# UNDERSTANDING MASS SPECTRA:

## A Basic Approach

SECOND EDITION

R. Martin Smith

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# PREFACE TO THE SECOND EDITION

Mass spectrometry (MS) has undergone a profound change over the past decade. Instrumentation and techniques related to the automated analysis of biomolecules and new drugs now account for a large percentage of the research and publications in this field. In comparison, gas chromatography/mass spectrometry (GC/MS) and electron ionization (EI) mass spectra of ''small'' molecules play a less important role than they once did. But GC/MS is far from dead, and EIMS continues to be the ionization method of choice for many laboratories that routinely analyze volatilizable low molecular mass compounds such as drugs, flavor and odor components, pesticides, and petroleum products. This situation seems unlikely to change in the near future.

The interpretation of EI mass spectra has always been a challenging subject to learn and to teach—especially to individuals who have not had the benefit of a graduate education in chemistry or who have been out of college for several years. The challenge is compounded by manufacturer-encouraged reliance on library search results for compound identification. Why learn anything about spectral interpretation when the computer can do all the work? The answer to this question is simple, as most conscientious users quickly realize. The library search often does not provide a realistic answer or (worse) may provide an answer that looks correct but is not. Even software programs that profess to ''interpret'' unknown spectra can only provide probable answers. After that, you are left to your own devices.

It was tempting to substantially increase the breadth and depth of the material that was covered in the first edition. However, my experience has been that an encyclopedic presentation of mass spectral interpretation does not give beginning mass spectrometrists what they need, which is a presentation that provides a few fundamental concepts in a logical, organized manner, without distracting and unnecessary detail. I wrote and revised this book for beginning mass spectrometrists, and I have retained the simplicity of its approach for that reason.

My own understanding of mass spectral interpretation has developed, and continues to develop, by trial and error. I am admittedly mostly self-taught. My knowledge of mass spectral literature has been limited by the nature of my career, whose primary focus was forensic science, not mass spectrometry. Some will see that as a

detriment. However, I believe that my naïveté allows me to present a different approach to this subject—one based on *learning* the subject, not on teaching it.

Although this edition has the same basic structure and content as the first, a number of significant changes have been made. In general, there are more references, especially for helping the reader gain access to in-depth information about specific subjects. Some Internet resources have also been included at the end of Chapter 1. I have tried to include examples from a broader range of chemical interests. There are still more forensic examples than other types, but I believe the molecules of forensic chemistry are not so unique that they cannot be used as a general teaching tool. Indeed, I hope that these examples are appealing because they come from a field that has captured the public interest and imagination.

Two of the more fundamental changes in content are the use of ionization energies (IEs) for determining the site of initial ionization and Stevenson's rule for determining retention of the charge in fragmentation products (Chapter 3). Fragmentation schemes for most compounds throughout the book have been altered to reflect these changes. Attention has been paid to differentiating between radical- and charge-induced fragmentations.

The material in several chapters—most notably in Chapters 2, 4, and 5—has been reorganized. The method for solving mass spectral unknowns has been placed in a separate chapter (Chapter 5), where it follows—rather than precedes—discussions of specific problem-solving tools such as neutral losses, low-mass ion series, and so forth. New problems and examples have been added to Chapters 2–4 that provide practice more specifically on the topics discussed in those chapters.

New material has been added to several chapters. Brief descriptions of newer techniques such as electrospray ionization (ESI) and MALDI are included in Chapter 1 simply because they are now so widespread that exposure to them is almost unavoidable. A derivation of the mass spectrometric equation for the time-of-flight (TOF) spectrometer is included for the same reason, as well as to provide a straightforward example of how $m/z$ values are related mathematically to physical variables in the spectrometer. Discussions of orbitals and bonds, the use of ionization energies, the nitrogen rule, and Stevenson's rule have all been added to Chapter 3, and new (and I hope better) examples have replaced some of the material in the chapter on rationalizing mass spectral fragmentations (Chapter 8 in this edition). I struggled with maintaining the mathematical derivations in Chapter 2 regarding the relationship between an ion's elemental composition and the relative sizes of the isotope peaks observed in the spectrum. I decided to keep them because many texts do not show where these equations come from.

The number of chapters describing specific types of fragmentation reactions is still limited (Chapters 5–7). A ''theme and variations'' approach is used, in order to emphasize the similarities—rather than the differences—between fragmentation types. Not all reaction types are covered, because I feel it is more important for the beginning reader to fully understand a few fragmentations that have wide applicability than to try to cover every possibility. Particular emphasis is placed on single-bond cleavage, fragmentations that eliminate small unsaturated molecules, and several well-known hydrogen rearrangements. I have tried to repeat these

fragmentations in as many contexts as possible throughout Chapters 4–9 to emphasize their utility and to facilitate committing them to memory.

Each time I have taught this material, and again as I was revising this book, I reached new levels of understanding of even some of the most basic concepts that are presented here. For most readers, I doubt that the contents of this book will be thoroughly digested in one reading. Rather, I would suggest studying it slowly, even repetitively. Try to understand the answers to each of the problems, practice writing down fragmentation mechanisms, then attempt to apply each concept to the spectra encountered in your own laboratory situation. The rewards will be well worth the effort.

*Madison, Wisconsin*
*January 2004*

R.M.S.

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There are many people I must thank for making this book a reality. Foremost among these are members of the Wisconsin Department of Justice, Division of Law Enforcement Services, without whose backing this book would probably never have become a reality. A special thanks goes to Jerry Geurts and Mike Roberts for their support and encouragement while I was in their employ. I am also grateful for the contributions of colleagues who provided me with interesting problem samples that found their way, directly or indirectly, into this book. The recent contributions of Casey Collins, Marty Koch, Mike Larson, John Nied, Joseph Wermeling, and Guang Zhang deserve special mention.

This edition was technically edited by someone who prefers not to be named. Although I will honor that request, I cannot in good conscience fail to acknowledge the invaluable contribution this individual made to the content, style, organization, and technical detail of this edition. No matter how far this book falls short of perfection, it is immensely closer to that goal than it was when this person was first given a copy of the manuscript.

My friend Mary Upshaw has worked in a laboratory for many years, but had only a general idea of what mass spectrometry was all about until I asked her to read the entire manuscript as a ''lay person''—no small request! Our subsequent discussions and her insightful comments lent much to the final organization and readability of this edition. (Her proofreading skills are great, too.) If you find this book easy to read, it is at least partly due to her efforts.

My editor Amy Romano deserves a medal for her patience. The revision ended up taking at least a year longer than either of us suspected it would (or wanted it to). I feel strongly—and I hope she does too—that the wait was well worth it.

Finally, a special word of thanks to John Allison, who seemed to believe in what I was doing and said the right things at the right times to keep me on track.

# ABBREVIATIONS AND NOTATIONS USED IN THIS BOOK

Atomic symbols, rather than names, of the elements are used throughout the book.

| | |
|---|---|
| $\sim$ and $\approx$ | Approximately equal to |
| $^{+\bullet}$ | Site of unpaired electron and positive charge (odd-electron ion) |
| $\Delta$ | Mass defect; also, site of double bond in organic compounds |
| CI | Chemical ionization |
| $EE^{+}$ | Even-electron ion |
| EI | Electron ionization |
| eV | Electron volt (1 eV = 23 kcal) |
| $\Delta G^{\ddagger}$ | Energy of activation (for a chemical reaction) |
| GC | Gas chromatography |
| IE | Ionization energy |
| LC | Liquid chromatography |
| M, M + 1, M − 15, etc. | Spectral peak with *m*/*z* value at, higher than, or lower than that of the molecular ion peak by a specified number of units |
| $M^{+\bullet}$ | Positively charged molecular ion |
| $\Delta M$ | Difference in mass or *m*/*z* values (mass or *m*/*z* discrimination) |
| MM | Molecular mass |
| MS | Mass spectrometry |
| *m*/*z* | Mass-to-charge ratio |
| $OE^{+\bullet}$ | Odd-electron ion |
| *P*(X) | Probability ($\leq$1) that an event will occur |
| QIT | Quadrupole ion trap |
| RTICC | Reconstructed total ion current chromatogram |
| SIM | Selected ion monitoring |

| | |
|---|---|
| TOF | Time-of-flight |
| u | Unified atomic mass unit |
| X, X + 1, X − 15, etc. | Peaks with *m*/*z* values at, higher than, or lower than that of some peak in the spectrum by a specified number of units |
| $X^+$, $(X + 1)^+$, $(X - 15)^+$, etc. | Ions having masses the same as, higher than, or lower than that of some ion in the spectrum by a specified number of units |
| [X] | Peak intensity for an ion having an *m*/*z* value of X |
| $[X^+]$ | Abundance of an ion having an *m*/*z* value of X |

# 1

# INSTRUMENTATION

## 1.1. INTRODUCTION

### 1.1.1. Overview

Mass spectrometry (MS) differs from other common forms of organic spectral analysis in that the sample does not absorb radiation such as infrared, ultraviolet, or radio waves from the electromagnetic spectrum. In contrast to infrared (IR) or nuclear magnetic resonance (NMR) spectrometry, both of which identify compounds with specificity comparable to that of mass spectrometry, MS is a destructive method of analysis—that is, the sample cannot be recovered after mass spectral analysis. On the other hand, MS is highly sensitive and requires less sample than either IR or NMR in order to provide more information about the structure of the analyte.

Mass spectrometers are typically not standalone instruments. Most often they are connected physically and electronically to a chromatograph as well as a computer. Figure 1.1 shows a typical arrangement of a chromatograph/mass spectrometer/computer system. The chromatograph separates mixtures and introduces the sample into the mass spectrometer. The mass spectrometer ionizes analyte molecules, then separates and detects the resulting ions. The computer system controls the operation of the chromatograph and the MS, and provides data manipulation and storage during and after data collection. For volatile samples, gas chromatography (GC) is

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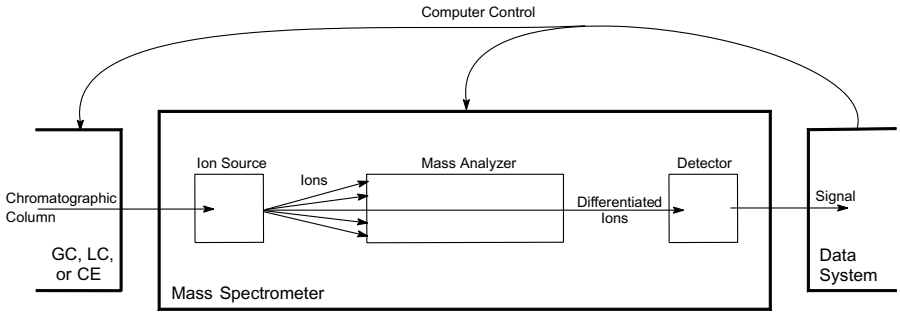


**Figure 1.1.** Block diagram of a chromatograph/MS/computer system.

used for mixture separation. For nonvolatile or thermally labile molecules, high pressure liquid chromatography (HPLC or just LC) is used. The abbreviated terms GC/MS and LC/MS are commonly used to describe the combination of these chromatographic techniques with MS.

In order to be analyzed by mass spectrometry, sample molecules must be ionized. In the case of electron ionization mass spectrometry (EIMS, the focus of this book), electrically neutral molecules are converted to molecular ions ($M^{+\bullet}$; see Section 3.1) by means of a beam of high-energy electrons. Ionization is followed almost immediately by fragmentation of the $M^{+\bullet}$ in which some bonds break, and in many instances new bonds form, in ways that are characteristic of the structure of the fragmenting ion. The product ions thus formed often undergo further characteristic fragmentation before leaving the ion source (Section 1.2), creating a cascade of ion-forming reactions. This is why mass spectrometry, especially when coupled with separation techniques such as GC or HPLC, is a highly specific way to identify organic compounds.

The components of the mass spectrometer that cause ion formation, separation, and detection are contained in an ultraclean housing usually kept at moderately high vacuum ($10^{-3}$–$10^{-6}$ torr[1]; some exceptions will be mentioned later). High vacuum ensures that, once the ions formed in the ion source begin to move toward the detector, they will not collide with other molecules because this could result in further fragmentation or deflect them from their desired path. Nearly all fragmentation reactions occurring under these conditions are *intramolecular* (involving only the decomposition of individual ions) rather than *intermolecular* (involving the reaction of ions with other species that may be present). High vacuum also protects the metal and oxide surfaces of the ion source, analyzer, and detector from corrosion by air and water vapor, which could compromise the spectrometer's ability to form, separate, and detect ions.

[1] 1 torr = 1 mm Hg, which is equivalent to ~133 pascal (Pa).

### 1.1.2. Sample Introduction

High sample purity is critical for unambiguous identification by mass spectrometry. The simultaneous presence of several different compounds in the ion source creates a situation in which ions from all these compounds are analyzed at the same time. This results in a composite mass spectrum that may be impossible to interpret. When capillary column GC is used for sample separation prior to introduction into the mass spectrometer, sample molecules can be introduced directly into the ion source of the spectrometer through the end of the capillary column. Carrier gas flow through a capillary column is low enough that the carrier gas can be removed by the vacuum system of the mass spectrometer. Helium (He) and hydrogen ($H_2$) are good choices as carrier gases for GC/MS work because their extremely low atomic and molecular masses (4 u and 2 u, respectively; 1 u = 1 unified atomic mass unit[2]) fall below those of all the ions normally seen in organic mass spectrometry.

HPLC has become increasingly important as an option for sample separation prior to mass spectral analysis—especially for compounds that are nonvolatile, thermally labile, or otherwise not amenable to analysis by GC. Capillary electrophoresis (CE) has also been coupled with mass spectrometry to separate and identify inherently ionic molecules such as amino acids, proteins, and DNA fragments. Whereas separation of sample and carrier gas is relatively straightforward in GC/MS, separating sample molecules from HPLC or CE solvents is more complex, so that combinations of these techniques with mass spectrometry for routine use have occurred only recently.

Other methods of sample introduction must be mentioned briefly. Analysis of a pure volatile liquid can be accomplished by placing the liquid in a small, evacuated glass bulb that is connected to the ion source with narrow metal or glass tubing and isolated from the MS vacuum system by a valve. Opening the valve causes the sample vapor to flow directly into the ion source. This method is used for introduction of the calibration and tuning standard perfluorotri-*n*-butylamine (PFTBA; see Section 1.5.1).

Samples that have low volatility or that may decompose during their passage through the GC can be placed on the tip of a probe that is inserted directly into the ion source. The probe tip containing the sample is inserted into a chamber that is isolated from the main vacuum system by a valve. This chamber is evacuated using an auxiliary vacuum pump, after which the valve is opened and the probe tip is inserted all the way into the ion source. Gentle heating of the probe tip provides volatilization of the sample and, in ideal cases, rudimentary fractional distillation of the desired compound. Nonetheless, sample purification prior to introduction by direct insertion probe is desirable. The added expense, potential for ion source

[2] There is currently a lack of consistency regarding the terms used for the atomic mass unit. The single term *amu* was used at one time, but it had different definitions in physics and chemistry, both involving $^{16}O$ as a standard mass. This term was discontinued when a unified standard mass was adopted. The International Union of Pure and Applied Chemistry (IUPAC) suggests the *unified atomic mass unit* (abbreviated u), which is based on $^{12}C$ (Section 2.1.2). The *dalton* (abbreviated Da) is identical in size to u and is the term used in biological and biochemical applications as well as for stoichimetric calculations.

contamination by introduction of too large a sample, and the versatility of modern chromatographic techniques have made these devices increasingly rare.

## 1.2. IONIZATION SOURCE

Sample molecules must be ionized in order to be analyzed and detected in mass spectrometry. Until fairly recently, volatile compounds were ionized primarily in the electron ionization (EI) source, which is still the most common ion source used in GC/MS work. Since the focus of this book is the interpretation of EI mass spectra, most of this section will describe the EI source. As the number of larger and less volatile molecules requiring analysis by mass spectrometry has grown, sample introduction and ionization techniques have been developed that produce detectable numbers of ions of these compounds. Some of these ionization techniques are now used so routinely that a brief description of them is warranted. A list of ionization methods and their application to various sample types is given in Table 1.1.

**Table 1.1. Molecular ionization methods in mass spectrometry**

| Type of Ionization | Ionizing Agent | Source Pressure | Uses |
|---|---|---|---|
| Electron ionization (EI) | 50–70 eV electrons | $10^{-4}$–$10^{-6}$ torr | Extensive fragmentation allows structure determination; GC/MS (Section 1.2.1) |
| Chemical ionization (CI) | Gaseous ions | ~1 torr | Molecular mass determination; GC/MS (Section 1.2.2) |
| Desorption ionization (DI) | | $10^{-5}$–$10^{-6}$ torr | Molecular mass and structures of high mass, nonvolatile compounds in condensed phase |
| Fast atom bombardment (FAB) | Energetic Ar or other neutral atoms | | |
| Laser desorption (LDI) and matrix-assisted LDI (MALDI) | Energetic photons | | Section 1.2.3.2 |
| Electrospray (ES) ionization | Electric field; ions in solution | Atmospheric or slightly reduced pressure | HPLC/MS and CE/MS (Section 1.2.3.1) |
| Atmospheric pressure chemical ionization (APCI) | Corona discharge; gaseous ions | Atmospheric | HPLC/MS |

### 1.2.1. Electron Ionization Source

Ion sources from different instrument manufacturers (and sometimes even different models from the same manufacturer) may differ from one another both in appearance and in names assigned to the component parts. However, most have the same basic design. A typical example is shown in Figure 1.2.

The EI source is most commonly a small chamber about 1 cc in volume, in which analyte molecules interact with a beam of highly energetic electrons that have typically been accelerated through a potential difference of 50–70 volts (V) across the volume of the ion source [50–70 electron volts (eV); 1 eV = 23 kcal]. This electron beam is produced by boiling electrons off a narrow strip or coil of wire made of a tungsten-rhenium alloy. Between the filament and the center of the ion source is a metal plate with a slit called the electron aperture. This slit limits the size of the electron beam and confines ionization to a small volume within the center of the ion source. Opposite the filament is the collector, a metal plate held at a positive electrical potential ($+V$ in Figure 1.2) that attracts and intercepts the electron beam after it has passed through the source. Surrounding the entire ion source

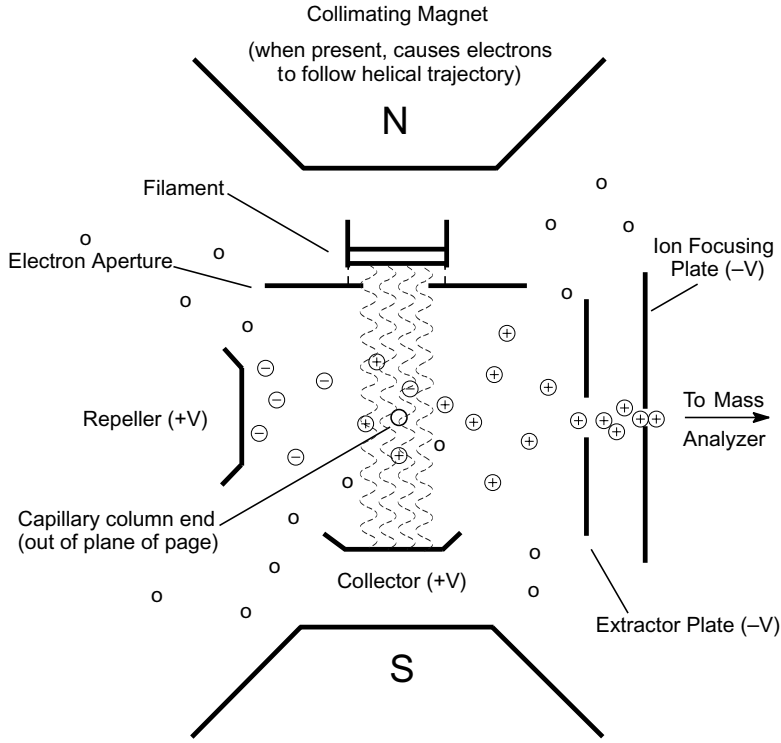


**Figure 1.2.** Schematic diagram of a typical electron ionization (EI) source. Samples can enter the source through a capillary GC column, a heated probe, or evacuated bulb through openings that are perpendicular to the plane of the page.

in some cases is a collimating magnet, which causes the electrons in the beam to travel in a helical path, as shown in Figure 1.2. Although this helical trajectory improves the probability that the electrons and molecules will interact, sample ionization is still very inefficient—less than one molecule in a thousand undergoes ionization.

What happens during ionization is complex. It is naïve to view electrons as literally smashing into sample molecules and knocking electrons out of orbitals. Instead, when an energetic electron approaches the electron density field of the molecule closely enough that sufficient energy is transferred quantum mechanically to overcome the ionization potential of the molecule, one electron is ejected from one of the bonding or nonbonding orbitals of the molecule (Section 3.3). Ionization energies (IE) for most organic compounds range from about 5–15 eV. Bond dissociation energies are even smaller, so this method of ionization not only causes molecules to expel one or more electrons, it also provides enough energy for substantial fragmentation of the first-formed ion (the molecular ion, $M^{+\bullet}$). Because of the excess energy present in 50–70 eV electrons, enough additional energy may be transferred to overcome the second, or even third, ionization potential of the molecule, leading to ions having +2 or +3 charges. The ionization process is discussed in more detail in Chapter 3.

Many different products form during ionization. Some of these are not positive ions. Table 1.2 lists the most important of these products. If the sample absorbs enough energy to raise an electron from the ground state to an excited state, but not enough to cause ejection of the electron, an ''excited molecule'' is formed (product *a* in Table 1.2). Excited molecules can return to their neutral ground state through thermal vibrations or the emission of light, and because no ions are formed in the process, they are simply pumped away from the ion source by the vacuum system.

**Table 1.2. Types of ionization reactions**

| | |
|---|---|
| | *a.* Excited molecule (not detected) |
| | *b.* Negative ion formation (not detected by positive EIMS): |
| | $(A{-}B{-}C)^{-\bullet}$ |
| | $(A{-}B)^{-} + C^{\bullet}$ and others |
| | *c.* Electron Ionization: $(\mathbf{A{-}B{-}C})^{+\bullet} + 2e^{-}$ |
| | *d.* Dissociative ionization: |
| $e^{-} + (A{-}B{-}C) \rightarrow$ | $A\cdot + (\mathbf{B{-}C})^{+} + 2e^{-}$ |
| | $(\mathbf{A{-}B})^{+} + C^{\bullet} + 2e^{-}$ |
| | $(A{-}B)^{\bullet} + \mathbf{C}^{+} + 2e^{-}$ and others |
| | *e.* Dissociative ionization with rearrangement: |
| | $(\mathbf{A{-}C})^{+\bullet} + B + 2e^{-}$ |
| | $(A{-}C) + \mathbf{B}^{+\bullet} + 2e^{-}$ |
| | *f.* Multiple ionization: |
| | $(\mathbf{A{-}B{-}C})^{2+} + 3e^{-}$ |
| | $(\mathbf{A{-}B})^{+} + \mathbf{C}^{+} + 3e^{-}$ and others |

Ions detected by positive ion EIMS are shown in boldface.

Sometimes the analyte molecule absorbs an electron and a negative ion is formed (Table 1.2, product *b*). In order to be absorbed by the molecule, the electron must be of very low energy (~0.1 eV), and there are few electrons of this energy in a standard EI source. By reversing the polarity of the repeller, ion focusing plate, and extractor plate in the ion source, and by altering the detector so that it will detect negative ions, a negative ion mass spectrum can be recorded. For most compounds negative ion MS offers few advantages over positive ion MS, and overall it tends to be less sensitive.[3] There are some specific applications, however, most notably with halogenated compounds. In this book only positive ion products and their fragmentations will be covered.

The remaining products listed in Table 1.2 are positive ions. The ion that is formed first results directly from ejection of a single electron from the neutral molecule (product *c*). This *molecular ion* ($M^{+\bullet}$) is very important because it has virtually the same mass as that of the analyte molecule (the small mass of the lost electron can be ignored). Indeed, mass spectrometry is one of the few analytical tools available for determining the molecular mass of a compound.

Ion products *d* and *e* in Table 1.2 are formed by unimolecular dissociation of $M^{+\bullet}$. In the first case a single bond is broken and a neutral group of atoms having an odd number of electrons (called a radical; see Section 3.1) is lost. The second process (dissociation with rearrangement) involves breaking some bonds while at the same time forming new ones. This results in expulsion of a fragment containing an even number of electrons, usually as a neutral molecule. The equations in Table 1.2 imply that such ions are formed in a concerted process in which ionization, bond making, and bond breaking all occur at about the same time. However, fragmentations that involve rearrangement of atoms usually occur in a stepwise fashion through one or more intermediates.

If more than one electron is ejected from the analyte molecule, ions having charges of +2, +3, or even +4 may be formed (Table 1.2, products *f*). Biopolymers such as peptides may have charge states of +10 or more from protonation of basic sites on the molecule. Since mass spectrometry actually measures the mass-to-charge ratio (*m*/*z*) of an ion, not its mass, an ion having a charge greater than +1 is found not at the *m*/*z* value corresponding to its mass (*m*), but rather at *m*/2, *m*/3, or *m*/4, depending on the number of charge states. Further, if *m* is not evenly divisible by the number of charges *z*, *m*/*z* will have a nonintegral value. For example, the double charged molecular ion ($M^{2+}$) of a compound having a molecular mass of 179 is found at *m*/*z* 179/2 = 89.5.

Most compounds do not produce multiple charge molecular ions in EI, but they may be formed in low abundance from small molecules that have few possible modes of fragmentation or from compounds with aromatic rings or large heteroatoms such as Cl, Br, or S. Mass spectrometers used for routine organic analysis

[3] A very sensitive and highly specific technique called resonance electron capture ionization (RECI) takes advantage of the low-energy electrons expelled during the EI of methane and results in the formation of negatively charged molecular ions ($M^{-\bullet}$).

often report $m/z$ values only to the nearest integral mass, or they may report only one peak for each $m/z$ value (Section 1.5.2). In such cases, detecting ions having nonintegral masses, even if they occur, is not always possible. Mass spectrometers with higher resolving power may be necessary to identify these ions with certainty.

The complex mixture of ionic and neutral products formed by any ionization method must be separated so that positive ion products travel in the direction of the $m/z$ analyzer, and negative ions and neutral products are left behind. Neutral products are removed by the vacuum system, because the electric and magnetic fields present in the ion source have no effect on their motion. Positive and negative ions, on the other hand, can be separated by appropriately placed charged surfaces in the ion source (Figure 1.2). To accomplish this, the repeller is kept at a positive potential ($+V$) both to attract and neutralize negative ion products and to repel positive ions. Conversely, the extractor plate and ion focusing plate (the ion optics) are both kept at a negative electrical potential ($-V$) to attract and accelerate the positive ions toward the $m/z$ analyzer. Slits in the extractor and ion focusing plates allow passage of the positive ions and help focus the ion beam as it approaches the analyzer.

When the filament is on and analyte molecules are flowing into the ion source, many reactive species are produced. Indeed, the intensity of the electron beam itself is sufficient to corrode metal surfaces in the ion source that are directly in its path—those on the electron aperture and collector. In addition, ion products may become electrically neutralized or undergo polymerization on the surfaces of the repeller, extractor plate, and ion focusing plate. Over time, the sensitivity of the instrument declines, as these surfaces are less able to maintain the potentials necessary for optimal ejection and focusing of positive ions from the source. Mechanical and chemical cleaning of the metal surfaces in the source is needed to restore sensitivity. The daily acquisition and evaluation of the spectrum of a standard compound whose ions' $m/z$ values and abundances are known help determine when tuning and source cleaning are necessary (Section 1.5.1).

Keeping the filament off when high concentrations of sample are present in the ion source (especially while solvents are eluting during a GC run) allows the source to remain usable for several months without cleaning. Chemical ionization (CI) mass spectrometry (Section 1.2.2), which depends on the presence of high ion concentrations in the source, leads to the deterioration of ion source performance more rapidly than EI under normal circumstances.

### 1.2.2. Chemical Ionization

Unlike EIMS, in which molecules are ionized through interaction with high-energy electrons, ionization in chemical ionization mass spectrometry (CIMS) depends on collisions of ions and molecules. In positive ion CIMS the sample is ionized by reaction with ions generated within a large excess of a relatively low molecular mass reagent gas such as methane (as $CH_5^+$), isobutane [as $(CH_3)_3C^+$], or ammonia

(as $NH_4^+$), at a pressure of about 1 torr. Although some reagent gas ions are themselves formed by ion/molecule reactions

$$CH_4 + e^- \rightarrow CH_4^{+\bullet} + 2e^-$$
$$CH_4^{+\bullet} + CH_4 \rightarrow CH_5^+ + {}^\bullet CH_3$$

others are formed by unimolecular decomposition of the $M^{+\bullet}$, for example,

$$(CH_3)_3CH + e^- \rightarrow (CH_3)_3CH^{+\bullet} + 2e^- \rightarrow (CH_3)_3C^+ + H^\bullet$$

In CIMS the concentration of analyte molecules (at approximately $10^{-3}$ torr) is small compared to that of reagent gas molecules. Thus, the electron beam, which is more energetic than that used in EIMS (~200 eV), preferentially ionizes the reagent gas. Analyte molecules are ionized through reaction with reagent gas ions, rather than by the electron beam. Most reagent gas ions are strong proton donors and form protonated molecules (sometimes incorrectly called pseudomolecular ions) that have a mass 1 u greater than that of the molecular mass of the original compound[4]:

$$M + CH_5^+ \rightarrow MH^+ + CH_4$$

This type of ion formation (often called *soft ionization*) imparts significantly less energy to analyte molecules than do interactions with high-energy electrons, so that the resulting ions have little excess internal energy. These ions therefore fragment less than those formed by EIMS. As a result, although CIMS is useful for determining the molecular mass of compounds that do not produce a detectable $M^{+\bullet}$ by EIMS (see Figure 1.3), CI mass spectra may show an insufficient number of fragment ion peaks to yield structural information. The protonated molecules produced during CIMS can be induced to undergo fragmentation by combining CI with product-ion mass spectrometry/mass spectrometry (MS/MS; see Section 1.3.4.1). This technique yields structural information similar to that obtained by fragmentation of the $M^{+\bullet}$ in EIMS.

The interpretation of CI spectra, as well as spectra produced by electrospray and desorption ionization methods (Section 1.2.3), will not be covered in this book.

### 1.2.3. Other Ionization Methods

***1.2.3.1. Electrospray Ionization.*** The conventional ion source shown in Figure 1.2 can be used for both EI and CI, provided the sample enters the ion source in the gaseous state. Although many organic compounds can be analyzed in this

[4] Some reagent gas ions may react with sample molecules by addition, rather than by proton donation. It is not unusual to observe weak intensity peaks at *m/z* values greater than that expected for the protonated molecule, corresponding to the addition of one or more reagent gas ions to the sample molecule. In some instances, CI can also result in hydride abstraction, thereby forming an $(M - H)^+$ ion, which has a mass 1 u less than the analyte molecule.

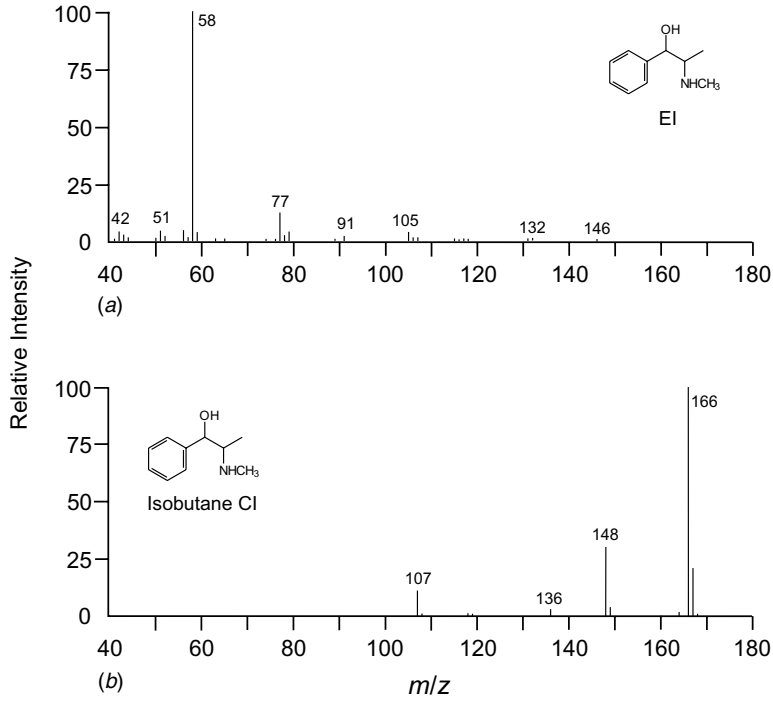


**Figure 1.3.** Mass spectra of ephedrine resulting from (*a*) EI and (*b*) chemical ionization (CI) using isobutane as the reagent gas (adapted with permission from Fales et al., 1975. Copyright American Chemical Society). The peak at *m*/*z* 166 in (*b*) corresponds to the protonated molecule.

manner, a large number of compounds, because of their inherent size and/or charge state, are nonvolatile or thermally labile. Many of these compounds are most easily separated by HPLC or CE, in which separation takes place in solvents that have an aqueous component. John B. Fenn and Koichi Tanaka shared the 2002 Nobel Prize in chemistry for their development of methods such as electrospray ionization (ESI) and desorption ionization in the analysis of large biological molecules.

The ESI source has allowed LC/MS and CE/MS to become routine analytical tools. Basically ESI works by converting the HPLC or CE effluent, already containing the sample in ionic form, into an aerosol and subjecting the resulting spray to high voltage in a chamber held near atmospheric pressure (Figure 1.4). This process creates a mist of charged droplets that flow toward the orifice of the capillary. In the configuration shown, the nebulizing needle, which creates the aerosol, is orthogonal (perpendicular) to the eventual direction of ion flow toward the *m*/*z* analyzer. Other geometric configurations are possible and have been used.

As the charged droplets travel toward the capillary opening, they are subjected to the counterflow of a drying gas, such as nitrogen ($N_2$), which causes evaporation of