007825) + (1 \times 15.994915) = 120.0575$ u.

The monoisotopic mass is meaningful for low-mass compounds because the elemental composition can be determined from a well-defined isotopic pattern of the molecular ion. The nominal and monoisotopic masses can both be correlated with the most abundant peak in the isotopic cluster. As the mass of a compound, however, increases, the isotopic pattern becomes more symmetrical and extends over many masses [21]. Also, the monoisotopic peak becomes difficult to identify. For high-mass compounds (e.g., proteins and oligonucleotides), the molecular ion profile measured coalesces and becomes a single asymmetric peak. For such compounds, the average mass value is accepted as the molecular mass.

1.5.1. Mass-to-Charge Ratio

As mentioned above, the mass spectrometry data are presented as the *mass-to-charge ratio*, which by definition is the mass of the ion (m) divided by the number of charges (z) the ion carries. The total charge on the ion is represented by $q = ze$, where e is the charge on an electron [$e = 1.602 \times 10^{-19}$ coulomb (C)]. The unit of the mass-to-charge ratio is the thomson (Th) [22]. However, use of the unitless term m/q is common practice in the literature, and that practice is followed in this book. In the past, m/e had been used in place of m/z. The term m/e assumes that all ions in the spectrum are singly charged, whereas z can be a multiple integer.

Multiply charged cations are formed by attachment of several protons. This process usually occurs for biomolecules in the ESI mode of ionization. The corresponding ions will appear at $[M + nH]^{n+}/n$, where M is the molecular mass of the biomolecule, n the number of protons it can accept, and H the mass of a proton. Thus, M and m/q can have two distinct values. These values are identical only for singly charged ions. This distinction is clearly explicable in Example 1.2.

► **Example 1.2** The nominal mass of acetophenone is 120 u (see Example 1.1). In the mass spectrum, $C_8H_8O^+$ ion will appear at $m/q = 120$ and $C_8H_8O^{2+}$ at $120/2 = 60$.

Similarly, a protein that has a mass of 50,000 Da and can accept 25 protons to produce an $[M + 25H]^{25+}$ ion displays an m/q at $(50{,}000 + 25 \times 1)/25 = 2001$.

1.6. GENERAL APPLICATIONS

Mass spectrometry plays a central role in almost every field of science. This distinction is the result of the high level of molecular specificity, detection sensitivity, and availability of ionization techniques for all classes of compounds. Some of the major areas of applications are:

- Physics:
  - Determination of the accurate masses of elements and abundances of isotopes
- Chemistry:
  - Accurate mass measurement of atoms and molecules
  - Structure analysis of organic compounds
  - Quantitative analysis of inorganic and organic compounds
  - Fundamentals of gas-phase ion chemistry
  - Measurement of physical properties of ions
  - Elemental analysis
  - Precise isotope ratio measurements
- Environmental science:
  - Analysis of environmental pollutants in air, water, and soil
  - Study of Earth's atmosphere and water resources (lakes, rivers, oceans)
- Medicine and life sciences:
  - Molecular mass measurement of large biological compounds
  - Simultaneous separation and detection of complex mixtures of biological compounds
  - Quantitative analysis of a variety of compound types in biological tissues and fluids
  - Amino acid sequence determination of proteins and peptides
  - Higher-order structures of proteins and peptides
  - Covalent complexes of biomolecules
  - Identification of specific diseases
  - Structural characterization of lipids and oligosaccharides
  - Sequence determination of oligonucleotides
  - Profiling of bacteria and viruses
  - Study of functional aspects of biomolecules
  - Clinical studies
  - Measurement of isotope ratios for biological tracer studies
- Pharmaceutical sciences:
  - Analysis of isolated and synthesized drugs
  - Pharmacodynamic and pharmacokinetic evaluation of new and old drugs

Geology:

Determination of age and composition of rocks and other geological species

Industry:

Monitoring of process streams such as a refinery stream in the petroleum industry

Forensic science:

Analysis of explosives and banned substances

Material science:

Analysis of metals, alloys, semiconductors, and polymers

This list is by no means complete. Mass spectrometry will continue to become an integral part of many diverse fields. With continued developments in the future, we will witness an expanded role for mass spectrometry in many unchartered territories, especially in offering new perspectives on solving real-world problems. With sensitive and faster analysis methods at hand, the role of mass spectrometry will expand to studies related to human health and safety.

OVERVIEW

In this introductory chapter, some basic concepts of mass spectrometry were discussed. A brief history of mass spectrometry was presented. Mass spectrometry has its roots in early work with the cathode-ray tube but now it is a more mature discipline and an indispensable analytical tool. It is used primarily to determine the mass of atomic and molecular species and to structurally characterize and quantify a very broad range of compounds. The major assets of this technique are specificity, sensitivity, and ability to analyze real-world samples.

Essential steps of mass spectrometric analysis are ionization, separation of ions on the basis of m/z ratio, and detection of the ion current of separated ions. To perform these functions, a mass spectrometer is made of an ion source, a mass analyzer, a detector, a data system, a vacuum system, and electronic control units. The data are presented in the form of a mass spectrum, which is a plot of m/z values on the x-axis versus their abundances on the y-axis. From this spectrum, the mass of the target species and its structure can be determined.

Depending on the size of the molecule, mass spectrometry provides information on the nominal, monoisotopic, and average masses. The nominal mass and monoisotopic mass information is obtained for low-mass compounds, whereas, for high-mass compounds, the average mass value is measured.

EXERCISES

1.1. List the basic steps involved in mass spectrometric analysis.

1.2. Why is mass spectrometric analysis performed under high vacuum?

1.3. What are the essential components of a mass spectrometer, and what is the function of each?

1.4. Calculate the nominal mass, monoisotopic mass, and average mass of the tranquilizer diazepam, $C_{16}H_{13}N_2OCl$.

1.5. The molecular mass of a peptide is 2051 Da. Calculate the m/z value of the triply protonated peptide.

1.6. Calculate the mass of diazepam in kg.

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